








Development of Nursery Techniques in Important Forest Tree Species - *Pterocarpus marsupium* Roxb. and *Santalum album* L.

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By

Rajkumar B. Barmukh

Under the Guidance of
Dr. T.D. Nikam M.Sc., Ph.D.

Department of Botany
University of Pune
Pune – 411 007 (India)

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UNIVERSITY OF PUNE
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DEPARTMENT OF BOTANY
UNIVERSITY OF PUNE
Ganeshkhind,
Pune-411 007 (INDIA).

Ref. No. **: BOT /**

Date **:**

Tel. No. : 25601219
Telex : 145-7719 UNIP IN
Gram : UNIPUNA
Fax : 020-25690498
E-mail : unipune.ernet.in

CERTIFICATE OF THE GUIDE

CERTIFIED that the work incorporated in the thesis 'Development of nursery techniques in important forest tree species *Pterocarpus marsupium* Roxb. and *Santalum album* L.' submitted by Mr. Rajkumar B Barmukh was carried out by the candidate under my guidance. Such material as has been obtained from other sources has been duly acknowledged in the thesis.

(T D Nikam)
Research Guide

DECLARATION BY THE CANDIDATE

I declare that the thesis entitled 'Development of nursery techniques in important forest tree species *Pterocarpus marsupium* Roxb. and *Santalum album* L.' submitted by me for the degree of Doctor of Philosophy is the record of work carried out by me during the period from December 1999 to July 2007 under the guidance of Dr T D Nikam, Reader, Department of Botany, University of Pune, and has not formed the basis for the award of any degree, diploma, associationship, fellowship, titles in this or any other University or other institution of Higher learning.

I further declare that the material obtained from other sources has been duly acknowledged in the thesis.

Date: 3rd August 2007.

Rajkumar Barmukh

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(Rajkumar B Barmukh)

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Chapter I

Introduction

Role of seeds in regeneration

The exponential rise in the human population is directly threatening the forest covers by such activities as urbanization, agriculture, industrialization, mining and increased demands for wood and other forest based products. The forest cover will decline by about 477 million hectares by the year 2030 and the largest area decline will occur in Asia and Africa (Hannerz, 2006). Tropical forests account for about 52% of the total forest area of the world and are well known as storehouses of biodiversity. India possesses almost the entire range of tropical forest, from dry deciduous to wet evergreen. However, surprisingly little information is available on the requirements at seed and seedling stages for the majority of the tropical species (Khurana and Singh, 2001) and at the same time, the studies on nursery germination were restricted to few species. To meet the increasing demands, there is need to work out the germination studies and development of seedlings of tropical forest species for conservation, reforestation, and for sustainable utilization.

Pterocarpus marsupium Roxb. and *Santalum album* L. are commercially important tropical forest tree species recognized for their quality timber and fragrant heartwood, respectively. These species also have medicinal importance in Ayurveda and other traditional systems of medicine. The *S. album* is a source of oldest known and highly acclaimed perfumery material. However, the ever increasing demands have lead to the mass scale illegal cutting and poaching. Therefore the natural stands of these species are declining at an alarming rate and if proper steps are not taken, materials derived from these trees will be hardly available in future. Recently, these plants are categorized as threatened (Das *et al.*, 2001; Tiwari *et al.* 2004). On the other hand, natural resurgence in these species is poor and unreliable and meager information is available on their nursery germination. Considering the importance and potential of these species as planting materials, the present investigation was undertaken with an objective to identify reliable methods to induce uniform nursery

germination for raising the quality seedlings for mass plantation of these species.

Seeds are unique in natural regeneration and propagation of higher plants. This uniqueness is attributed to seed's exclusive genetic composition that results from mixing parental genetic material. The diverse genetic compositions offer wider range of ecological adaptability. As compared to vegetative propagules, seeds are much more resistant to damage and environmental stress and are usually produced in large numbers and are readily available each year or at longer intervals. More importantly, seeds of many species can be stored for longer periods under suitable conditions.

The habit of producing seeds for regeneration developed in ancient gymnosperms some 300 million years ago. The principal roles of the seed are to give protection to the embryo, to be dispersed into a new environment, and to provide nutrients for the embryo during its germination and establishment.

Using seeds is a valuable propagation method because it reduces the risk of injuring the parent plant, minimizes the impact on natural settings, and makes it possible to grow a large number of seedlings. In some cases, seed propagation is the only practical means of propagating a plant, because vegetative means have not been explored or have been unsuccessful. The fundamental objective of seed propagation is to promote rapid and uniform germination and establishment of seedlings. To achieve this successfully the knowledge on seed germination and seedling growth is, therefore, necessary for the success of efforts on augmentation, introduction and re-introduction of tree populations (Khurana and Singh, 2001).

Structure of seed

A seed is composed of three basic parts: the embryo, the food storage tissue, and the seed covering. A mature viable seed needs enough stored food or energy source for germination and early seedling growth. Seeds of some species have a mass of food reserve called endosperm

surrounding or in contact with the embryo while in other species, the reserved food is stored in the cotyledons attached to the embryo. Seed coverings can consist of the seed coat and parts of the fruit or seedpod. These structures protect the embryo and food reserve inside the seed. They can also inhibit germination until conditions are suitable for germination and seedling development.

Seed germination

The process of germination commences with the uptake of water by imbibition of the dry seed, followed by embryo expansion growth. Water uptake triggers the activation of certain biochemical processes that result in the synthesis of the building blocks (nucleic acids, amino acids, enzymes, etc.) for growth and development. This usually culminates in rupture of the covering layers and emergence of the radicle is generally considered as the completion of germination. Initiation of cell elongation in embryonic axes has been recognized as a key factor in seed germination (Evenari *et al.,* 1957; Hegarty, 1977; Mohr and Schopfer, 1995). Dormant embryos restrict their own water uptake even if free water is available (Boubriak *et al.,* 1997) suggesting that the germinability positively correlates with the water uptake capacity of the embryo or the embryonic axes. Water uptake of the germinating embryo is controlled by cell wall loosening rather than by changes of osmotic pressure (Schopfer and Plachy, 1985)

Seeds can exhibit notoriously idiosyncratic germination behavior. Delayed and irregular germination in the nursery is a severe restriction on efficient nursery management (Bonner *et al.*, 1974). Man has shown a strong interest in managing seed germination and much research has therefore gone into devising effective artificial treatments to remove dormancy and/or to ensure that the seeds quick and even germination in the nursery beds. Dormant seeds have the potential to germinate but are prevented from doing so by some mechanism.

Seed dormancy is a temporary failure or block of a viable seed to complete germination under physical conditions that normally favor the process (Hilhorst, 1995; Bewley, 1997; Koornneef *et al.*, 2002). It is nature's way of setting a time clock that allows seeds to initiate germination when conditions are normally favorable for germination and survival of the seedlings. Plants are endowed with several mechanisms of dormancy to optimize the time of seed germination (Rees, 1997; Jones, 1999; Funes and Venier, 2006) in order to secure the successful establishment of their seedlings in natural populations (Bewley and Black, 1994; Li and Foley, 1997; Foley, 2001). Although it is an efficient mechanism to guarantee the survival and perpetuation of the species, seed dormancy is an important limiting factor for plant propagation in nurseries (Malavasi and Malavasi, 2004).

The phenomenon of plant seed dormancy is of great economical importance in modern agriculture and horticulture. Greatly valued are the seeds that germinate evenly, fast and with high efficiency in order to establish a vigorous and uniform population of plants. Even though all the proper growth conditions are present, dormant seeds do not germinate unless their dormancy mechanism has been overcome. Balancing the usefulness of limited levels of dormancy with the need to induce germination upon demand has consumed the efforts of physiologists, geneticists, and breeders for generations.

Seed dormancy is often caused by structures surrounding the embryo, the physiological state of the embryo itself, or a combination of these factors (Tian *et al.*, 2002). It can be caused by a hard seed coat that is impermeable to water or gases, or resistant to embryo expansion. Seed dormancy in many species is caused by the inhibitory influence of structures covering the embryo rather than by factors within the embryo itself (Toole *et al.*, 1956). Nikolaeva (1977) divided dormancy types into those that are "endogenous" and due to properties of the embryo and those that are "exogenous" and result from properties of the endosperm or any

other tissues of the seed or fruit. Kigel (1995) defined 'hard seeds' as those with an impermeable seed coat, and thus are unable to hydrate and germinate even when in contact with free water.

***Pterocarpus marsupium* Roxb.**

Pterocarpus marsupium Roxb., (Bija or Bijasar or Bijasal, Fabacaeae) is a deciduous tree that can reach the height up to 30 m and girth of about 2.5 m. The tree has a straight clean bole. Earlier it was reported to be commonly found in hilly regions throughout the Deccan Peninsula and extending to the states of Gujarat, Madhya Pradesh, Uttar Pradesh, Bihar and Orissa (Anonymous, 1998). The gray bark is rough, scaly and longitudinally fissured. Old trees exude a blood red gum resin. Leaves are imparipinnate with usually 5-7 oblong leaflets. Flowers are yellowish, fragrant and produced in large panicles. Fruit is orbicular winged pod (samara) about 5 cm in diameter. Each pod bears 1-2 seeds (Plate III-2 and Plate IV-1).

Pterocarpus marsupium Roxb., is a significant element of Indian forests. Its timber is highly valued in the international market for its quality (Kirtikar and Basu, 1999; Husain and Shahzad, 2007). In India, this timber is rated next to that of teak. It is chiefly used for building purposes as a substitute for teak after suitable seasoning and treatment. The wood is used for crafting doors, window frames, rafters, beams, and posts. It is also used in the construction of railway carriages, wagons, carts and boats and occasionally for shipbuilding.

Apart from this, *P. marsupium* is a medicinally important species as well. The gum exude 'Kino' obtained from this tree has antipyretic, antihelminthic properties and is used as an astringent (Singh *et al.*,, 1965). The bark is used for the treatment of stomachache, cholera, dysentery, urinary complaints, tongue diseases and toothache. The wood is of great importance as infusion or decoction of it is taken for diabetes. Its

bitter flowers improve the appetite and cause flatulence (Tiwari *et al.*, 2004).

The seeds of *P. marsupium* are encased in a hard and impermeable fruit coat. Fruits remain indehiscent at maturity, and require physical abrasion or other mechanisms to open the fruit and allow seeds to imbibe and germination to proceed. This characteristic of legume is a major reason for seed dormancy leading to uneven germination which is a major set back in reforestation programmes (Aduradola, 2005). The regeneration of leguminous trees in natural habitats is low (Dewan *et al.*, 1992). Similar to many other leguminous taxa, the seed germination in *P. marsupium*, has been reported to be very poor (Lakhsmi *et al.*, 1992; Das and Chatterjee, 1993). Its propagation through stem cuttings has been tried but has revealed a feeble response (Tiwari *et al.*, 2004). The poor propagation coupled with overexploitation for commercial and pharmaceutical use is gradually widening the gap between demand and supply and thus putting further pressure on the species. The indiscriminate exploitation of *Pterocarpus marsupium* Roxb. has resulted in its inclusion in the list of depleted plant species (Chaudhari and Sarkar, 2002). According to Tiwari *et al.* (2004), the depletion of this species from its natural habitat has brought it at the verge of extinction and will extinct soon if proper steps are not taken for its conservation.

***Santalum album* L.**

Santalum album L., (Indian Sandalwood, Santalaceae) is an important tree species that is regarded to be native to Peninsular India (Fischer, 1938). It is a small to medium-sized, semi-parasitic evergreen tree that bears slender branches. A healthy specimen can reach up to 18 m in height and 2-4 m in girth. It is commonly found in the comparatively dry regions of peninsular India from Vindhya Mountains southwards, especially in Mysore and Tamil Nadu. It has also been introduced into Rajasthan, parts of Uttar Pradesh, Madhya Pradesh and Orissa, where it has

become naturalized at some places. Bark is reddish, rough with deep vertical cracks on old trees. Leaves are glabrous, thin, elliptic-ovate or ovate-lanceolate. Flowers are straw-colored, brownish purple, reddish purple, or violet, unscented and are produced in terminal and axillary paniculate cymes. The fruit is globose drupe, about 1 cm in diameter, purple-black. The endocarp is ribbed. Seeds are globose or obovoid.

Sandalwood tree is deeply connected with many events of rich cultural heritage in India and is highly acclaimed worldwide. It is among the oldest known perfumery material (Srinivasan *et al.*, 1992). Owing to its unique persistent sweet and woody fragrance, its heartwood oil (sandalwood oil) is widely used in internationally reputed brands of perfumes (Srinivasan *et al.*, 1992) and in cosmetic and aromatherapy industries (Kim *et al.*, 2006).

The sandalwood oil has been reported to have various biological properties such as antiviral, anti-carcinogenesis and antitumor effects (Kim *et al.*, 2006). Banerjee *et al.* (1993) have reported that sandalwood oil shows an indirect antioxidant activity by increasing the glutathione-*S*-transferase (GST) activity and the acid soluble SH level in the liver. The heartwood of mature trees (□ 10 years old) contains essential oils, chiefly the sesquiterpen alcohols *cis*-α-santalol (Verghese *et al.*, 1990), α-*trans*-bergamotol, *epi-cis*-β-santalol along with small quantities of *trans*-β-santalol and *cis*-lanceol (Howes *et al.*, 2004). Among the reported constituents, α-santalol is one of the major components in many species of the *Santalum* genus, is responsible for most of the activity of the oil (Kim *et al.*, 2006).

The estimated global annual requirement is about 10000 tonnes of sandalwood (equivalent to 200 tonnes oil), involving a trade of about $125 million (Das *et al.*, 2001). The heartwood has commercial importance due its close grains, making it highest quality wood for carving in fabricating costly handicraft items that are fragrant, elegant looking and are largely self-protective from termites and woodborers (Anonymous,

1999). The demand for sandalwood is ever increasing but the supply is decreasing steadily. The sandalwood tree is suited for aforestation programs in the draught-prone and arid-prone areas of Indian peninsula, yet very less effort on this line have been made so far. Moreover, this important tree species is under the threat of extinction due to spike disease and illegal poaching (Das *et al.*, 2001). The natural resurgence in this species is from seeds but the seeds have poor and delayed germination.

Seed and forest regeneration

According to the report on Global Forest Resource Assessment- 2005 by FAO (FAO, 2005), the total forest area in 2005 was just under 4 billion hectares corresponding to an average of 0.62 ha per capita. This land under forests corresponds to about 30% of the total land area. However, the area of forest is unevenly distributed. Among the top ten countries with the largest forest area, the same report ranked India as 10^{th} country with about 68 million ha of forest cover. Hannerz (2006) quoted that by the year 2030 the forest cover will decline by about 477 million ha and the largest area decline will occur in Asia and Africa. The forest cover in India has been estimated to be 678,333 km^2, which constitutes only 20.64% of the country's geographical area. To maintain a stable environment and sustainable development at least about 33% of the land should be covered by forest. Since the forest and forest trees are not an infinite resource, the indiscriminate use of forest resources is gradually limiting the supply of traditional woods and other forest products imperative in national as well as international trade. Forest regeneration systems are therefore being encouraged in most of the developing countries (Aduradola *et al.*, 2005) to ensure that rate of production of wood and other forest resources meet the demand for various forest products.

With a few exceptions like the poplars and willows and some tropical species of *Casuarina*, tree species are usually propagated from

seed. With the exception of few renowned species such as *Tectona grandis*, exploration on tropical forest seeds has been insufficient in comparison with the severity of the problems and the number of species with potential value for plantations. In most regeneration systems, the quality of seed is a key input in the production process (Nwoboshi, 1982).

Seed propagation is an important mode of propagation in silviculture in the temperate and tropical regions alike. Seed germination and early seedling development stages are critical periods for the establishment of plant species. Good forestry projects start with good seeds and forestry industries also have a requirement for predictable and uniform seedling establishment. The knowledge of seed biology is also essential for understanding the process and patterns within a given plant community, such as the establishment of plants, succession and natural regeneration (Vázquez Yanes and Orozco-Segovia, 1993).

The seed propagation is easy, fast and a reliable method for rapid multiplication of species. However, successful seed germination depends on numerous internal and external factors and seeds that are difficult to germinate are generally considered to have some form of dormancy. There are several methods to free seeds from dormancy and initiate early growth, such as stratification, scarification, growth regulators etc. Gibberellic acid has been used for promoting germination of many kinds of seeds. Moreover, gibberellic acid can largely replace cold and light requirements, and scarification needed by some seeds for germination. The exogenous application of growth regulators such as auxin, gibberellins, cytokinins and chemicals such as potassium nitrate or thiourea have been found to improve the seed germination in many species.

The *Pterocarpus marsupium* Roxb. and *Santalum album* L. are multipurpose trees with very high market value. The existing natural stands of these trees are reducing at an alarming rate. Recently these species are reported as threatened and therefore there is need for their conservation. The natural resurgence is from seeds but the seeds possess

some kind of dormancy principle leading to delayed and poor germination. This has been a major constraint in nursery germination and preparation of seedlings. The vegetative methods of propagation have revealed a feeble response and biotechnological approaches such as tissue culture are neither cost effective nor easy to handle by nurserymen.

In the present investigation, therefore, the attempts were made to acquire the basic knowledge about seed harvesting, processing, assessing viability, dormancy, germination, seed storage, seedling vigor and seed storage physiology and to induce quick, reliable and uniform nursery germination by employing various dormancy braking methods like physical scarification, acid scarification, application of germination stimulants and plant growth regulators for raising the quality seedlings, and maintenance of the seedlings in the nursery for mass plantation of *Pterocarpus marsupium* Roxb. and *Santalum album* L.

Chapter II

Review of Literature

2.1 Seed biology of *Pterocarpus* species and other Fabaceae

Plant species of fifteen different families produce seeds whose coats are temporarily impermeable to water and, possibly, gases. Such seeds do not germinate even if exposed to conditions ideal for germination; they are called "hard seeds". Among these families is the Fabaceae, of which most species produce hard seeds (Crocker & Barton, 1957; Baskin & Baskin, 2001). In terms of economic importance the Leguminosae is the most important family in the Dicotyledonae (Harborne, 1994). Among the three subfamilies of Leguminose, Fabaceae (Papilionaceae) is the most useful to man and provides a very large number of important crop plants. The fruit is a pod, often a legume.

The impermeability of seed coat to water, otherwise known as hardseededness, is a common mechanism of dormancy in Leguminosae (Rolston, 1978). Dormancy due to hardseededness is also found in several other families viz., Anacardiaceae, Bixaceae, Cannaceae, Convolvulaceae, Ebenaceae, Geraniaceae, Liliaceae, Malvaceae, Myrtaceae, Rhamaceae, Sapindaceae, Solanaceae and Zingiberaceae (Ballard, 1973; Atwater, 1980).

With few exceptions, seeds from Fabaceae were reported to be having innate or secondary dormancy and hardseededness has been a major problem preventing or delaying seed germination. The presence of hardseededness contributes to heterogeneous seedling emergence (Woodstock 1988; Argel & Patton 1999).

The majority of treatments listed as dormancy-breaking treatments were in fact treatments to remove hardseededness. Seeds of members of the Fabaceae have a region of the testa described as the strophiole through which the imbibition of initially hard seeds may occur if treated appropriately. Therefore the treatments that created a gap (the strophiolar cleft) in the impermeable testa through which moisture can enter seeds of species within this tribe have proven effective in enhancing seed germination.

Imbibition injury was a further barrier in the seed germination studies in the family Leguminosae. Imbibition injury is damage caused by very rapid imbibition of water by very dry seeds when they are set to germinate.

As with many woody species, drying of seeds may induce seed coat dormancy in seeds that would normally germinate without pretreatment (Dirr and Heuser 1987).

Several efficient pretreatments to overcome dormancy of Leguminosae seeds are documented in the literature (Schmidt, 2000; Baskin and Baskin, 2001), of which, the physical scarification has been most commonly used. Cruz *et al.* (2001) scarified the seeds of *Hymenaea intermedia* to give 96% germination in 26 days, while non-scarified seeds reached similar value only after 418 days.

In *Bowdichia virgiloides*, 79% of germination was obtained when seeds were scarified, as against 21% in the non-scarified seeds (Smiderle and Souza, 2003). In *Sesbania sesban* germination was 82% after seed scarification as compared to 68% in non-scarified seeds (Veasey and Freitas, 2002). Exposing the seeds to hot water (60 to 100 °C) is another competent method of overcoming hardseededness (Bianchetti, 1981; Bianchetti and Ramos, 1981; Bianchetti and Ramos, 1982; Baskin and Baskin, 2001).

Seeds of *Dimorphandra mollis* immersed in boiling water (100 °C) for two minutes showed 64% germination contrasting 12% germination in untreated seeds (Hermansen *et al.*, 2000). In *Dinizia excels*, 62% germination was recorded in seeds immersed in water at 80 °C for 10 minutes as against 7% in the control treatment (Vastano Júnior *et al.*, 1983). Seeds of *Mimosa scabrella* immersed in water at 90 °C showed 79% germination, whereas only 17% germination was observed in untreated seeds (Bianchetti, 1981).

The seeds of *Lupinus montanus* have physical dormancy and the germination improved after the application of pretreatments that soften

the seed coat (Rodriguez and Rojo, 1997). In *Lupinus sulphureus ssp. Kincaidii,* only 9% seeds without stratification or scarification showed germination. Scarification alone resulted in 45% germination, while stratification alone yielded 17% and 23% mean germination after pre-chilling for 4 and 8 weeks respectively.

Amorpha californica Nutt. and *Amorpha canescens* Pursh. germinated completely without treatment (Mirov and Kraebel 1939). *Amorpha canescens* Pursh. seeds stratified at 3 to 4 ^{0}C for 2 and 8 weeks showed increased rate of germination due to possible reduction in seed coat impermeability, while 30 minutes of scarification in sulphuric acid reduced germination by 50% (Cox and Klett 1984). Light scarification of *A. fruticosa* L. seeds and soaking seed in sulphuric acid for 5 to 8 minutes has been used to stimulate germination (Brinkman, 1974; Dirr and Heuser, 1987).

Most of the *Acacia* species produce seeds with hard seed coats and therefore have poor germination unless they are first scarified by briefly treating them with sulphuric acid or soaking in hot water (Kumar and Purkayastha 1972; Natarajan and Rai 1988; Rana and Nautiyal 1989; Gunn 1990). ISTA (1993) has recommended clipping, nicking, or filing through the seedcoats followed by soaking in water for 3 hours, or soaking seeds in concentrated sulphuric acid for 1 hour followed by a thorough rinsing for testing the germination in acacias.

In India, the genus *Pterocarpus* L. is represented by two important forest tree species, *P. marsupium* Roxb., the species under investigation and *P. santalinus*, commonly called as red sanders or red sandalwood. Various seed germination studies on the red sanders had been undertaken but the literature on seed germination in Bijasal is very sparse.

The seed germination in *Pterocarpus marsupium* Roxb. was reported to be very poor (Lakshmi *et al.,* 1992; Das and Chatterjee, 1993, Kalimuthu and Lakshamanan, 1994). Studies on the effect of shade on seed germination in *P. marsupium* showed that seed germination percentage was

improved on shaded nursery beds. Kalimuthu and Lakshamanan (1994, 1995) have reported better germination in the pods of *Pterocarpus santalinus* and *P. marsupium* soaked in 40% HCl for 24 hours. Das (1993) reported poor *in vivo* as well as *in vitro* seed germination percentage in *P. marsupium.* Low germination was also observed when the fruit coat and seed coat were removed manually.

Naidu and Mastan (2001) have reported improved seed germination in *P. santalinus* when the seeds were soaked in cow-dung slurry (2-6 days), soaked in boiled water (80-100 ^{0}C), when placed under running tap water (2-6 days), exposed to alternate soaking and drying (4-8days) and seeds soaked continuously in normal water for 4-8 days. Rai *et al.* (1986) suggested that application of cow dung slurry to the pods attracted termites which feed on the fibrous seed covering of *P. santalinus* that resulted in elevated seed germination.

Pods of *P. santalinus* treated with up to 500 ppm kinetin for different durations from 10-50 hours resulted in improved percentage of germination in all imbibitional periods and maximum germination percentage (80%) was observed in pods treated with 400 ppm kinetin for 10 hours. The germination percentage decreased with increased duration of soaking in 500 ppm kinetin concentration (Naidu and Rajendrudu, 2001).

Naidu (2001) studied the influence of gibberellic acid, IBA and IAA on seed germination in *P. santalinus.* Soaking of pods in IAA, IBA and GA_3 solutions of 200 and 800 ppm was effective to induce pod germination and GA3 was reported as more effective in improving pod germination than either IBA or IAA.

Tiwari *et al.,* (1999) studied the effects of IAA, IBA, and GA_3 at 6 concentrations (1, 2, 5, 10, 20, and 100 ppm) on germination percentage in *P. marsupium.* IAA at lower concentrations (1-20 ppm) had only marginal effects on seed germination and higher concentrations of IAA had an inhibitory effect while IBA promoted only marginal increase

in germination at lower concentrations and was inhibitory at higher concentrations. Effects of GA_3 were similar to IBA.

Dayanand and Lohidas (1998) treated the pods of *P. santalinus* by soaking in tap water for 4 or 8 days, with the water changed every 24 hours; soaking in 1% sulphuric acid for 4 days, dipping in concentrated sulphuric acid for 5 minutes, scarifying by rubbing with sand and separating seeds with a sharp knife or scalpel. All the treatments except the acid treatments increased germination. The best germination (83.5%) occurred from seeds, followed by pods soaked in tap water for 8 days (75.0%), scarification of pods (58.5%) and soaking of pods in tap water for 4 days (51.5%).

Anuradha and Pullaiah (1999) have reported a protocol for *in vitro* shoot multiplication of *Pterocarpus santalinus* where the highest shoot bud regeneration frequency (10-15%) was achieved by culturing mesocotyl explants on B5 medium fortified with 3 mg/l 6-Benzylaminopurine (BAP) + 1 mg/l NAA within six week culture period. Shoots treated with 1 mg/l IAA or NAA or IBA prior to transferring them to the rooting medium exhibited better rooting than those with no prior treatment.

In vitro seed culture was used for induction of enhanced axillary branching in *Pterocarpus marsupium* and *P. santalinus* (Anuradha and Pullaiah, 1999b). Recently, Anis *et al.* (2005) and Husain and Shahzad (2007) have reported a successful approach for *in vitro* propagation of *P. marsupium.*

Various treatments like physical scarification, overnight soaking in cold water, HCl or HCl + alcohol and drying, or 2 hour soaking in HCl + alcohol and drying were tried by Anuradha and Pullaiah (1998) for efficient pod breakage in *P. santalinus.* Soaking of pods for 2 hours in HCl + alcohol and drying was the best but only gave 10% seed germination (the other methods gave even less).

The highest percentage of healthy seedlings (42-70%) was obtained from seeds presoaked in water for 24 hours followed by inoculation on half strength B5 medium + 0.05% activated charcoal + 4% agar + 0.1 mg/l BAP. Germination on 0.9% agar alone was 10- 12%. Multiple shoots were obtained when the medium was supplemented with 3 mg/l BAP (Anuradha and Pullaiah, 1998).

Jain (1996) studied factors important for improving natural regeneration of *P. santalinus* and found that uprooting of *Cymbopogon coloratus* grass as soon as it started to come up after the rains, was useful for natural regeneration of *P. santalinus,* since this grass is highly prone to burning when it dries and the consequent annual forest fires kill most of the regenerating *P. santalinus* seedlings and saplings. Further, removal of other interfering species such as *Ziziphus horrida, Bauhinia racemosa* and *Acacia sandra,* which compete for light, improved natural regeneration by 15.8-21.5%.

Vegetative propagation of elite trees of *P. santalinus* by grafting, air layering, and shoot cuttings was described by Reddy and Srivasuki (1990). None of the methods described had a high success rate, but the use of shoot cuttings was the most promising for large scale propagation where rooting was induced by immersing the lower end of the cuttings in IAA, IBA and IPA for 24 hours. Concentrations of growth regulators <75 ppm induced rooting, with 25% rooting (the maximum achieved) found with IBA.

Sarita *et al.,* (1988) obtained *in vitro* shoot multiplication from single node and terminal cuttings derived from aseptic seedlings and shoots differentiated from cotyledon callus of *P. santalinus.* Murashige and Skoog (MS) medium (1/4 salts) + BAP (3 μM) + adenine (0.4 mM) was suitable for micropropagation. Adventitious roots were formed from the cotyledonary callus cultured on MS + BAP (3 μM) at 28±2 ^{0}C.

2.2 Seed biology of *Santalum* species and other Santalaceae

Baskin and Baskin (2001) have included 17 genera of the family Santalaceae under the category of rooted hemiparasites whose seeds do not require a host stimulus for germination. The seeds of Santalaceae do not have a palisade layer of macrosclerides in the seed coat and they do not have physical dormancy (Corner, 1976). Seeds of at least some members of the family Santalaceae, including *Buckleya, Comandra,* and *Pyrularia* (Martin, 1946) and *Santalum* (Rangaswamy and Rao, 1963), have underdeveloped and linear embryos and thus potentially have morphological or morphophysiological dormancy. Because seeds of *Comandra* (Piehl, 1965), *Santalum album* (Srimathi and Rao, 1969), and *Buckleya* (Musselman and Mann, 1979) require long periods of cold or warm stratification before they germinate, Baskin and Baskin (2001) concluded that the embryos are physiologically dormant and that the seeds have morphophysiological dormancy.

Natural regeneration of sandal (*Santalum album* L.) is poor because of low germination (10-20%), scavenging of germinated seeds by squirrels and rodents, and browsing and trampling of young seedlings by wildlife and cattle (Nagarajaiah and Rao, 1993). Micropropagation through multiple shoot induction followed by rooting does not work well for most tree species, including sandalwood (Das *et al.*, 2001).

The delayed germination affects nursery management and increases the cost. The sandal seeds normally take a minimum period of 30 days after the dormancy period for germination to start and more than 140-150 days for obtaining 80% germination (Nagaveni and Shrimathi 1981).

To breakdown the dormancy period and promote faster germination, Toole *et al.*, (1956) used nitrates and nitrites on the seeds of 85 plant species belonging to 15 families. Potassium nitrate was the only chemical approved under international rules for breaking dormancy until the finding of GA and other chemicals. According to Akamine (1947), the

mechanical restraint of the tough seed coat that prevents the embryo from expanding to its maximum imbibitional capacity resulted in the poor germination of sandal seed. This hard seed coat is either less permeable to water imbibition or possesses some principal making the seed liable to delayed germination (Nagaveni and Shrimathi, 1981).

Nagaveni and Shrimathi (1981) have reported that mechanical removal of the hard seed coat results in increased germination percentage in *Santalum album.* Such incising of seeds had significantly accelerated germination over non-incised seeds (Effendi *et al.,* 1996). The germination rate, germination value, and germination percentage reached the optimum in the 36-hour soaking treatment for incised seeds and in the 72-hour soaking treatment for non-incised seed.

Bagchi and Kulkarni (1985) have reported significant differences in the germination behavior of sandal seeds collected from different trees. Germination ranged from 25.0 to 61.0% (av. 35%) and seedling survival (as a proportion of original seeds) ranged from 31.0 to 58.0% (av. 42.4%), 62 days after sowing.

Ananthapadmanabha *et al.,* (1988) studied the effect of NaOH, HCl and GA leachates of sandal seeds on germination in green gram (*Vigna radiata*), Bengal gram (*Cicer arietinum*), wheat (*Triticum aestivum*), *Trigonella foenum graecum* and decoated seeds of sandal. NaOH, HCl and GA leachates inhibited sandal germination almost completely for 30 days and germination was still low after 60 days (20-26% compared with 70% in untreated control seeds). For the other species, all leachates were inhibitory in the order HCl > GA > NaOH.

Surendran *et al.,* (1998) reported early germination after treating the seeds with GA (0.05%). Manohar *et al.,* (1999) have reported improved germination in sandal after 50-250 ppm GA_3 treatments for 16 hours and 250 ppm GA_3 for 16 hours gave best germination (78%) when the seeds were sown in soil/sand/organic manure (1:1:2) in trays kept in the greenhouse.

Ramaswamy (1949) treated the sandal seeds with coconut water to hasten germination, but there was no effect by this treatment. The findings of Manohar *et al.,* (1999), however, contradicted the results of Ramaswamy (1949) and claimed that soaking the sandal seeds in coconut water for 24 hours enhanced germination over the control. Rangaswamy and Rao (1963) reported sandal seed germination within 10 days on modified White's medium supplemented with 20% coconut milk and 400-ppm casein hydrolysate.

Manonmani and Vanangamudi (2001) reported that the fruit characteristics, viz., 100 fruit weight, fruit moisture content, 100 seed weight, seed length and seed breadth were closely related to the level of fruit maturity. The green fruits were not able to germinate while 29.0, 68.2 and 73.2% of greenish red, reddish brown and black fruits germinated. The brown fruits recorded the lowest percentage of reduction in germination after 14 months of storage. These results strongly supported the collection of sandal fruits when they are black in color.

Manonmani and Vanangamudi (2002) also studied the effect of seed size on the seed germination capacity and seedling vigor in sandal and suggested that it is best to collect only big-sized seeds in order to obtain maximum germination percentage and increased seedling vigor.

Nagaveni and Ananthapadmanabha (1986) however, reported that small seeds (up to 0.1 g) germinated more quickly (starting after 15 days and reaching a maximum at 70 days) than the medium (0.1-0.2 g) and large seeds (0.2 g) which started germinating after 30 days and reached a maximum at 90 days. But the seedling growth and survival was reported to have increased with seed size: survival of seedlings from small seeds was only 55-60%, that from medium seeds 70-75%, and from large seeds 90-95%. The use of seeds of weight >0.1 g was therefore recommended.

The seed germination percentages in *Santalum album* decreased with the increasing altitude from where the seeds were collected (Susila *et al.*, 1995).

Seeds collected from the bird droppings showed higher percent germination (85%) than the seeds collected from the fruits (70%) (Masano, 1986). Nagaveni and Srimathi (1985) tested the viability and germination percent of floating and sinking seeds and reported that there were viable seeds amongst floaters and non-viable amongst sinkers after 72 h and even after 15 days of soaking in water. As the time of soaking increased, germination capacity in sunken seeds decreased due to deterioration of the seed, which suggested that soaking for longer durations makes the seeds non-viable.

Chauhan and Chandra (2003) cultivated sandal hydroponically in full strength Hoagland nutrient medium without the association of a host. The seedlings survived for 18 months and continued to grow into fully developed trees when transferred to normal soil even without a host but failed to survive beyond 6-7 months after transfer.

From the literature it is evident that both the important tropical forest tree species *Pterocarpus marsupium* Roxb. and *Santalum album* L. have poor seed germination and constraints in raising the seedlings. These species did not respond well to vegetative propagation and a standardized method for fast and effective nursery germination is not available. These species are threatened due to overexploitation and deforestation and are therefore required to be conserved. Moreover, they are highly suitable as planting material for reforestation programs. In view of this, the attempts made in the present investigation towards the induction of uniform and fast nursery germination for raising the quality seedlings of these species are important.

Chapter III

Seed Source, Seed Quality and Design of Experiments

3.1 Introduction

Seeds are the most essential basic resource material for raising successful plantations. However, obtaining supplies of high quality, healthy seed has been a great problem for ever-increasing afforestation programs. Though the higher plants produce seeds usually in abundance, all of them seldom germinate and produce seedling due to either inherent constraint or some external factors. The importance of seeds in nature has resulted in studies on many aspects of seed biology such as seed formation, seed structure and dispersal, seed viability, germination behavior, chemistry of individual seed and the accumulation of seeds in the soil seed banks, all obviously related to the morphology, physiology, and ecology of seeds (Grübb, 1977). Knowledge of seed biology is also essential to understand community processes like plant establishment, succession, and natural regeneration (Vazquez-Yanes and Orozco-Segovia, 1993) and the study of these aspects is becoming increasingly important to understand seed behavior and predicting the regeneration capacity of the trees and the forest. Typical problems of tropical forest tree seeds which need more study include collection of seeds, cleaning, storage, and pre-treatment for germination (Bonner, 1992). To alleviate these problems, investigation related to seed source, seed testing including seed germination behavior and the identification of appropriate pre-treatments to improve seed germinability are of great importance for an efficient preservation of tropical tree species. Moreover, the knowledge of seed characteristics and seed germination behavior of any species forms the baseline for strategies to improve germination pattern. The meager information available on seed biology of *P. marsupium* and *S. album* is a major problem for the sustainable management and implementation of successful reforestation programs using these important tree species. Therefore the present chapter describes the aspects related to seed source, seed testing and germination behavior studies on seeds of *P. marsupium* and *S. album.*

3.2. *Pterocarpus marsupium* Roxb.

3.2.1 Source of seeds

A naturally growing mature tree of *Pterocarpus marsupium* Roxb. of about 15 m height located near the foothills of Sinhgad Fort, about 35 km South-West of Pune city, India, was selected as a seed source (Plate III-1). The tree species was identified and authenticated as *Pterocarpus marsupium var. acuminata* Prain. by the Botanical Survey of India (BSI), Western Circle, Pune. The authorities from BSI, Western Circle, Pune, commented that this particular variety has a very restricted distribution in the state of Maharashtra and suggested to raise the seedlings and ecorestoration of the species (personal communication).

3.2.2 Storage of pods

The dispersal unit (diaspore) in *Pterocarpus marsupium* is an orbicular winged pod (samara) where seeds remain enclosed inside a hard seed enclosure (Plate III-2). The pods were collected in the month of March 2004, 2005, 2006 and 2007. The healthy, fully matured and disease-free pods were collected from off the ground. These pods were air dried under shade for one week in the laboratory (temperature: 30±3 ^{0}C, relative humidity: 40%). The pods were cleaned and stored in polythene bags. Each bag contained 500 pods. The bags containing pods were placed in corrugated box and stored under ambient conditions.

3.2.3 Preparation of seeds for experiments

From the standpoint of seed handling, it is not always possible to separate the fruit and seed, since they are sometimes jointed in a single unit in some species (Hartmann *et al.*, 2002). The term 'seeds' therefore refers both to true seeds and to fruits and other dispersal units (Taylorson and Hendricks, 1977).

In *P. marsupium* the seed(s) remain enclosed inside a hard and stony non-separable pericarp with orbicular wing. This orbicular wing

Plate III-1: Source of seeds of *Pterocarpus marsupium* Roxb.

a. Tree in the vegetative phase

b. Vegetative branch

c. Tree laden with pods – Reproductive phase

d. Pods on branch

Plate III-1

Plate III-2: Fruits and seeds of *Pterocarpus marsupium* Roxb.

a. Winged pods (Samara) (Bar = 5 cm)

b. Dewinged pods (Bar = 5 cm)

c. True seeds (seeds removed from pericarp)
(Bar = 1 cm)

Plate III-2

Plate III-2b: Stages in the germination

A. Germination in the pod of *Pterocarpus marsupium* Roxb.
(Bar = 1 cm)
   a. Five days after sowing
   b. Eight days after sowing
   c. Twelve days after sowing
   d. Fifteen days after sowing
   e. Eighteen days after sowing

B. Germination in the seed of *Santalum album* L.
(Bar = 1 cm)
   a. Germinating seed after 20 days of sowing
   b. Germinating seed after 22 days of sowing
   c. Germinating seed after 25 days of sowing
   d. Germinating seed after 27 days of sowing
   e. Germinating seed after 30 days of sowing
   f. Germinating seed after 35 days of sowing
   g. Germinating seed after 40 days of sowing
   h. Germinating seed after 50 days of sowing

Plate III-2b

A

B

of the fruit was excised with sharp scissors. **Such dewinged pods hereafter in the thesis are refereed to as 'seeds' (Plate III-2).** The experiments to enhance seed germination were conducted with such dewinged pods, unless mentioned otherwise.

3.3 *Santalum album* L.

3.3.1 Source of seeds

The fruits of *Santalum album* L. were collected in the month of May 2004, 2005, 2006 and 2007 from the selected naturally growing trees in the campus of University of Pune, India (Plate III-3). The ripened, black colored drupes that were naturally fallen off were collected from off the ground.

The seed-source plant specimen was identified and authenticated from the Botanical Survey of India, Western Circle, Pune, India.

3.3.2 Storage of drupes

The drupes of *S. album* were thoroughly screened and intact and disease-free drupes were separated from the bulk. These drupes were air dried under shade for one week in the laboratory (temperature: 30±3 ^{0}C, relative humidity: 40%). The drupes were cleaned and stored in polythene bags under ambient conditions. Each bag contained 100 drupes.

3.3.3 Preparation of seeds for experiments

Seeds of *Santalum album* were separated from the fruits by following the method described by Pair and Khatamian (1982). Fruits were soaked overnight in distilled water to allow the pulp lightly ferment for slipping free from the seed. Such fruits were then rubbed in a cotton screen to separate pulp from the stony endocarp and finally washed thrice with distilled water.

Plate III-3: Source of seeds of *Santalum album* L.

a. Tree in the vegetative phase

b. Vegetative branch

c. Flowering twig

d. Ripened black drupes on branch

Plate III-3

The seeds still enclosed by stony endocarps were handpicked and air-dried. **The true seed enclosed in the stony endocarp is considered as seed and such seeds were used for the germination experiments, unless mentioned otherwise (Plate III-4).**

3.4 Design of experiments

All the experiments were performed at least in triplicate on the seeds collected in the year 2004, 2005, 2006 and 2007. The experiments were arranged in the completely randomized design. Each experiment was performed on the randomly selected 50 seeds. The concentrations of different acids, germination stimulants, and plant growth regulators used for enhancing seed germination and duration of presoaking the seeds in these chemicals was decided on the basis of preliminary laboratory trials. The nursery trials were also conducted in a completely randomized design in a net-house (temperature: 26.8 ± 5.8 °C, RH: 81.53 ± 15.43). The seeding trays and polythene bags containing seedlings were watered every alternate day and volume of water used was adjusted so as to keep the soil moist. Each experiment was performed separately unless mentioned otherwise.

3.5 Collection of data

For design of experiments, recording the observations and concluding the experiments on seed germination, the guidelines described by Baskin and Baskin (2001) and Baskin *et al.* (2006) were followed.

The primary data on seed germination was collected at regular intervals. Since these experiments were aimed at early, uniform and enhanced seed germination, in *P. marsupium*, the seed germination was counted for 15 days at the interval of 5 days. The experiments were concluded on the 30th day after sowing (DAS). The final germination percentage (FGP) was calculated from the total seeds that germinated on the 30th day out of those planted at the start.

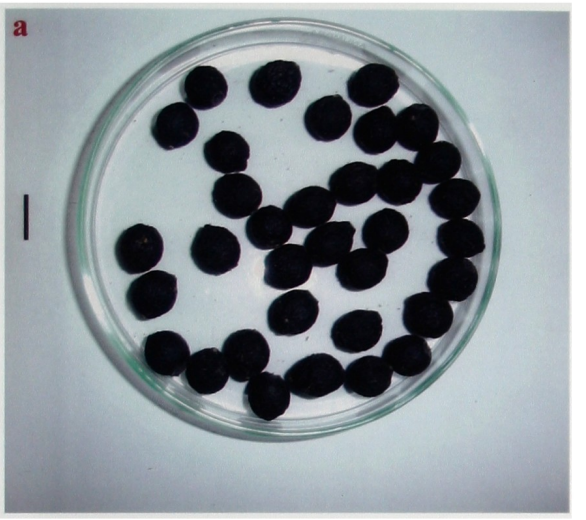
Plate III-4: Fruits and seeds of *Santalum album* L.

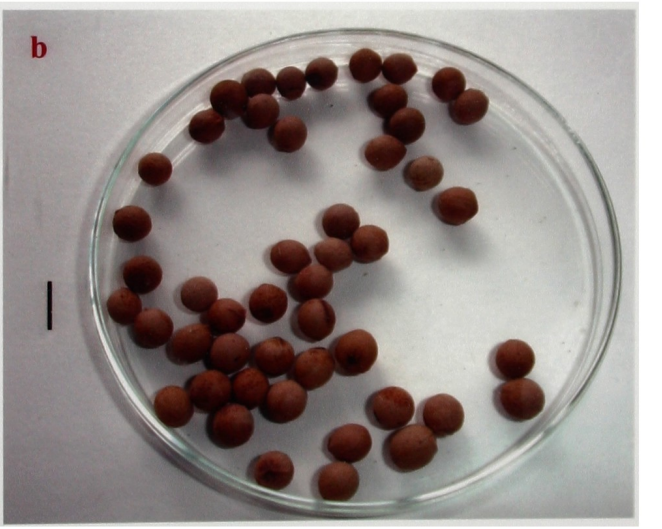
a) Drupes (Type of fruit) (Bar = 1 cm)

b) Seeds separated from drupes (Bar = 1 cm)
(Covered by endocarp, considered as seeds in the present investigation)

c) True seeds (seeds removed from endocarp)
(Bar = 1 cm)

Plate III-4





In *S. album* the seed germination was counted for 30 days at an interval of 10 days and the experiments were concluded on the 60th day after sowing (DAS) by counting the total number of seeds that germinated and expressing it in terms of final germination percentage (FGP).

The effectiveness of a seed treatment on total germination was assessed by estimating cumulative percentage germination (CPG), daily germination speed (DGS) and mean germination time (MGT) at each time interval during the germination run.

The cumulative percentage germination (CPG) was calculated using the method of Younsheng and Sziklai (1985).

(CPG) = (Total number of seeds germinated since the beginning of the experiment ÷ 50) × 100

The CPG was calculated for each time interval.

The effectiveness of the treatments was judged by comparing the CPG on the 15th day in *Pterocarpus marsupium* (CPG T15) and CPG on the 30th day in *Santalum album* (CPG T30). The treatments were also compared with reference to the mean CPG value computed as average of CPG on 5th, 10th and 15th day in *Pterocarpus marsupium* and CPG on 10th, 20th and 30th day in *Santalum album.*

Daily germination speed (DGS) was computed at each time interval by dividing CPG by number of days since beginning of the test (Muhammad and Amusa, 2003). For comparing the effectiveness of the treatments, mean DGS was used that was computed by taking average of DGSs computed at each time interval.

To assess the germination rate and spread, the mean germination time (MGT) was calculated using the method of Younsheng and Sziklai (1985) and Hartmann *et al.*, (2002) by using the relation:

$MGT = \Sigma n_i d_i / n$

Where:

n = total number of seeds germinated during 15-day experimental period for *Pterocarpus marsupium* and 30-day period for *Santalum album*

n_i = number of seeds germinated on day d_i;

d_i = day during germination period (5^{th}, 10^{th} and 15^{th} for *Pterocarpus marsupium* and 10^{th}, 20^{th} and 30^{th} for *Santalum album*).

Realizing the importance of dry-matter accumulation in seedling health and low MGT as indication of seed vigor and uniform seedling, a method was adopted (Butola and Badola, 2004) to determine the seedling vigor index (SVI) as,

SVI = dry weight per seedling/MGT × 100.

Five randomly selected seedlings of *Pterocarpus marsupium* and *Santalum album* generated from each experiment were carefully uprooted from the soil after one month from the date of sowing the seeds (for *Santalum,* 2 months after sowing). The root system was carefully washed to remove the associated soil particles. The fresh weight was noted and the seedlings were dried in the oven at 60 ^{0}C till constant weight was obtained (Sestak, 1971). The average dry weight of seedling was used for the calculation of SVI.

3.6 Data presentation

The results from the experiments in the years 2004, 2005, 2006 and 2007 were pulled together. The data was presented in the tables and figures as mean values of the data generated in these experiments, unless mentioned otherwise.

3.7 Statistical analysis of data

Each experiment was performed in a completely randomized design. Germination percentages were normalized by arcsine transformation (arcsin·√%) before performing statistical analysis (Zar, 1996). The data were analyzed for variance by performing ANOVA test in MS Excel program. Analysis of variance was applied to all the experiments unless mentioned otherwise.

The treatment means were compared with the best control response mean by following Dunnett's (1964) test at p = 0.05. Where the difference between the best control response mean and treatment mean was more than the critical difference (CD), the treatment mean was declared significantly different from the control mean. The critical difference was calculated from the following relation (Montgomery, 2001),

$$CD = d_{\alpha}(a\text{-}1, f)\sqrt{\{MS_E(1/n_i + 1/n_a)\}}$$

Where,

α	= level of significance (0.05)
$d_{\alpha}(a\text{-}1, f)$	=Constant (obtained from Montgomery, 2001b)
a-1	= Number of comparisons to be made
f	= degrees of freedom from ANOVA
MS_E	= Error of mean square (MS)
n_i	= No. of replicates of treatment
n_a	= No. of replicates of control

The treatment means were also compared to identify relative effectiveness of the treatment and treatment means were separated by Duncan's Multiple Range Test (DMRT) (Duncan,1955) at p = 0.05. The data were represented as mean ± standard deviation.

3.8 Seed characteristics

3.8.1 *Pterocarpus marsupium* Roxb.

3.8.1.1 Pod size

To characterize the size of diaspore, the diameter of 1000 randomly selected diaspores was measured with the help of ordinary scale. The diameter was expressed in centimeter.

3.8.1.2 Moisture content (MC)

To determine the moisture content (MC), the fresh weight of 10 batches each of 100 pods was measured on electronic balance (Contech India Ltd., Model CA 120). The pods were then dried in the hot air oven set to 60 ^{0}C till the constant weight of pods was obtained (Sestak, 1971). The MC was expressed as percentage of fresh weight.

Likewise, the MC in the seeds separated from the diaspore was also determined. The weight of 10 batches each of 100 seeds was measured and averaged to determine the 1000 seed weight.

3.8.1.3 Imbibition kinetics

To study the kinetics of imbibition, three batches each of 100 g pods were soaked in distilled water and increase in the weight was recorded at the interval of 1 h for the period of 24 h. The increase in the weight was expressed as percentage of original weight.

In the second experiment, the wing associated with the pod was removed with sharp scissors and the central seed case was retained. The water uptake by 100 g such processed pod was monitored and compared with that of entire pods.

3.8.2 *Santalum album* L.

The seed characteristics were observed and recorded. Following experiments were performed at least in triplicate on the healthy and disease free endocarps (referred to as seeds).

III-1. Pod characteristics in *Pterocarpus marsupium* Roxb.

Pod character	Seed lot 2004	Seed lot 2005	Seed lot 2006	Seed lot 2007
Colour	Dark brown	Dark brown	Dark brown	Dark brown
Pod diameter (cm)	4.6 ± 1.3	4.4 ± 1.2	4.4 ± 1.3	4.5 ± 2.1
1000 pod weight (g)	742.5 ± 21.9	750.1±19.5	745.5±12.9	758.5±25.1
1000 seed weight (g)	128.6 ± 34.2	119.9 ± 42.2	122.4 ± 23.2	124.5 ± 25.4
Number of pods per kg	1347±32.9	1315±12.2	1340±10.1	1320±14.4
Number of seed per kg	7800 ± 22.1	8400 ± 34.7	8170 ± 45.9	8070 ± 56.9
% Moisture content				
Pod	14.11 ± 3.2	14.62±2.9	14.86±3.9	14.41±3.9
Seed	11.23 ± 1.2	10.12 ± 1.4	12.11 ± 2.4	11.45 ± 2.2
% viability (TZ test)	96%	92%	93%	95%

The values represent (mean± SD)

III-2: Seed viability in *Pterocarpus marsupium* Roxb.

DAS	Number of Seeds Germinated during time interval	Percentage Germination during time interval	Total number of Seeds Germinated since DAS	Cumulative percentage germination	MGT (Days)	Mean DGS (% seeds per day)	Final Percentage Germination (Viability)
5	1.33 ± 0.0	2.67 ± 0.0	1.33 ± 0.0	2.67 ± 0.0	-	-	-
10	12.33 ± 2.0	24.67 ± 4.0	13.67 ± 2.0	27.33 ± 4.0	-	-	-
15	4.67 ± 1.7	9.33 ± 3.5	18.33 ± 2.7	36.67 ± 5.3	10.88	1.90	42.00 ± 4.0

The values represent (mean± SD) of three independent experiments, each experiment performed on 50 seeds.

Plate III-5: Testing seed viability in soil media

a) *Pterocarpus marsupium* Roxb.

b) *Santalum album* L.

Plate III-5

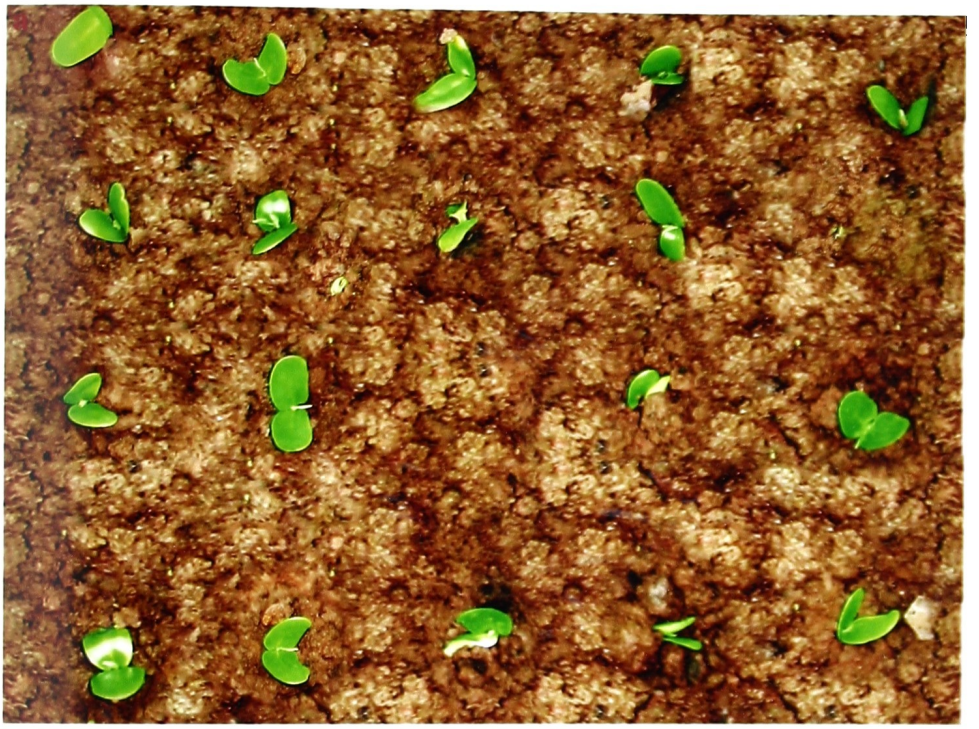


Plate III-6: Testing seed viability by TZ test in *Pterocarpus marsupium* Roxb.

a) Viable seeds with TZ positive test
Longitudinal split (Bar = 1 cm)

b) Non-viable seeds with TZ negative test
Longitudinal split (Bar = 1 cm)

Plate III-6

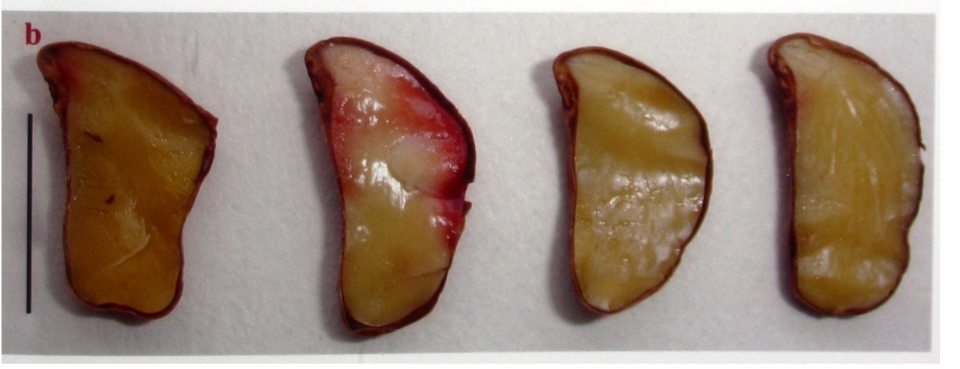


Plate III-7: Testing seed viability by TZ test in *Santalum album* L.

A. Viable seeds with TZ positive test

a. Seeds in longitudinal section (Bar = 1 cm)

b. Seeds in transverse section (Bar = 1 cm)

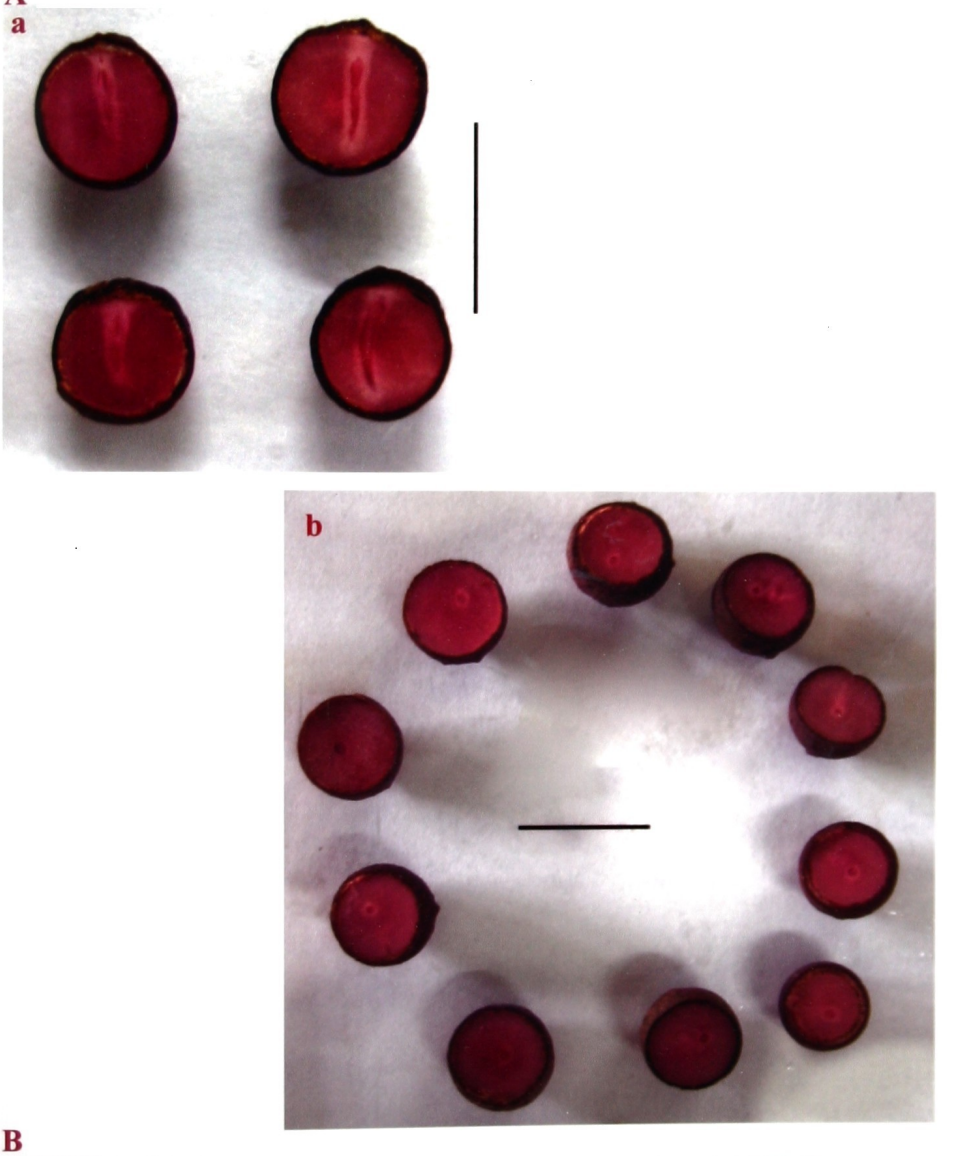
B. Non-viable seeds with TZ negative test

a. Seeds in longitudinal section

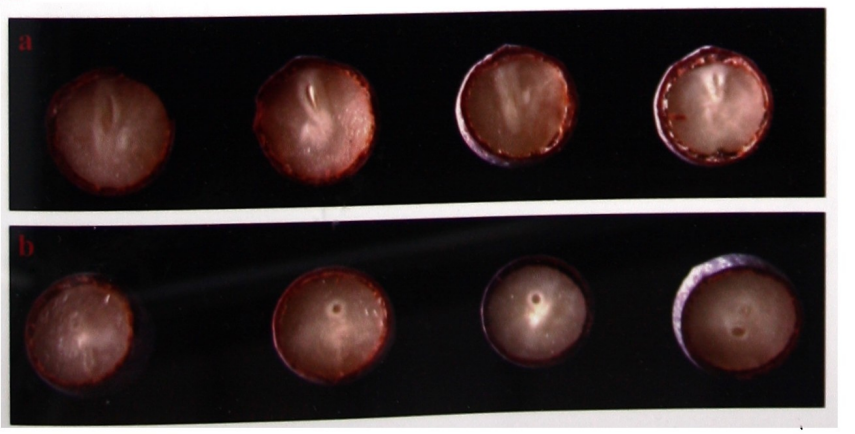
b. Seeds in transverse section

Plate III-7

A



B



3.8.2.1 Size of the endocarp

The diameter of 1000 randomly selected seeds was measured with micrometer screw gauge. The diameter was expressed in millimeter.

3.8.2.2 Moisture content (MC)

Ten batches of 100 randomly selected seeds (endocarps) were used to record 100 seed weight which were then averaged to determine 1000 seed weight. The weight of 1000 seeds separated from the endocarp was determined by following the same procedure. The moisture content (MC) in the seeds was determined by following Sestak (1971). Hundred seeds were dried at 60 ^{0}C in hot air oven till constant weight was obtained. The MC was expressed as percentage on fresh weight basis.

3.8.2.3 Imbibition kinetics

To study the kinetics of imbibition, three batches each of 100 g endocarps were soaked in distilled water and increase in the weight was recorded at the interval of 1 h for the period of 24 h. The increase in the weight was expressed as percentage of original weight.

The endocarp was subjected to gentle hammering so as to introduce crack in it. The imbibition rate in 100 g such cracked endocarps was monitored and compared with the intact endocarps.

3.9 Testing of viability

3.9.1 Direct viability tests

3.9.1.1 Laboratory experiments

Although there are many indirect, rapid tests for evaluating seed quality, they are not always reliable for assessing seed viability. For evaluating the germinability of seeds, the only dependable technique is the direct growth method or germination tests (Smith *et al.*, 1990). In most forest and conservation nurseries, the germination test is the best performance test of a seed lot and therefore is used as the operational test of seed viability (Smith *et al.*, 1990).

The easiest way to determine the germinability of seed is by the direct method of observing germination under controlled conditions of light, temperature and moisture.

The randomly selected seeds of *P. marsupium* and *S. album* were hydrated in distilled water for one hour. The seeds were surface sterilized with 0.1% (w/v) $HgCl_2$ for 5 minutes and washed thrice with sterile distilled water. Seeds were blotted dry and 25 seeds were space-layered in a semitransparent plastic tray containing moist germination paper over-layered on moist absorbent cotton. In order to discourage the spread of fungal molds, a general recommendation of Bonner (1974) was followed to leave space twice the normal seed width or diameter between seeds. The assessment of seed germination was done in triplicate with 50 seeds per replica.

The observations on seed germination in *P. marsupium* were made for 15 days at the interval of 5 days whereas in *S. album,* observations were made for 30 days at the interval of 10 days. Germination was defined as the emergence and development from the seed embryo of those essential structures which were indicative of the seed's capacity to produce a normal plant under favorable conditions in soil.

The seeds from which the radicle emerged out were scored as viable. Seed viability was expressed as the percentage of seeds germinated. The ungerminated seeds were subjected to TZ test at the end of the germination experiment.

3.9.1.2 Germination in soil

The raised nursery beds of 1 m × 1.5 m × 0.3 m were used for testing the viability of seeds. The seed samples of *P. marsupium* and *S. album* were hydrated for about 1 h in distilled water. The hydrated seeds were sown at the depth of 2 cm and space planted at a distance of about 5 cm. The seed beds were irrigated after every 3 days.

The observations on seed germination in *P. marsupium* were made for 15 days at the interval of 5 days and the experiments were concluded on 30th day after sowing (DAS) by recording the final germination count.

In *S. album*, observations were made for 30 days at the interval of 10 days and the experiments were concluded on 60th day after sowing (DAS) by recording the final germination count.

Seeds were scored as germinated after seedling emergence which was defined as emergence of the coteledonary leaves in *Pterocarpus marsupium* and hypocotyl hook in *Santalum album*. The ungerminated seeds were subjected to TZ test at the end of the germination experiment.

The experiments on seed germination on seed bed were conducted under natural photoperiod and irradiance.

3.9.2 Indirect viability tests

The seeds of *Santalum album* were bisected longitudinally with a razor blade to expose the main structures of the embryo. The tetrazolium test was conducted by incubating 100 seed halves in 50ml of 1% (w/v) solution of 2, 4, 5-triphenyl tetrazolium chloride (TTC) prepared in 0.1 M Sorensen's buffer (pH 7.0). The seed halves were incubated in the TTC solution for 24 hours at 28 °C. After the incubation period, the seed halves were bleached in 5% hypochlorite for 3 min. The seeds wherein embryos turned reddish-pink were scored as viable (Porter *et al.*, 1947; Mitter, 1993) and seeds that remained light yellow were scored as non-viable (Eplee & Norris, 1987).

The seeds of *Pterocarpus marsupium* were extracted from the pods. Hundred seeds were incubated in 50 ml of 1% TTC solution for 24 h at 28 °C. After the incubation period, the seeds were bleached in 5% hypochlorite for 3 min. The seeds were bisected and the embryo was observed. The TTC test was interpreted as described for *Santalum album*.

3.10 Results

3.10.1 *Pterocarpus marsupium* Roxb.

The seed characteristics are presented in Table III-1.

Some of the stages of pod germination in *P. marsupium* are presented in Plate III-2b. Under laboratory conditions, about 35% pods germinated within the period of 30 days whereas under the field conditions, there was only 28% germination within the same period. After sowing the seeds in soil, the pods did not germinate for first 10-12 days, after which the radicle protruded out and reached the length of about 2 mm in the next 3-4 days. The embryo elongated and the cotyledons slipped out of the central seed case. The germination was epigeal and the first structure to surface the soil was a pair of cotyledons followed by plumule. The germination percentage was poor and seedling growth was slow. After about one month from the onset of germination trial, the seedling reached the height of about 9-11 cm and produced 4 leaves.

The imbibition kinetics revealed that the rate of imbibition is very slow in this species (Fig III-1a) and removal of the associated wing improves the imbibition.

The results on the tests to analyze percentage germination of seeds are presented in Table III-2. The cumulative percentage germination was monitored till 15 DAS and the final percentage germination was recorded on 30th DAS.

The germinability in the seeds as inferred from direct germination test was 42% (Plate III-5). The remaining seeds were healthy and intact, showed positive TZ test but did not show any sign of germination. The CPG T15 recorded in these seeds was 36.67%. The mean time to complete germination was 10.88 days and the seeds germinated at the speed of 1.9% seeds per day.

Among the seeds of *Pterocarpus marsupium* incubated in 1% TTC solution for 24 hours at 28 °C followed by bleaching in

5% hypochlorite for 3 min, the embryo in up to 96% seeds appeared red in color indicating that percentage of seeds were viable (Plate III-6).

3.10.2 *Santalum album* L.

The seed characteristics are presented in Table III-3.

Fresh seeds of *S. album* did not germinate for up to 1.5 – 2 months after collection of fruits indicating the presence of some dormancy principle. The seeds germinated only after this resting period and the seed germination was epigeal. The stages in the germination of true seed are presented in Plate III-2b. The radicle emerged out after about 25-35 days after initiation of germination run. A pronounced arching was observed in the hypocotyl during germination. In case of seeds sown in the soil, the lush-green loop of hypocotyl appeared above ground while the cotyledons remained underground. A considerable swelling was observed in the lower portion of hypocotyl, probably due to translocation of nutrients. After this phase, the hypocotyl became erect and pulled the cotyledons above the ground. The seed still remained attached to the seedling, either felled off or dried up after few days. The seedlings were sensitive to draught since quick drying of seedlings was observed if they were occasionally water stressed.

The imbibition kinetics recorded in the Fig. III-1b clearly revealed that the process of imbibition is relatively slow in the seeds of this species. The cracking of the endocarp slightly improved the rate of imbibition thereby indicating the role of endocarp in regulating the process.

The results on the viability in the seeds of *S. album* as reflected by seed germination are presented in Table III-4 and Plate III-5. The cumulative percentage germination was observed for the period of 30 days from sowing the seeds and the final percentage germination was determined 60 days after sowing the seeds. The results presented are mean values of the viability tests performed each year on the seeds collected in the month of April 2004, 2005, 2006 and 2007.

III-3. Seed (endocarps) characteristics in *Santalum album* L.

	Seed lot 2004	Seed lot 2005	Seed lot 2006	Seed lot 2007
Colour	Light brown	Light brown	Light brown	Light brown
diameter (mm)	6.2 ± 1.2	6.4 ± 2.2	6.3 ± 2.3	6.5 ± 1.1
1000 endocarp weight (g)	147.8 ±11.9	159.1±9.5	155.5±8.9	158.5±5.1
1000 seed weight (g)	99.60 ± 4.9	105.23±7.9	101 ±14.9	102 ±6.6
Number of endocarps per kg	6775.5 ± 12.2	6300.4±32.2	6500.3±26.4	6450.4±34.8
Number of seed per kg	10045 ± 115.2	9545 ± 98.2	9815 ± 78.7	9712 ± 68.7
% Moisture content				
With endocarp	7.23 ± 3.2	7.26 ± 2.9	7.43 ± 3.9	7.12 ± 3.9
True seed	7.87± 3.1	7.67 ± 3.2	7.92 ± 3.3	7.74 ± 3.1
% viability (TZ test)	94%	95%	95%	95%

The values represent (mean± SD).

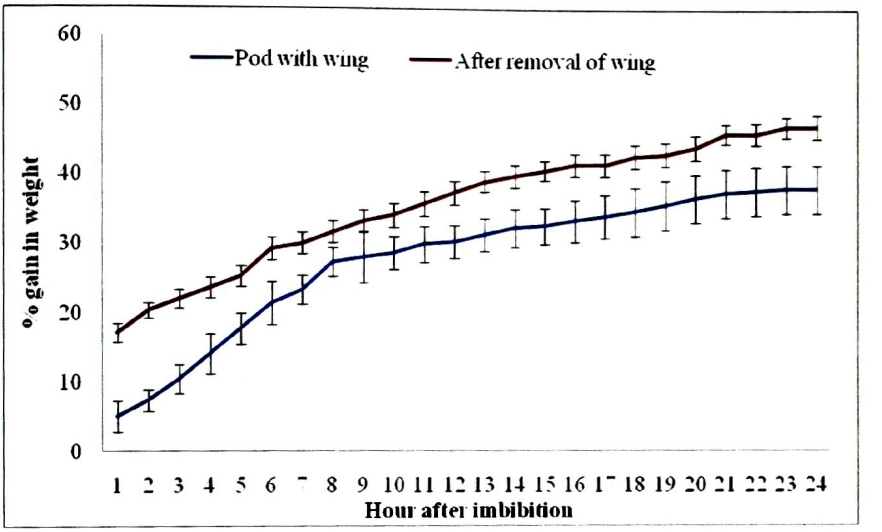
III-4: Seed viability in *Santalum album* L.

DAS	Number of Seeds Germinated during time interval	Percentage Germination during time interval	Total number of Seeds Germinated since DAS	Cumulative percentage germination	MGT (Days)	Mean DGS (% seeds per day)	Final Percentage Germination (Viability)
10	1.33 ± 0.9	2.67 ± 1.2	1.33 ± 0.6	2.67 ± 1.2	-	-	-
20	4.33 ± 1.5	8.67 ± 3.1	5.67 ± 2.1	11.33 ± 4.2	-	-	-
30	6.00 ± 2.0	12.00 ± 4.0	11.67 ± 4.1	23.33 ± 8.1	24.01	0.54	32.00 ± 3.5

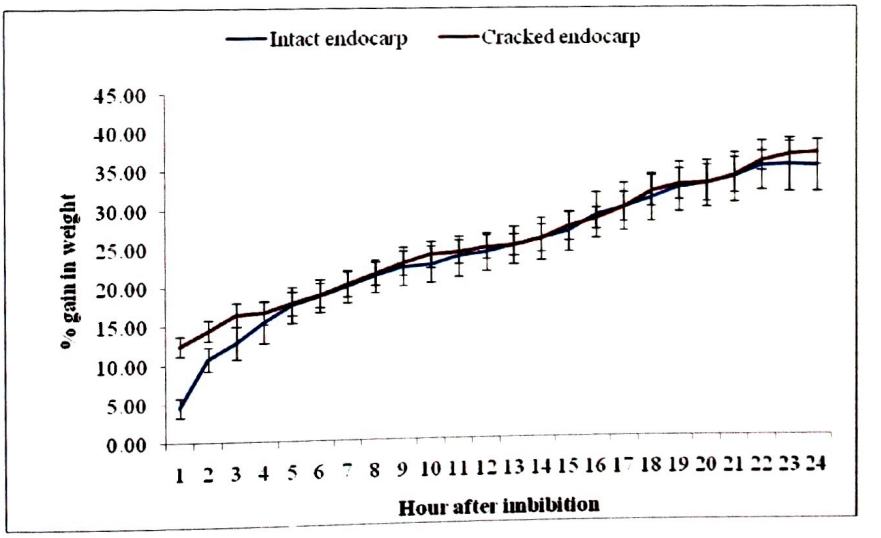
The values represent (mean± SD) of three independent experiments, each experiment performed on 50 seeds.

III-1 Imbibition kinetics

a. *Pterocarpus marsupium* Roxb.



b. *Santalum album* L.



The germinability in the seeds of *Santalum album* was 32% as indicated by the final germination percentage (FGP). The remaining seeds were healthy and intact but failed to germinate. The seeds germinated at the speed of 0.54% seeds per day, showed 23.33% CPG T30 and showed MGT of 24.01 days.

From the seeds of *Santalum album* used for TZ test, up to 95% seeds tested positive i.e., in the seeds incubated in 1% TTC solution for 24 hours at 28 °C followed by bleaching in 5% hypochlorite for 3 min, the embryonic axis turned red in color in 94% of seeds, indicating viability in these seeds was 94% (Plate III-7).

3.11 Discussion

A viable seed contains an embryo that is alive and capable of germination if proper stimuli are provided. Direct germination (expressed as percentage germination) and tetrazolium (TZ) percentage are two common measures of seed viability (Scianna, 2001). Seed quality is of prime importance for growing high-quality seedlings. In crop species and forest trees species the loss of viability and vigor compromises the usefulness of seeds (Baskin and Baskin, 2001; Cortelazzo *et al.*, 2005).

The quality of the seeds used governs the efficiency and success in raising plants in the nursery and also in their subsequent establishment in forest plantations. Therefore an accurate estimate of the quality of the seeds which form the basis of afforestation projects is of prime importance (Turnbull, 1975). Similarly, it is necessary to have an accurate estimate of germination so as to accomplish planting targets while at the same time avoiding the waste of valuable seed caused by over sowing. It is always advisable to perform viability tests on the seed lots collected so as to avoid the waste of time and labor and the cost thereof. In nurseries and reforestation programmes, the rapid, uniform and total germination is

important. The total germination depends largely on the viability and vigor of the seeds used. Therefore, it necessary to analyze the viability of any seed lot to be used for raisings the seedlings.

The easiest way to determine the viability of seed is by the direct method of observing germination under controlled conditions of light, temperature and moisture. TZ tests are also used to determine the viability of ungerminated seeds at the end of germination tests. The tetrazolium test (TZ test) is especially useful for determining the viability of very dormant seeds that would otherwise require long pretreatments before germination tests could be conducted (Stein *et al.*, 1986).

Percent germination indicates the percentage of normal seedlings produced by a sample of pure seeds under controlled conditions. In the seeds of tree and shrub species, TZ viability is an indication of maximum potential germination under optimum propagation conditions. Actual nursery germination which is usually lower than the TZ viability provides some indication of propagation success relative to the maximum potential for germination (Scianna, 2001). The results of the present investigation are on the same line. The seeds of *P. marsupium* showed 42% viability and *Santalum album* showed 32% viability in the direct germination test. However, the TZ test indicated higher viability of 96% in *P. marsupium*. The seeds of *Santalum album* showed 94% viability in TZ test.

Viability determined by tetrazolium or excised embryo test reveals the seeds' maximum potential and generally is higher than indicated by a germination test (Flemion 1941; Hamilton and Carpenter 1975). TZ test provided an accurate assessment of viability for many species. It was particularly useful for species with dormancy i.e. low recorded germination but high estimated viability by TZ test (Daws *et al.*, 2004).

The data on seed characteristics of seeds collected in four successive seasons did not show considerable variation. The process of imbibition occurred at a slower pace in both the species. This suggests that the fruit structures covering the true seed might be involved in the delayed germination and therefore some degree of physical dormancy exists in these species. The TZ test indicated more than 90% viability in the seeds of *P. marsupium* and *S. album* but the actual seed germination in soil was < 45% in both the species. To achieve this level of germination a longer duration of one month for *P. marsupium* and two months for *S. album* was required. This indicated that the seeds of these species are difficult to germinate and therefore some dormancy principle is operative.

Chapter IV

Induction of Germination By Physical Methods

4.1 Introduction

The seed coat acts as a first barrier interacting with the environmental factors before the process of germination begins and has a great influence on the ability of many seeds to germinate (Wareing, 1969; Come, 1970; Wareing and Saunders, 1971) and may impart coat-imposed dormancy (Taiz and Zeiger, 2006). The seed coat regulates germinability by imposing a permeability barrier and by interfering with the vital processes like uptake of water required for imbibition and subsequent radicle emergence, gaseous exchange required for respiration and other oxidative processes, and the outward diffusion of endogenous germination inhibitors, if any (Taiz and Zeiger, 2006). Seed dormancy in many species is caused by the inhibitory influence of structures covering the embryo rather than by factors within the embryo itself (Toole *et al.*, 1956). The seed coat may intervene in the preservation of seed dormancy but some effects may also influence early stages of germination (Mayer, 1974).

The true seed of *P. marsupium* is encased in hard, fibrous and lignified seed case which is very difficult to open so as to expose the seed. In *S. album* also the true seed remains enclosed in a hard endocarp. Though the embryo viability is very high (from TZ test) in these species, these surrounding structures might be acting as mechanical barriers thereby preventing or delaying seed germination in these species. Therefore the influence of weakening the hard and compact coat surrounding the true seed by application of physical methods is presented in this chapter.

4.2 Materials and Methods

4.2.1 Germination of seeds enclosed in diaspore

To assess the germination behavior of intact seeds (the diaspore- winged samara) in *Pterocarpus marsupium*, 50 seeds were soaked in 500 ml distilled water for 30 min and were sown at the depth of about 2 cm in cavity seeding trays containing moist garden soil. Such seeds were referred as intact seeds (IS).

In the second set, the wing of the diaspore was cut with the help of sharp scissors and the central seed case was isolated. Such dewinged seeds (DS) were immersed in 500 ml distilled water for 30 min and were sown at the depth of about 2 cm in cavity seeding trays containing garden soil.

The true seeds are fragile and easily cracked even by application of slight pressure. It was observed that extracting the seeds from samara and subsequent hydration of true seeds resulted in the rapid imbibition stress (Plate IV-1). Upon imbibition, the testa and the cotyledons were found broken. The embryonic axis was also disturbed and germination percentage was very low. In nature, the surrounding fruit structure might be preventing the rapid imbibition and the physical damage caused by imbibition. Secondly, this pod is indehiscent and separation of seed from pod was difficult, tedious and time intensive. To overcome these difficulties and to prevent imbibition damage, the physical treatments were applied to the dewinged pods (DS), referred as 'seeds' in the thesis.

4.2.2 Effect of physical treatments on seed germination

4.2.2.1 Induction of seed germination by hot water treatment

Fifty randomly selected seeds of *Pterocarpus marsupium* were wrapped in a clean cotton cloth and then completely submerged in 500 ml hot water set to 60 ^{0}C in 1000 ml beaker. The temperature was maintained at the set point during the course of the treatment by keeping the beaker containing seeds in a temperature-controlled water bath. The hot water treatment was given separately for 20, 40 and 60 min.

The seeds of *Santalum album* were selected randomly and used for the experiment. For each hot water treatment, 50 seeds were wrapped in a clean cotton cloth and submerged separately for 20, 40 and 60 min in 500 ml hot water set to 60 ^{0}C. The temperature was maintained at the set point during the course of the treatment by keeping the beaker containing seeds in a temperature- controlled water bath.

After the treatment for specified duration, the seeds were blotted dry and immediately sown at the depth of about 2 cm in the plastic bags (6" height × 4" width) containing moist garden soil or cavity seeding trays containing moist garden soil. After seed sowing the bags as well as trays were watered thoroughly and maintained in the shade-net house. The details of culture conditions in the shade-net house are described in the Chapter III.

4.2.2.2 Induction of seed germination by boiling water treatment

The randomly selected 50 seeds of *P. marsupium* and *S. album* were immersed in 500ml of water brought to boiling temperature. The seeds were immersed in water till the temperature of boiling water dropped to room temperature. The seeds were then blotted dry and immediately sown at the depth of about 2 cm in cavity seeding trays or plastic bags containing moist garden soil.

After seed sowing the trays as well as bags were watered thoroughly and maintained in the shade-net house. The details of culture conditions in the shade-net house are described in the Chapter III.

4.2.2.3 Induction of seed germination by physical scarification

In *P. marsupium* the central seed-case of the dewinged seed was incised with sharp secateurs so as to cut the outside edge of seed-case by about 1-2 mm and thereby exposing the true seed inside. The incised seeds were allowed to imbibe for 30 min in water. The seeds were blotted dry and immediately sown at the depth of about 2 cm in the plastic bags (6" height × 4" width) and cavity seeding trays containing moist garden soil (Plate IV-1).

The seeds of *S. album* were surface scarified by cracking the endocarp (Plate IV-1). The seeds were gently hammered in a mortar with pestle to crack the endocarp. The scarified seeds were submerged in water

for one hour, blotted dry and immediately sown at the depth of about 2 cm in cavity seeding trays and plastic bags containing moist garden soil.

After seed sowing the trays as well as bags were watered thoroughly and maintained in the shade-net house. The details of culture conditions in the shade-net house are described in the Chapter III.

4.2.2.4 Germination of seeds separated from diaspore

The central seed case of dewinged samara in *Pterocarpus marsupium* was incised with sharp secateurs so as to cut the outside edge of seed case. The cut opened area was widened up with scalpel to take out the seed(s). Randomly selected 50 seeds were sown at the depth of about 2 cm in the plastic bags (4" × 6") and cavity seeding trays containing moist garden soil.

The seeds of *S. album* were gently hammered with pestle in a mortar to crack the endocarp. Then the stony endocarp was completely removed manually (Plate IV-1). The de-endocarp seeds were sown at the depth of about 2 cm in cavity seeding trays and plastic bags containing moist garden soil (Plate IV-1).

After seed sowing the trays as well as bags were watered thoroughly and maintained in the shade-net house. The details of culture conditions in the shade-net house are described in the Chapter III.

4.3 Results

4.3.1 *Pterocarpus marsupium* Roxb.

4.3.1.1 Germination of seeds enclosed in diaspore

The results on seed germination pattern in the winged intact seeds (IS) and dewinged seeds (DS) are presented in Table IV-1 and Fig.IV-1a-f.

The winged seeds showed low cumulative percentage germination on 15th day after sowing (CPG T15= 22.67%). The germination was spread over a longer duration (MGT = 12.26 days), with

Plate IV-1: Physical scarification of seeds and germination

A. *Pterocarpus marsupium* Roxb.

a. Seeds with emerging radicle (Bar = 1 cm)
(Single seeded pod)

b. Seeds with emerging radicle (Bar = 1 cm)
(Double seeded pod)

c. Imbibition injury in the true seeds

d. Cavity seeding trays with seedlings

B. *Santalum album* L.

a. Intact seeds (Bar = 1 cm)

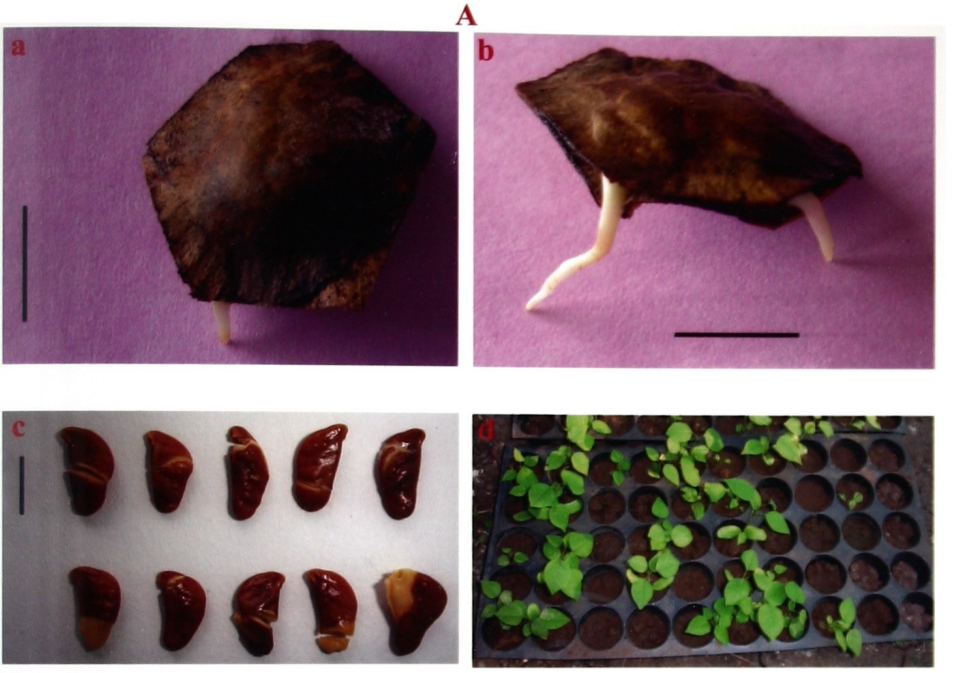
b. Seeds with physical scarification-
Cracked endocarp

c. Germinating seeds

d. Seedlings of *Santalum album* L. in polythene bags

Plate IV-1

A



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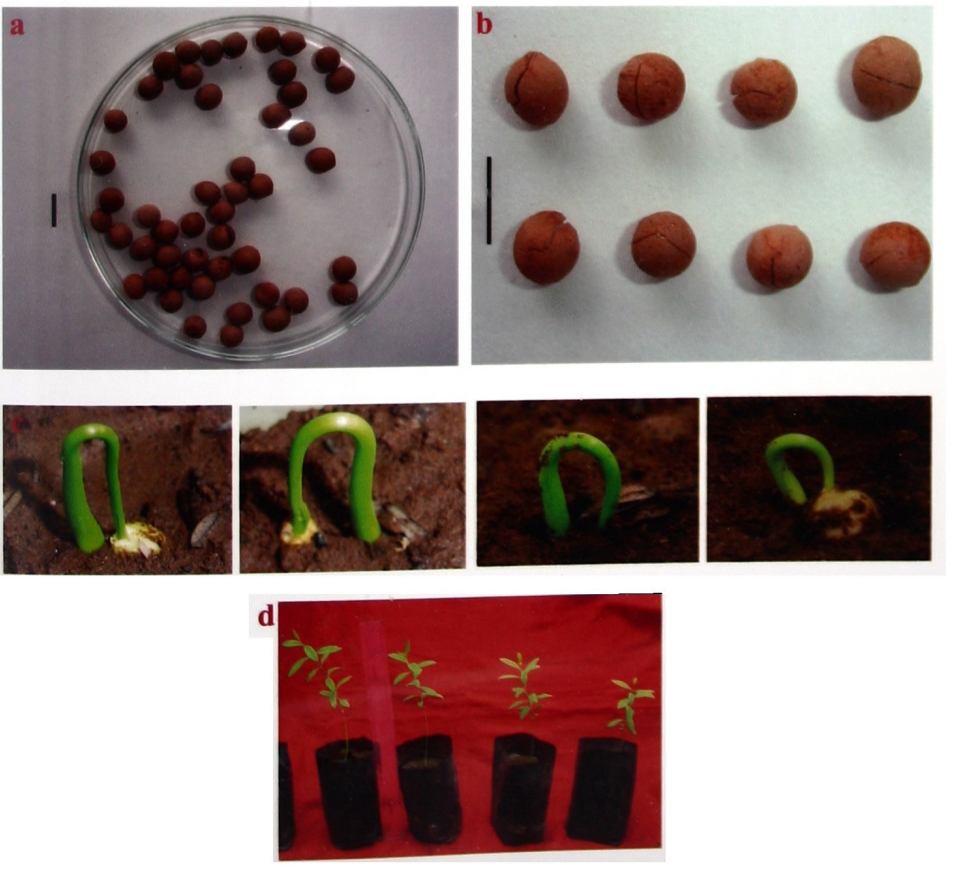


IV-1: Germination pattern of seeds enclosed in diaspore of *Pterocarpus marsupium* Roxb.

Germination scored on DAS	Type of Diaspore	Number of Seeds Germinated during time interval	Percentage Germination during time interval	Total number of Seeds Germinated since DAS	Cumulative percentage germination
5th	Intact seeds	0.33 ± 0.6	0.67 ± 1.1	0.33 ± 0.6	0.67 ± 1.1
	Dewinged seeds	2.33 ± 1.1	4.67 ± 2.2	2.33 ± 1.1	4.67 ± 2.2
10th	Intact seeds	5.67 ± 1.5	11.33 ± 3.0	6.00 ± 2.0	12.00 ± 4.0
	Dewinged seeds	11.33 ± 2.0	24.67 ± 4.0	14.67 ± 2.0	28.33 ± 4.0
15th	Intact seeds	5.33 ± 1.2	10.67 ± 2.3	11.33 ± 2.3	22.67 ± 4.6
	Dewinged seeds	4.57 ± 1.7	8.33 ± 3.5	17.33 ± 2.7	35.67 ± 5.3

Intact seeds = Seeds enclosed in the diaspore (i.e. winged samara fruit)
Dewinged seeds = Seeds enclosed in the diaspore in which the wing has been removed
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

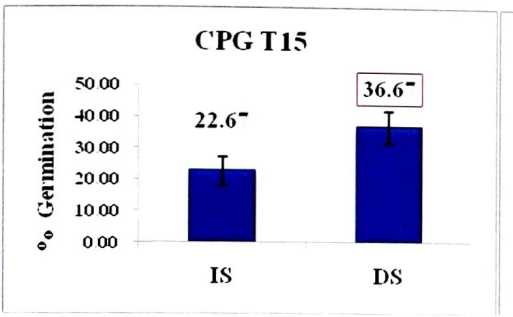
IV-2: Effect of hot water, boiling water and physical scarification on seed germination in *Pterocarpus marsupium* Roxb.

DAS	Treatment	Number of Seeds Germinated	Percentage Germination	Total number of Seeds Germinated	Cumulative percentage germination
5 Days	DS	1.00 ± 0.0	2.00 ± 0.0	1.00 ± 0.0	2.00 ± 0.0
	20 min HW	1.67 ± 1.5	3.33 ± 3.0	1.67 ± 1.5	3.33 ± 3.0
	40 min HW	3.33 ± 1.2	6.67 ± 2.3	3.33 ± 1.2	6.67 ± 2.3
	60 min HW	3.00 ± 1.0	6.00 ± 2.0	3.00 ± 1.0	6.00 ± 2.0
	BW	7.33 ± 1.5	14.67 ± 3.0	7.33 ± 1.5	14.67 ± 3.1
	PS	11.33 ± 2.3	22.67 ± 4.6	11.33 ± 2.3	22.67 ± 4.5
10 Days	DS	12.00 ± 2.0	24.00 ± 4.0	13.00 ± 2.0	26.00 ± 4.0
	20 min HW	12.33 ± 2.5	24.67 ± 5.0	14.00 ± 1.7	28.00 ± 3.5
	40 min HW	14.67 ± 1.7	29.33 ± 3.5	18.00 ± 2.5	36.00 ± 5.0
	60 min HW	13.00 ± 1.0	26.00 ± 2.0	16.00 ± 1.7	32.00 ± 3.4
	BW	13.67 ± 1.5	27.33 ± 3.1	21.00 ± 0.0	42.00 ± 0.0
	PS	13.67 ± 1.2	27.33 ± 2.3	25.00 ± 2.0	50.00 ± 4.0
15 Days	DS	5.00 ± 1.7	10.00 ± 3.5	18.00 ± 2.7	36.00 ± 5.3
	20 min HW	6.00 ± 1.0	12.00 ± 2.0	20.00 ± 1.0	40.00 ± 2.0
	40 min HW	4.33 ± 3.5	8.67 ± 7.0	22.33 ± 3.2	44.67 ± 6.4
	60 min HW	1.00 ± 1.0	2.00 ± 2.0	17.00 ± 2.0	34.00 ± 4.0
	BW	5.00 ± 1.7	10.00 ± 3.5	26.00 ± 1.7	52.00 ± 3.5
	PS	2.67 ± 1.5	5.33 ± 3.1	27.67 ± 1.5	55.33 ± 3.1

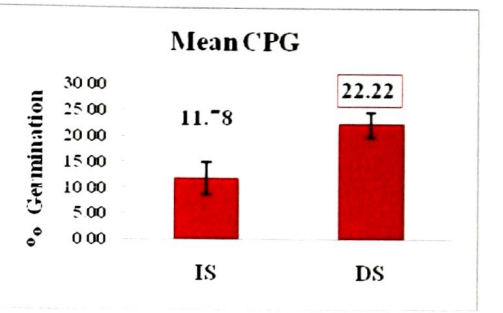
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
DS = Dewinged seeds, HW = Hot water (60^{0}C), BW = Boiling water (100^{0}C),
PS = Physical Scarification

IV-1 Germination pattern of seeds enclosed in diaspore of *Pterocarpus marsupium* Roxb.

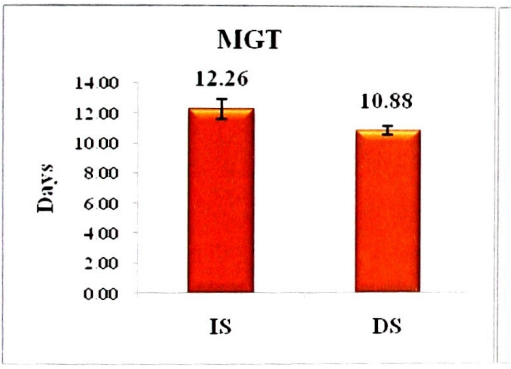
a) Cumulative percentage germination on 15th day



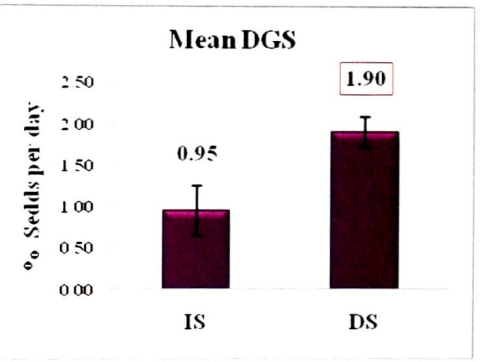
b) Mean Cumulative percentage germination



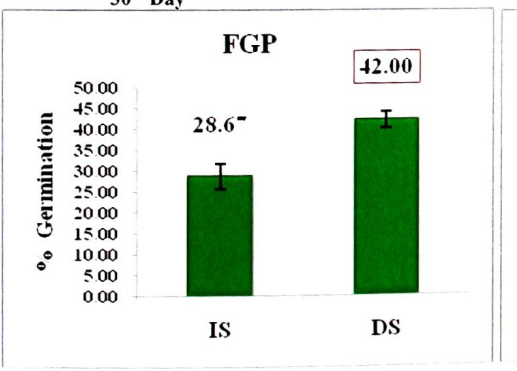
c) Mean germination time



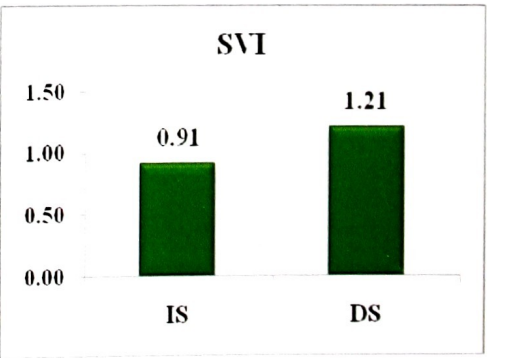
d) Mean daily germination speed



e) Final percentage germination on 30th Day



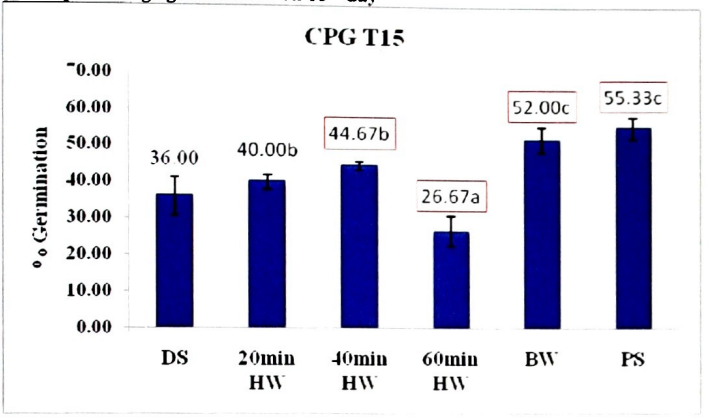
f) Seedling vigor



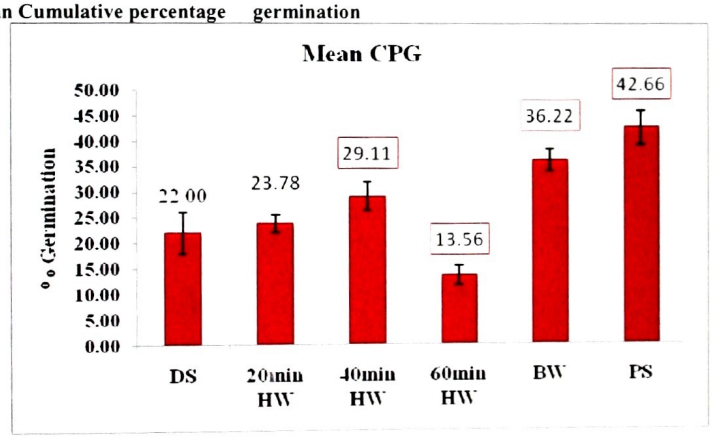
Intact seeds = Seeds enclosed in the diaspore (i.e. winged samara fruit)
Dewinged seeds = Seeds enclosed in the diaspore in which the wing has been removed
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

IV-2 Effect of physical treatments on seed germination in *Pterocarpus marsupium* Roxb.

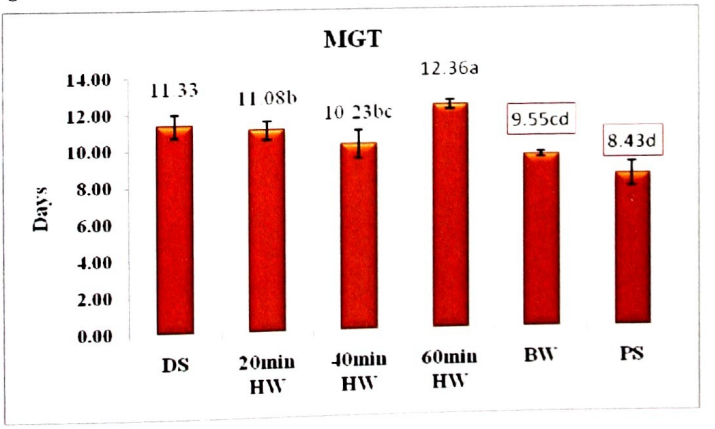
a) Cumulative percentage germination on 15th day



b) Mean Cumulative percentage germination



c) Mean germination time



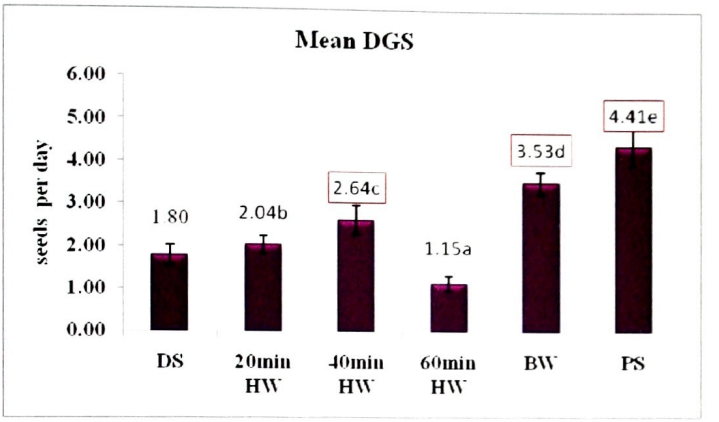
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.

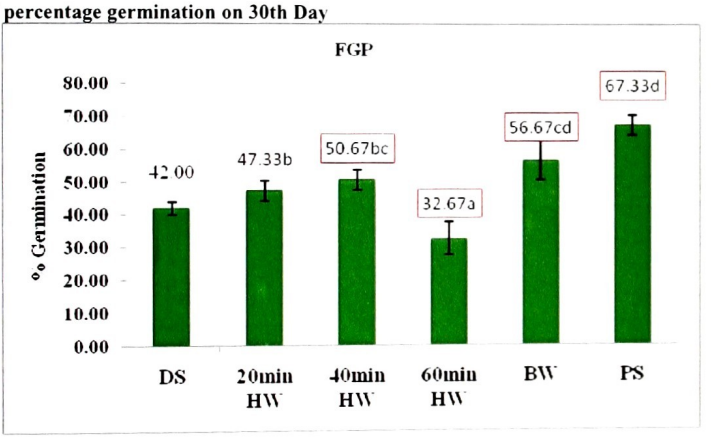
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

IV-2 Effect of physical treatments on seed germination in *Pterocarpus marsupium* Roxb.

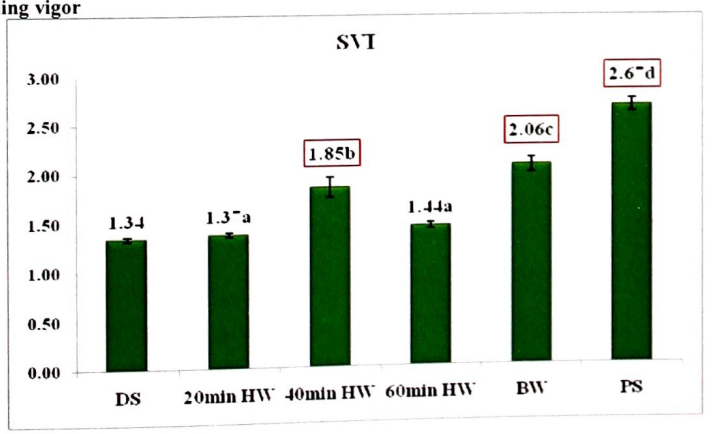
d) Mean daily germination speed



e) Final percentage germination on 30th Day



f) Seedling vigor



The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.

Treatment means with similar letters are not significantly different as per DMRT at p=0.05

least mean daily germination speed (mean DGS = 0.95% seeds per day) that culminated in the final percentage germination (FGP) of 28.67%.

When the wing of the seed was removed, significantly improved germination was observed within 15 days (CPG T15= 36.67%) as compared to winged seeds (CPG T15 = 22.67%). The mean CPG (22.22%) in the dewinged seeds also increased significantly as compared to winged seeds (mean CPG = 11.78%). The mean DGS was doubled (1.9% seeds per day) as compared to winged seeds (0.95% seeds per day) giving 42% final germination percentage (FGP) as against 28.67% in winged seeds. The spread of germination was reduced only by 11% in dewinged seeds (MGT = 10.88 days) from that of winged seeds (MGT = 12.26days).

4.3.1.2 Effect of hot water treatment on seed germination

The results on effect of hot water treatment on seed germination in *P. marsupium* are depicted in Table IV-2 and Fig IV-2. The seeds were exposed to hot water (60 ^{0}C) for 20, 40 and 60 minutes; the treatment for 40 minutes was found to be beneficial for seed germination as revealed by 24% and 32% increase in CPG T15 and mean CPG respectively and 47% more mean DGS (Fig IV-1d). The FGP (50.67%) was significantly improved over untreated seeds.

The seeds treated with hot water for shorter duration of 20 min showed only marginal improvement in seed germination pattern over the best control response (Table IV-2 and Fig IV-2). At the same time, longer exposure (60 min) to the hot water significantly reduced the CPG T15 (26.67%) as compared to untreated seeds (CPG T15 = 36%), (Fig IV-1a). Such seeds not only showed poor germination rate and spread in terms of higher mean germination time (MGT = 12.36 days, Fig IV-1c) but also revealed restrained germination by showing reduced final germination percentage (FGP, 32.67%) as compared to the best control response in dewinged seeds (FGP- 42%, Fig IV-1e).

4.3.1.3 Effect of boiling water treatment on seed germination

The results recorded in the Table IV-2 and Fig IV-2 on the influence of boiling water treatment on seed germination showed a significant improvement over the best control response in all the parameters computed. The germination was quicker as indicated by higher CPG T15 and mean CPG values over the best control response. Germination was spread (MGT) over a significantly short range of 9.55 days as compared to 11.33 days for dewinged seeds. The seeds germinated with 3.53% mean DGS that ultimately culminated in 38% increase in final percentage germination (FGP = 58%) over dewinged seeds (42%) at the end of the trial.

4.3.1.4 Effect of physical scarification on seed germination

The results on the effect of physical scarification on seed germination in *P. marsupium* are depicted in Table IV-2 and Fig IV-2.

Physical scarification resulted in the 55.33% CPG T15 (Fig IV-2a) that culminated in 67.33% FGP (Fig IV-e) which was significantly improved over dewinged pods (FGP = 42%). The germination spread (MGT, 8.43 days, Fig IV-2c) was lessened by about 26% than untreated seeds (11.33 days) with significantly improved mean DGS of 4.41% seeds per day as compared to 1.8% seeds per day in untreated seeds (Fig IV-d).

There was not much difference in the seedling vigor in the seedlings obtained from intact seeds and the dewinged seeds. However, in the seedlings obtained from seeds treated with hot water for 40 min (1.85), seeds subjected to boiling water (2.06) and the physically scarified seeds (2.67), seedling vigor differed significantly from that shown by seedlings from dewinged seeds (1.34) (Fig IV-2f).

4.3.2 Discussion

One of the major barriers preventing the seed germination includes seed coat and other enclosing tissues such as endosperm, pericarp or extra-floral organs (Taiz and Zeiger, 2006). Physical dormancy is mainly the result of seed coat impermeability regarding water uptake and oxygen diffusion into the embryo (Morris *et al.*, 2000; Baes *et al.*, 2002; Olvera-Carrillo *et al.*, 2003; Briggs *et al.*, 2005). Such a barrier should be removed in some way before seeds can germinate. The diaspore in *Pterocarpus marsupium* is an orbicular winged pod (samara) where seed remains enclosed inside a hard seed enclosure. Artificial techniques may improve permeability in hard-coated seeds (Rolston 1978), some of which like subjecting the seeds to chemical or physical scarification and application of thermal or pressure techniques simulate natural conditions, for example during wildfires, mechanical abrasion, digestion by birds and mammals, or soil temperature changes (Bewley and Black, 1994; Morris, 2000; Baskin and Baskin, 2001; Manzano *et al.*, 2005). A variety of pre-sowing treatments such as hot water, sulphuric acid and mechanical scarification has successfully been used to overcome seed coat-imposed dormancy (Teketay, 1996; Teketay and Tigabu, 1996; Tigabu and Odéon, 2001; Ren and Tao, 2004; Bhatia *et al.*, 2005; Phartyal et al., 2005).

The results of the present investigation suggest that the germination in *P. marsupium* improves after cutting the wing of the seed (Table 4.1 and Fig 4.1). The removal of wing has created an easy passage for water to enter earlier than in the winged seeds. This is evident from the better germination behavior in terms of higher CPG T15, lower MGT, faster germination (higher mean DGS) with more FGP than winged seeds.

Pterocarpus marsupium is a member of Leguminosae and is included under the subfamily Fabaceae. In Leguminosae, hot water overcomes physical dormancy by creating tension which consequently causes cracking of the macrosclerid layer (Brant *et al.*, 1971), or by affecting the strophiolar plug (Dell, 1980). The most widely used pre-

germination treatments for damaging the coat or breaking dormancy in legumes are short lasting soaking of the seeds in hot water or physical scarification or piercing of the coat (Rolston, 1978).

The results of the present investigation on seed germination in *Pterocarpus marsupium* by hot and boiling water treatment indicate that these treatments improved seed germination pattern wherein the boiling water treatment was better as compared to hot water treatment. This was evident by 15% decrease in MGT, 96% increase in mean DGS and improved FGP by 35% in the seeds treated with boiling water over the best control response. These results on the use of boiling water to improve the seed germination are in agreement with the results on *Pterocarpus santalinus* (Naidu and Mastan, 2001), wherein the effects of different pretreatment methods including soaking in boiling water (80–100 ^{0}C) for different durations on seed germination were tested and better germination percentage compared to the best control response was reported. Further they suggested that the method can be adopted for raising the seedlings on large scale in the nursery practices for plantation. Kalimuthu and Lakshmanan (1995), however, reported contrasting results with the treatment of hot water indicating unimproved germination in the seeds of *P. santalinus* as well as *P. marsupium* soaked in hot water for 24 h. This contrasting result might by due to the prolonged exposure of seeds to the hot water which might have damaged the embryo and reduced the chances of its germination.

The use of hot water treatment to improve seed germination has also been reported in other members from Fabaceae, viz., *Cercis, Robinia, Parkia* and *Cytisus*. A 24-hour soak in water; a boiling water dip for 15 seconds or more followed by a 24-hour soak in cooler water; or immersion overnight in 88 ^{0}C hot water that gradually cools; has been generally used to overcome seed-coat dormancy in *Cercis* (Heit, 1967; Robertson, 1976; Frett and Dirr, 1979; Smith, 1986; Raulston, 1990) and *Robinia* species (Wilson, 1944). The seeds of *Parkia biglobosa* exposed to

boiling water for 4 seconds gave maximum percentage germination (42.9%) whereas untreated seeds (the best control response) did not germinate during the eight week experimentation period (Aliero, 2004). Abdullah *et al.* (1989) reported that repeated brief (3-second) immersion in boiling water resulted in complete elimination of hard-seededness in *Cytisus scoparius* but low germination percentage was indicative of some damage done to the embryo.

An increase in temperature triggers germination by changing the internal enzymatic kinetics and thus the biochemistry of seed cells or by melting the suberized layer in seed coat sclerenchyma or at micropyle, allowing the seed to take up water (Agboola and Etejere, 1991). The sudden exposure of dry seeds to boiling water may rupture the seed coat, thereby allowing water to permeate the seed tissues causing physiological changes and subsequent germination of the embryo (Agboola and Adedire, 1998). The results of the present investigation on the use of boiling water to facilitate early germination in *P. marsupium* and similar earlier reports in other genera of Fabaceae are suggestive of the similar mechanism of action of hot/boiling water treatment on seed germination.

Mechanical scarification is a simple and one of the very effective methods to overcome seed-coat-imposed dormancy. Rupturing the seed coat by physical scarification has enhanced germination in many tropical seeds (Aduradola *et al.*, 2005). Hard seeded nature of legumes is an important reason for seed dormancy or delayed germination in legumes.

In the present investigation the seeds of a legume *P. marsupium,* were scarified to produce a narrow opening in the seed case. In such scarified seeds the germination was significantly improved over the best control response, not only in terms of higher FGP (67.33%) than the best control response (42%), but also with shorter spread (MGT = 8.43 days) and higher germination rate (mean DGS = 4.41% seeds per day) as compared to the best control response (11.33 days and 1.8% seeds per day respectively). The rapid germination of scarified seeds was probably due to

water and gases entering the embryo early through the cracks that fired a sequence of biochemical reactions resulting in the transformation of the embryo into a seedling early enough than other physical treatments. These results are analogous to those reported by Dayanand and Lohidas (1988) in *Pterocarpus santalinus* wherein the seeds were scarified by rubbing them with sand. The untreated seeds showed only 36.5% germination whereas scarification improved the germination up to 58.5%.

The rupturing of the seed coat is a mechanism which has triggered germination in many hard-seeded species with impermeable seed coats (Ballard 1973; Baskin & Baskin 1989; Bewley & Black 1994; Rolston 1978; Tigabu and Oden 2001). Such methods to rupture seed coat can change the percentage of germinated seeds from less than 20% to more than 90% (Rolston 1978). It is also referred as nicking and has been effective in releasing the dormancy in many genera like *Acacia tortilis, A. seyal, Albizia gummifera, Brachystegia spiciformis, Delonix elata, Faidherbia albida, Leucaena leucocephala, Maesopsis eminii, and Terminalia* (Msanga 1998, Wolf and Kamondo 1993). Kaye and Kuykendall (2001a) found physical scarification as an effective method for promoting germination in *Lupinus sulphureus* ssp. *Kincaidii.* The germination percentage in *Enterolobium contortisiliquum* was 2% and 92% in the non-scarified and scarified seeds respectively (Malavasi and Malavasi, 2004).

Most of the members of Leguminosae show delayed and uneven germination behavior because of presence of hard, water impermeable seed/fruit coat. This general conclusion is also applicable to *P. marsupium* since more germination was observed in dewinged pods of *P. marsupium* than in the winged pods. Further, the physical scarification substantially improved seed germination pattern, which can be taken as an indicative of existence of physical dormancy in *P. marsupium.*

4.3.3 *Santalum album* L.

4.3.3.1 Effect of hot water and boiling water treatment on seed germination

The results on the effect of hot water treatment (60 ^{0}C) on seed germination in *Santalum album* are presented in Table IV-3 and Fig.IV-3. The seeds treated with hot water for 20 and 40 min showed CPG T30 as 28.67% and 31.33% respectively. These values of CPG T30 were marginally higher than the best control response (CPG T30 = 23.33%, Fig IV-3a). The seeds exposed to hot water for 40 min showed better germination behavior compared to seeds exposed to hot water treatment for 20 min. This was evident by higher value of mean CPG (16.88%), lowered MGT (23.88 days), improved mean DGS (0.73% seeds per day) and better FGP (28%) than the values obtained in seeds exposed to hot water for 20 min as well the untreated seeds. The germination was delayed in the seeds exposed to hot water for 60 min as evident by lower CPG T30 (22.67%), lower mean CPG (12.44%), higher MGT (23.71 days), less mean DGS (0.51% seeds per day) and low FGP (26%).

The results recorded on effect of boiling water (100 ^{0}C) treatment on seed germination in Table 4.3 and Fig.IV-3 suggested the hindered germination pattern in the treated seeds. This is reflected by lower CPG T30 (22%), mean CPG (12%) and FGP (24%) as compared to the best control response. In untreated seeds the higher values of CPG T30, mean CPG and FGP were 23.33%, 12.44% and 25.33% respectively. However, the germination in terms of MGT and mean DGS did not differ significantly in the treated and the best control response seeds. In untreated as well as treated seeds the germination spread in terms of MGT remained in the range of 23-24 days and germination speed in terms of mean DGS remained in the range of 0.49 – 0.54% seeds per day

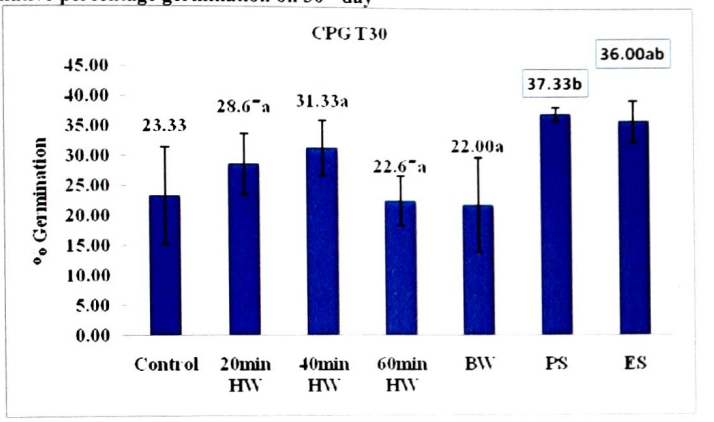
IV-3: Effect of hot water, boiling water and physical scarification on seed germination in *Santalum album* L.

DAS	Treatment	Seeds Germinated During Time Interval	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
	Control	1.33 ± 0.9	2.80 ± 1.2	1.33 ± 0.6	2.80 ± 1.2
	20 min HW	1.67 ± 0.9	3.33 ± 1.2	1.67 ± 0.6	3.33 ± 1.2
	40 min HW	1.67 ± 0.0	3.33 ± 0.0	1.67 ± 0.0	3.33 ± 0.0
10	60 min HW	0.33 ± 1.0	1.33 ± 2.0	0.33 ± 1.0	0.67 ± 2.0
Days	BW	0.33 ± 0.6	0.67 ± 1.2	0.33 ± 0.6	0.67 ± 1.2
	PS	2.67 ± 0.6	5.33 ± 1.2	2.67 ± 0.6	5.33 ± 1.2
	ES	2.67 ± 1.5	5.33 ± 3.1	2.67 ± 1.5	5.33 ± 3.1
	Control	5.33 ± 1.5	9.67 ± 3.1	6.67 ± 2.1	12.33 ± 4.2
	20 min HW	6.00 ± 1.0	12.00 ± 2.0	7.67 ± 1.2	15.33 ± 2.3
	40 min HW	6.33 ± 0.6	12.67 ± 1.2	8.00 ± 0.6	16.00 ± 1.2
20	60 min HW	6.67 ± 1.0	13.33 ± 2.0	7.00 ± 1.0	14.00 ± 2.0
Days	BW	6.33 ± 1.2	12.67 ± 2.3	6.67 ± 1.5	13.33 ± 3.0
	PS	7.67 ± 1.2	15.33 ± 2.3	10.33 ± 1.0	20.67 ± 2.0
	ES	7.33 ± 0.6	14.67 ± 1.2	10.00 ± 1.7	20.00 ± 3.5
	Control	7.00 ± 2.0	14.45 ± 4.0	14.67 ± 4.1	23.33 ± 8.1
	20 min HW	6.67 ± 1.5	13.33 ± 3.1	14.33 ± 2.5	28.67 ± 5.0
	40 min HW	7.67 ± 1.2	15.33 ± 2.3	15.67 ± 0.6	31.33 ± 1.2
30	60 min HW	4.33 ± 0.6	8.67 ± 1.2	11.33 ± 1.2	22.67 ± 2.3
Days	BW	4.33 ± 3.2	8.67 ± 6.4	11.00 ± 4.0	22.00 ± 8.0
	PS	8.33 ± 0.6	16.67 ± 1.2	18.67 ± 0.6	37.33 ± 1.2
	ES	8.00 ± 0.0	16.00 ± 0.0	18.00 ± 1.7	36.00 ± 3.5

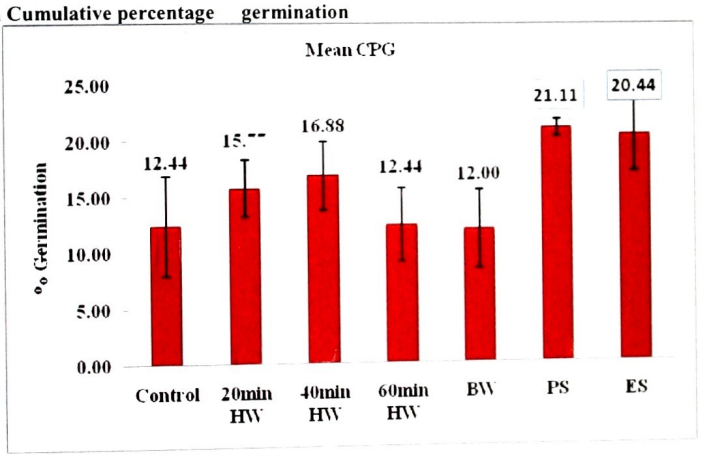
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
HW = Hot water (60°C), BW = Boiling water (100°C), PS = Physical scarification
ES = Extracted seeds

IV-3 Effect of physical treatments on seed germination in *Santalum album* L.

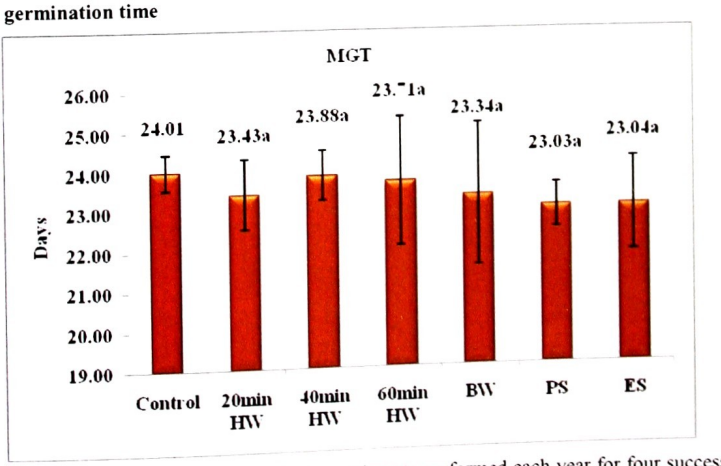
a) Cumulative percentage germination on 30th day



b) Mean Cumulative percentage germination



c) Mean germination time



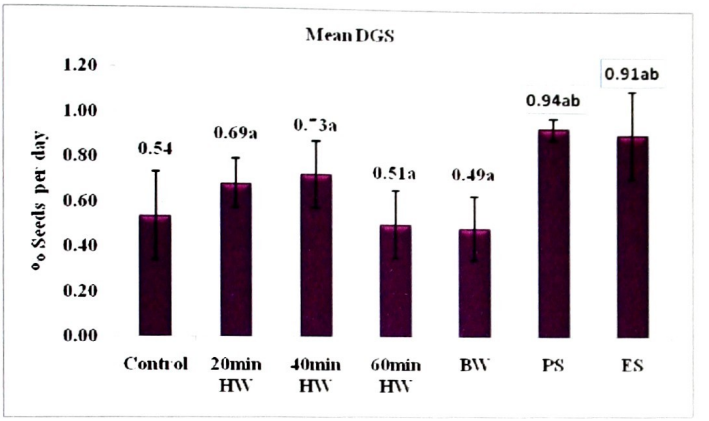
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.

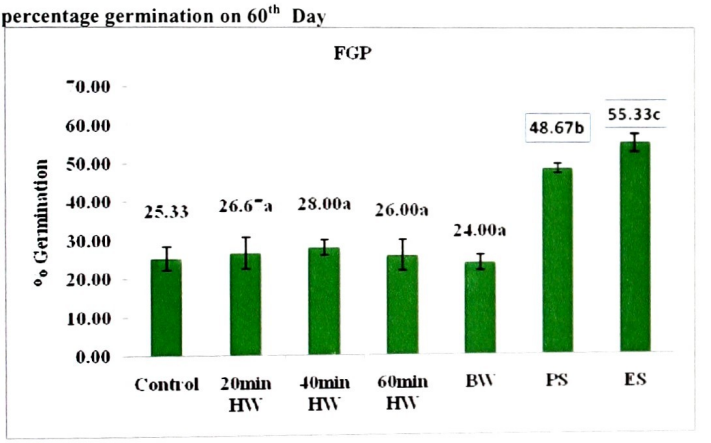
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

IV-3 Effect of physical treatments on seed germination in *Santalum album* L.

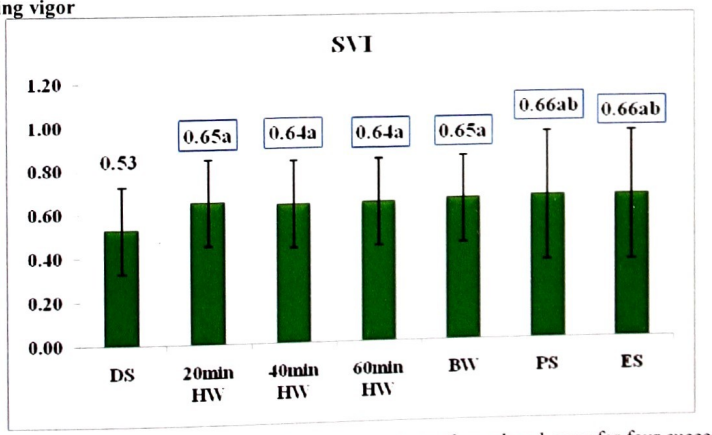
d) Mean daily germination speed



e) Final percentage germination on 60th Day



f) Seedling vigor



The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

4.3.3.2 Effect of physical scarification and complete removal of seed coat on seed germination

The physical scarification (PS) by cracking the stony endocarp resulted in improved germination (Table IV-3 and Fig.IV-3a-f). The cracking of endocarp was helpful to enhance the germination process that consequently produced significant improvement in all the parameters computed. The CPG T30, mean CPG and FGP showed 60%, 70% and 45% increment over the best control response respectively. The spread of germination in terms of MGT was marginally decreased to 23.03 days from 24.01days in the best control response. The germination was significantly quicker as reveled by 74% increment over the best control response in mean DGS.

The germination in the seeds without endocarp showed 54% increase in CPG over the best control response (CPG T15 = 23.33%) however, the spread of germination was only marginally reduced (MGT = 23.04 days) as compared to the best control response (MGT = 24.01 days) (Table IV-3 and Fig IV-3c). On the other hand, the speed of germination was significantly faster in the seeds devoid of stony endocarp as reflected in mean DGS of 0.91% seeds per day. This was significantly faster than the best control response in which the mean DGS recorded was only 0.54% seeds per day. In the best control response the FGP was 25.33% only whereas in the seeds devoid of endocarp it was significantly boosted to 41.33%, i.e., about 63% increment in germination over the best control response was recorded.

All the physical treatments applied for enhanced germination in *Santalum album* also improved the seedling vigor significantly compared to vigor computed in the seedlings obtained from non-treated seeds. However, seedling vigor was more or less same in the seedlings obtained from different seed treatments (Fig IV-3f).

4.3.4 Discussion

Sandalwood tree is deeply connected with many events of rich cultural heritage in India and is highly acclaimed worldwide. It is among the oldest known perfumery material (Srinivasan *et al.*, 1992). The ever increasing demand for sandalwood and products derived from it has resulted in exhausting their natural stands. On the other hand, its propagation through seeds has limitations due to poor rate of seed germination (Nagarajaiah and Rao, 1993). The diaspore in *Santalum album* is a seed enclosed in the hard, stony endocarp; in general this total structure is considered as 'seed'.

The seeds were exposed to physical treatments viz., immersion in hot water for different durations, boiling water treatment, physical scarification and complete removal of endocarp and the seeds were observed for germination pattern. The seeds initially hydrated in distilled water (control) have shown poor germination behavior in terms of lower mean CPG, longer spread of germination (higher MGT), less mean DGS and lower FGP. This result is in consonant with that obtained by Manohar *et al.* (1999) in *S. album*. They reported unimproved germination in seeds of *S. album* subjected to soaking treatments in tap water for different durations.

The hot water treatments applied to sandal seeds only marginally improved the germination behavior. These results concur with those by Nagaveni and Srimathi (1981), where soaking the sandal seeds in warm water (80 ^{0}C) was reported to be ineffective in improving seed germination.

Cracking of the hard endocarp in sandal seeds not only improved the FGP (44% more than the best control response), but also resulted in faster germination as indicated by lower MGT (6% lower than the best control response) and higher mean DGS (74% more than the best control response). These results on improved seed germination in *S. album*

after cracking the endocarp are on the same line of those reported by Akamine (1947), Srimathi and Rao (1969), Effendi *et al.*(1996) and Woodall (2004).

Akamine (1947) commented that the only effective method of hastening the germination of seed of *S. album* was to remove the seed coat partially or preferably wholly, by hand and hot water was either ineffective or injurious. Effendi *et al* (1996) also reported significantly accelerated germination in the seeds of *S. album* after incising the endocarp followed by soaking in water for 0, 12, 24, 36, 48, 60, or 72 hours. Seed germination in *S. spicatum* was enhanced, in both field as well as pot studies, when the woody endocarp was fractured and a simple wetting and rapid drying procedure to crack large amounts of sandalwood nuts prior to sowing in the field was suggested (Woodall, 2004).

However, the results on the germination after physical scarification (cracking the endocarp) of sandal seeds of the present investigation do not agree with Nagaveni and Srimathi (1981), where physical scarification was reported to be ineffective.

Thus, the results of the present investigation on seed germination in *S. album* and earlier reports on *S. album* and other species of *Santalum* are suggestive of facilitated germination due to cracking the endocarp.

This investigation reports improved seed germination behavior after complete removal of the stony endocarp. The stony endocarp was first cracked opened by gently hammering with pestle in a mortar. The cracked endocarp was then separated to liberate the seed. The seeds without endocarp were sown in the soil and observed for germination (Table IV-3 and Fig IV-3). Such seeds showed improved germination pattern in terms of elevated CPG T30, mean CPG and FGP along with higher mean DGS and less MGT as compared to seeds enclosed in the endocarp. However, the results recorded in the present investigation were suggestive of no difference in the seed germination pattern observed in the

seeds completely devoid of endocarp and the seeds in which the endocarp was cracked open. Removal of the mesocarp of *S. album* seed results in 85-100% germination within 30 days, under laboratory as well as in the nursery conditions (Srimathi and Rao, 1969).

The influence of physical scarification and/or warm water treatments, however, appears to be species specific, i.e., some species may not react equally to these treatments and may not show enhanced germination after application of these treatments.

Dehgan *et al.*, (2003) reported that physical scarification with sandpaper failed to improve seedling emergence in *Lupinus diffuses* and immersing seeds in 90 ^{0}C water which was then allowed to cool for 24 h, either killed or severely injured the embryos as no seedlings emerged from treated seeds. On the contrary, seeds of *Lupinus texensis* placed in 85^{0}C water improved seedling emergence but the treatment was not as effective as physical scarification (Davis *et al.*, 1991) while Hosokawa *et al.* (2001) recommended hot water treatment scarification for *Lupinus sericeus* seeds.

The germination percentages increased in *Apeiba aspera,* after submerging the seeds in warm water (70 ^{0}C) for 10 minutes but the same treatment did not improve germination of seeds of *A. tibourbou* (Sautu *et al.*, 2006).

In *Ulex europaeus* L., none of the hot water treatments (70, 75, 80, 85, 90, 95 or 100°C for 1, 2 and 5 min) could significantly improve germination over the control seeds while physical scarification gave a germination of 35%, which was significantly different ($P<0.001$) from unscarified seed, which had a germination of 10% (Sixtus *et al.*, 2003).

In *Senna occidentalis*, Demel and Teketay (1996) demonstrated 82 to 100% germination after physical scarification and immersing in boiling water.

From the results of the present investigation on *Santalum album,* the germination pattern observed after complete removal of stony

endocarp was not much different than the pattern observed in cracked seeds (physical scarification) but was significantly improved over the best control response. Nicolás *et al.,* (1996, 1997), Thomsen (1997) and Shen & Odén (2002) showed that in European beechnuts, removal or physical scarification of the endocarp resulted in alleviation of germination capacity and dormancy release rates. Seed coat/pericarp as constrain in the germination of seeds is widespread in tree and shrub species and is often referred to as physical dormancy (Baskin and Baskin, 2001).

The results of the present investigation and earlier reports in *Santalum* and other genera are suggestive of the role of the endocarp in modifying dormancy. The hard endocarp is acting as a mechanical barrier restricting entry of water, and protrusion of the radicle, thereby delaying germination.

Chapter V

Breaking of Dormancy By Acid Scarification

5.1 Introduction

Controlled seed germination is one of the necessary aspects in the natural perpetuation of some species for which there exist some critical checkpoints at the transitions from dormancy to germination and from germination to growth (Kermode, 2005). The forest industries rely on seeds that exhibit high rates of germination and vigorous, synchronous growth after germination; hence dormancy is sometimes considered as an undesirable trait (Kermode, 2005). Scarification is any process of breaking, scratching, mechanically altering, or softening the seed coverings to make them permeable to water and gases to ensure early and uniform germination (Hartmann, 1997b). Mineral acids soften the seed coat thereby removing barriers to moisture uptake, gas exchange, swelling of the embryo, and may create easy passage for radicle emergence.

The pronounced dormancy in the seeds of some forest tree species leads to a non-uniform germination and poor seedling vigor that create inconvenience for the forest nurseries. The germination in *P. marsupium* and *S. album* was very low even when the TZ test indicated more than 90% viability. The diaspore in *P. marsupium* has hard and woody pericarp while that of *S. album* has hard woody endocarp. As acid scarification improves the permeability in such structures and help to alleviate germination percentage, the effects of acid scarification on the germination in seeds of these species were analyzed and are described in the present chapter.

5.2 Materials and methods

The seeds for the acid scarification experiments were selected randomly. Fifty seeds were allocated to each treatment. Other details on the design of experiments, collection of data and statistical analysis of data are described in the Chapter III.

The hydrochloric acid (HCl), sulphuric acid (H_2SO_4) and nitric acid (HNO_3) used in this study were of analytical grade and procured from Sisco Research Laboratory (SRL), India.

The seeds of *P. marsupium* were acid scarified in a 500ml Borosil beaker by soaking the seeds in concentrated H_2SO_4 (99%, 36N), concentrated HCl (35%, 11N) and concentrated HNO_3 (70%, 15.6 N) separately for 15, 30 and 45 min. The control was maintained by submerging the seeds in distilled water for 15, 30 and 45 min. The volume of acid for the treatment was used as recommended by Hartmann *et al.*, (2002). The seeds were exposed to acid treatment in the ratio of 2:1:: Acid : seed (v/v). The acid–seed mixture was sparingly stirred by using glass rod. After acid scarification, the acid was decanted and the seeds were washed under running tap water for 2 h. The seeds were then sown in the plastic bags (4" × 6") and cavity seeding trays containing moist garden soil and observed for germination pattern on 5^{th}, 10^{th} and 15^{th} day after sowing (DAS) and for seedling vigor.

Samples of 50 seeds of *S. album* were used for acid scarification with concentrated H_2SO_4 (99%, 36N), concentrated HCl (35%, 11N) and concentrated HNO_3 (70%, 15.6 N) separately for 15, 30 and 45 min. At the same time, the samples of 50 seeds were placed in distilled water (control) for 15, 30 and 45 min. The other details in the acid scarification method were similar to those for seeds of *P. marsupium* described above.

The seeds were then sown in the plastic bags (4" × 6") and cavity seeding trays containing moist garden soil and observed for germination pattern on 10^{th}, 20^{th} and 30^{th} day after sowing (DAS) and for seedling vigor.

5.3 Results

5.3.1 *Pterocarpus marsupium* Roxb.

5.3.1.1 Effect of concentrated HCl

The results on effect of concentrated HCl on seed germination in *P. marsupium* are depicted in Table V-1 and Fig V-1a-f.

In the seeds treated for 15 min, 16% seeds germinated within first five days while 34% seeds took 5-10 days to germinate and only 2% seeds germinated from 10-15days after sowing. The seeds treated for 30 min showed 17.33%, 27.33% and 8% germination during 0-5, 5-10 and 10-15 days respectively. The seeds treated for 45 min showed delayed germination wherein 2.67%, 29.33% and 6.67% seeds germinated during 0-5, 5-10 and 10-15 days respectively. On the basis of these results, it is concluded that the treatment for 30 min duration was more beneficial than from 15 and 45 min treatments for improving seed germination in *P. marsupium.* This was also evident from various germination parameters computed from this data.

Seeds of *P. marsupium* scarified with concentrated HCl for 15 and 30 minutes showed significantly improved germination that was reflected in CPG T15 (52.00 and 52.67% respectively, Fig V-1a) which was significantly improved over the best control response (CPG T15 =24.67%). Similar trend was observed in the mean CPG (Fig V-1b) which was also significantly improved in the seeds treated with concentrated HCl for 15 and 30 min.

The germination rate was equally faster in the seeds treated with concentrated HCl for 15 min (mean DGS = 3.89% seeds per day, Fig V-1d) and 30 min (mean DGS = 3.81), but the seeds treated for 30 min showed significantly less MGT (9.11 days, Fig V-1c) as compared to untreated seeds (MGT = 11.43 days). The percentage germination in the seeds treated with concentrated HCl for 30 minutes was significantly improved (FGP = 61.33%) over control (FGP = 43.33%), (Fig V-1e).

V-1: Effect of scarification with concentrated HCl (11 N) on seed germination in *Pterocarpus marsupium* Roxb.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
5 Days	Control	3.33 ±1.5	2.67 ± 2.3	3.33±1.5	2.67± 2.3
	11 N HCl 15 min	8.00 ± 2.0	16.0 ± 4.0	8.00 ± 2.0	16.0 ± 4.0
	11 N HCl 30 min	8.67 ± 5.3	17.33 ± 10.6	8.67 ± 5.3	17.33 ± 10.6
	11 N HCl 45 min	1.33 ± 0.6	2.67 ± 1.2	1.33 ± 0.6	2.67 ± 1.2
10 Days	Control	9.33 ± 2.5	18.67 ± 5.0	12.67 ± 1.2	25.33 ± 2.3
	11 N HCl 15 min	17.00 ± 1.7	34.00 ± 3.5	25.00 ± 2.7	50.00 ± 2.3
	11 N HCl 30 min	13.67 ± 3.1	27.33 ± 6.1	22.33 ± 4.2	44.67 ± 8.3
	11 N HCl 45 min	14.67 ± 2.5	29.33 ± 5.0	16.00 ± 2.0	32.00 ± 4.0
15 Days	Control	7.00 ± 2.0	14.00 ± 4.0	19.67 ± 1.2	39.33 ± 2.3
	11 N HCl 15 min	1.00 ± 1.0	2.00 ± 2.0	26.00 ± 6.6	52.00 ± 7.2
	11 N HCl 30 min	4.00 ± 3.5	8.00 ± 7.0	26.33 ± 3.21	52.67 ± 6.4
	11 N HCl 45 min	3.33 ± 1.2	6.67 ± 2.3	19.33 ± 2.31	34.00 ± 4.6

The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

V-2: Effect of scarification with concentrated H_2SO_4 (36 N) on seed germination in *Pterocarpus marsupium* Roxb.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
	Control	3.33 ± 1.5	6.67 ± 3.1	3.33 ± 1.5	6.67 ± 3.1
	36 N H_2SO_4 15 min	10.67 ± 4.0	21.33 ± 8.1	10.67 ± 4.0	21.33 ± 8.1
5 Days	36 N H_2SO_4 30 min	15.33 ± 3.6	30.67 ± 7.2	15.33 ± 3.6	30.67 ± 7.2
	36 N H_2SO_4 45 min	17.33 ± 2.5	34.67 ± 5.1	17.33 ± 2.5	34.67 ± 5.0
	Control	9.33 ± 2.5	18.67 ± 5.1	12.67 ± 1.2	25.33 ± 2.3
	36 N H_2SO_4 15 min	16.00 ± 4.6	32.00 ± 9.2	26.67 ± 1.5	53.33 ±3.1
10 Days	36 N H_2SO_4 30 min	22.00 ± 3.5	44.00 ± 7.1	37.33 ± 4.1	74.67 ± 8.1
	36 N H_2SO_4 45 min	22.67 ± 1.2	45.33 ± 2.3	40.00 ± 2.7	80.00 ± 5.3
	Control	7.00 ± 2.0	14.00 ± 4.0	19.67 ± 1.1	39.33 ± 2.3
	36 N H_2SO_4 15 min	2.00 ± 1.0	4.00 ± 2.0	28.67 ± 2.3	57.33 ± 4.6
15 Days	36 N H_2SO_4 30 min	2.00 ± 1.5	4.00 ± 3.1	39.33 ± 2.5	78.67 ± 5.0
	36 N H_2SO_4 45 min	2.00 ± 1.7	4.00 ± 3.5	42.00 ± 1.0	84.00 ± 2.0

The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

Scarification for longer duration (45min, Fig 5.1a-e) with concentrated HCl though was apparently effective for seed germination in *P. marsupium* as evident from improved CPG T15 (38.67%) as compared to the best control response (24.67%), better MGT of 10.50 days and higher FGP (56%), the seedlings were physically damaged and there was high rate of mortality among the seedlings. The physical damage was evident in the burnt cotyledonary leaves in these seedlings. These seedlings were slow growing and showed only 22% survival.

5.3.1.2 Influence of concentrated H_2SO_4

The results on effect of scarification with concentrated H_2SO_4 on seed germination in *P. marsupium* are presented in Table V-2 and Fig V-1a-e and Plate V-1. From the recorded data on effect of presoaking in acid for different durations (15, 30 and 45min) on seed germination; presoaking for 45 min was best to improve percentage germination. However, the boost in percentage germination was accompanied with severe seedling injury.

There was gradual increment in the CPG T15 in the seeds treated for 15min (57.33%), 30 min (78.67%) and 45 minutes (84%), (Fig V-1a). A similar trend was observed in the mean CPG values (Fig V-1b). The spread of germination was gradually reduced from 11.43 days in untreated seeds to 8.47 days, 8.30 days and 8.18 days in the seeds treated for 15, 30 and 45 minutes respectively (Fig V-1c). The speed of seed germination (mean DGS, Fig V-1d) was improved from 1.21% seeds per day (the best control response) to 4.46%, 6.24% and 6.84% seed per day in the seeds treated for 15, 30 and 45 minutes respectively.

The FGP (Fig V-1e) was significantly improved by 52% in seeds treated for 15 minutes, 96% in seeds treated for 30 minutes and 100% in seeds treated for 45 minutes as compared to the best control response (FGP = 43.33%).

Plate V-1: Acid scarification of seeds and germination in *Pterocarpus marsupium* Roxb.

a. Unscarified seeds
b. Seeds scarified with concentrated H_2SO_4
c. Damaged seedling due to soaking the seeds in concentrated H_2SO_4 for 60 min
d. Seedlings in polythene bags

Plate V-1

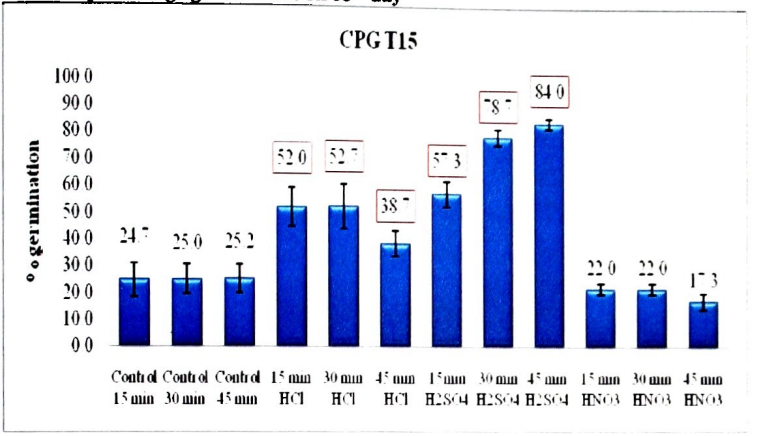
V-3: Effect of scarification with concentrated HNO_3 (15 N) on seed germination in *Pterocarpus marsupium* Roxb.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
5 Days	Control	3.33 ± 1.5	2.67 ± 2.3	3.33 ± 1.5	2.67 ± 2.3
	11 N HNO_3 15 min	1.33 ± 1.2	21.33 ± 8.1	10.67 ± 4.0	21.33 ± 8.1
	11 N HNO_3 30 min	4.00 ± 2.0	8.00 ± 4.0	4.00 ± 2.0	8.00 ± 4.0
	11 N HNO_3 45 min	2.33 ± 0.6	4.67 ± 1.2	2.33 ± 0.6	4.67 ± 1.2
10 Days	Control	9.33 ± 2.5	18.67 ± 5.0	12.67 ± 1.2	25.33 ± 2.3
	11 N HNO_3 15 min	12.67 ± 1.2	25.33 ± 2.3	14.00 ± 2.0	28.00 ± 4.0
	11 N HNO_3 30 min	10.67 ± 3.1	21.33 ± 6.1	14.67 ± 4.6	29.33 ± 9.2
	11 N HNO_3 45 min	5.67 ± 1.2	11.33 ± 2.3	8.00 ± 1.0	16.00 ± 2.0
15 Days	Control	7.00 ± 2.0	14.00 ± 4.0	19.67 ± 1.2	39.33 ± 2.3
	11 N HNO_3 15 min	4.33 ± 1.5	8.67 ± 3.1	18.33 ± 2.9	36.67 ± 5.8
	11 N HNO_3 30 min	5.00 ± 0.6	10.00 ±1.2	19.67 ± 4.9	39.33 ± 9.9
	11 N HNO_3 45 min	3.00 ± 1.0	6.00 ± 2.0	11.00 ± 1.0	22.00 ± 2.0

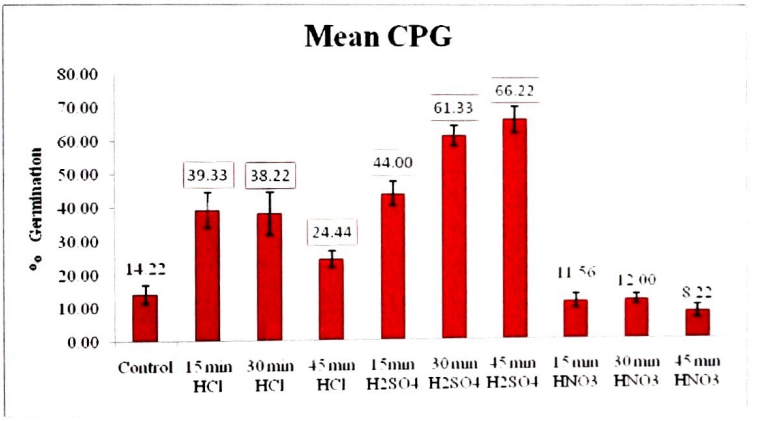
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

V-1 Effect of acid scarification on seed germination in *P. marsupium*

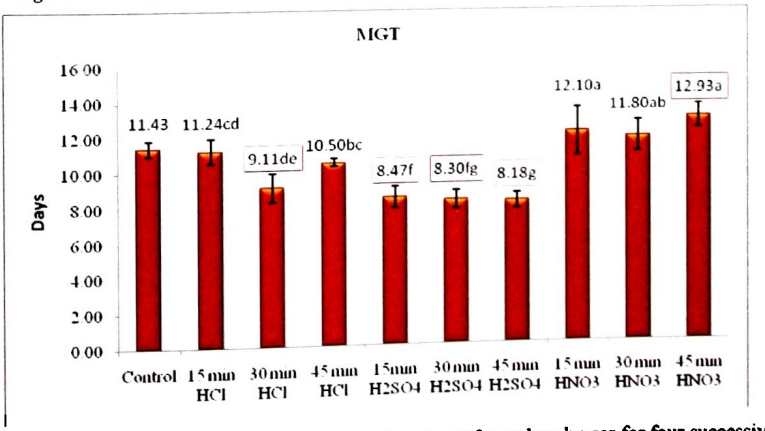
a) Cumulative percentage germination on 15th day



b) Mean Cumulative percentage germination



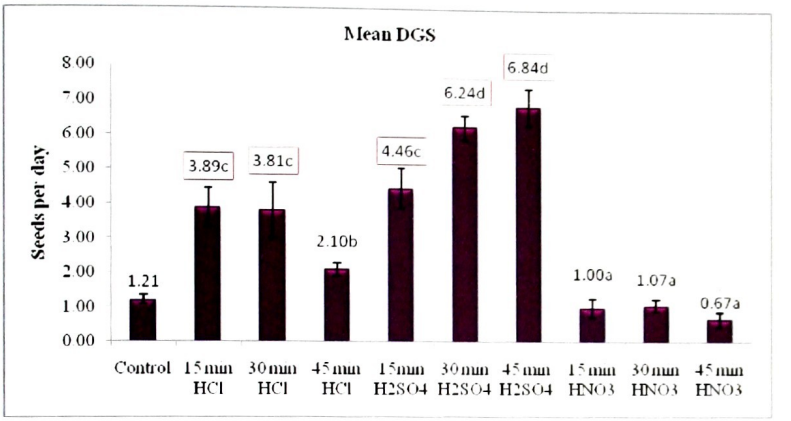
c) Mean germination time



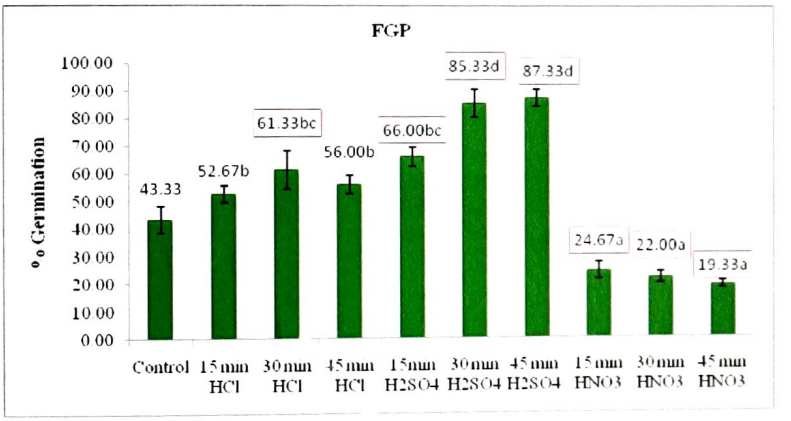
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

V-1 Effect of acid scarification on seed germination in *P. marsupium*

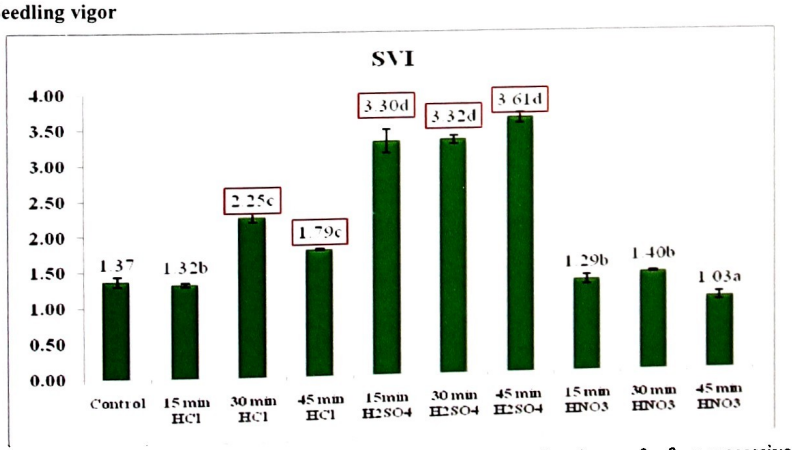
d) Mean daily germination speed



e) Final percentage germination on 30th Day



f) Seedling vigor



The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

However, physical injury was observed in the seedlings obtained from the seeds scarified for 45 min (PlateV-1). The cotyledonory leaves showed chlorosis and necrotic margins. About 80% seedlings from this experiment died after 10-12 days of emergence. The seeds scarified for 60 min did not germinate.

5.3.1.3 Effect of concentrated HNO_3

The seeds treated with concentrated HNO_3 for 15, 30 and 45 minutes showed poor seed germination pattern as compared to untreated (the best control response) seeds (Table V-3 and Fig V-1a-e).With increase in the duration, the CPG T15 decreased from 24.67% through 22% (15 and 30 min treatment) to 17.33% (45 min treatment, Fig V-1a). The spread of germination in the seeds scarified for 45 min was maximum (MGT = 12.93 days, Fig V-1c) among all acid scarification treatments and significantly more than untreated seeds. The mean DGS (0.67% seeds per day in 45 min treatment) was less than the best control response (1.21% seeds per day, Fig V-1d).

The seeds treated with concentrated HNO_3 for all durations significantly decreased the FGP to 24.67% (15 min), 22% (30 min) and 19.33% (45 min) from 43.33% in untreated seeds, indicating inhibitory effect of HNO_3 on seed germination (Fig V-1e).

The enhanced seed germination due to acid scarification of the seeds also produced boost in the seedling vigor. Seeds scarified with HCl and H_2SO_4 for 30 min produced seedlings with significantly higher vigor than the non scarified seeds, while the seedlings obtained through the scarification of seeds with concentrated HNO_3 were less vigorous as that of seedlings from non-scarified seeds (Fig V-1f).

5.3.2 Discussion

The strong mineral acids hydrolyze the organic structural components in the fruit or seed coat thereby making these structures weak and permeable. The seeds are initially treated with acid which scarifies the coat, but the treatment is stopped well before acid penetrates the pericarp or seed-coat. After presoaking in acid for particular duration, the seeds are washed thoroughly to remove the traces of acid. This procedure facilitates the seed germination by breaking the seed-coat-imposed dormancy (Gordon and Rowe, 1982). The seed coat and/or pericarp in many taxa contain or produce or are suspected to produce an inhibitory substance(s). The hard seed coat and pericarp may also act as mechanical barriers to seed germination. Acid scarification leads to partial or complete removal of inhibitory substance(s) and weakening of the hard seed coat or pericarp (Mayer and Poljakoff-Mayber, 1963). This process has been shown to give significant improvement in germination time in many genera (Dehgan and Johnson 1982; Dehgan and Schutzman 1983; Keeley and Fortheringham 2000; Baskin and Baskin, 2001).

In the present investigation, the seeds of *P. marsupium* were separately scarified with concentrated HCl, H_2SO_4 and HNO_3 for 15, 30 and 45 min. Among these treatments, better germination behavior was observed in seeds scarification with concentrated HCl and H_2SO_4 for 30 min. The germination was enhanced in terms of higher CPG T15, MGT, mean DGS, higher FGP. The seedlings showed normal morphology and survival, which is more important in seedlings obtained through acid scarification of seeds.

In the earlier report on *Pterocarpus santalinus* and *P. marsupium,* enhanced germination was reported with different acid scarification pretreatments (Kalimuthu and Lakshmanan, 1995). The treatments included soaking in concentrated H_2SO_4, HCl or HNO_3 for 10 or 15 min, or in 40% HCl for 24 h. The highest germination percentage was observed in seeds soaked in 40% HCl for 24 h (75.8% in *P. santalinus* as

compared with 37.5% in control and 84.5% in *P. marsupium* as compared with 38.5% in the the best control response). In the present investigation, presoaking the seeds in concentrated HCl for 30 min (FGP = 61.33%) was beneficial for improving seed germination in *P. marsupium.*

Scarification of *P. marsupium* seeds with concentrated HNO_3 resulted in suppressed germination. These results are on the same line of results reported by Kalimuthu and Lakshmanan (1995), wherein the treatment of concentrated HNO_3 was reported to be inhibitory for seed germination in *P. marsupium* as well as *P. santalinus.*

According to Kalimuthu and Lakshmanan (1995) the treatment with concentrated H_2SO_4 for 10 or 15 min failed to alleviate germination in *Pterocarpus santalinus* and *P. marsupium.* Contradictory results were observed in the present investigation, wherein the seeds scarified with concentrated H_2SO_4 showed improved germination and enhanced germination pattern.

The pods of *Pterocarpus santalinus* exposed to 1% sulphuric acid for 4 days or dipped in concentrated sulphuric acid for 5 min showed germination similar to that of the best control response (Dayanand and Lohidas, 1988). On the contrary in the present investigation, the seeds of *P. marsupium* treated for 30 min with concentrated H_2SO_4 showed significantly improved FGP (85.33%) over the best control response (43.33%)

To soften seed coat and/or remove chemical inhibitors from the testa, the use of sulphuric acid (H_2SO_4) scarification is well-known in literature on germination studies of many species (Baskin and Baskin, 2001).

The traditional pretreatment for improving seed germination in the shrub genus *Arctostaphylos,* (manzanita nutlets) is sulphuric acid scarification for 3 to 15 hours (Berg, 1974; Carlson and Sharp, 1975; Belcher, 1985).

The best germination in *Gymnocladus dioicus* (Fabaceae) have been obtained by treating the seeds with concentrated sulphuric acid for periods of 2 to 4 hours (Dirr and Heuser 1987; Liu *et al.*, 1981).

Germination rate of *Echinocactus grusonii* Link and Otto, Hildman and Monats (Cactaceae) and *Echinocactus platyacanthus* Link and Otto seeds improved significantly when seeds were treated with concentrated sulphuric acid (De La Rosa-Ibarra and Garcia 1994). Significant improvement in germination rate of *Pachycereus hollianus* (Weber) Buxb. seeds (Cactaceae) following sulphuric acid treatment were documented (Godínez-Alvarez and Valiente-Banuet, 1998).

Germination in seeds of *Chrysophyllum albidum* decreased with increasing sulphuric acid concentration (10, 50 and 98%) and treatment time (10, 30 and 60 minutes) (Aduradola *et al.*, 2005). Highest percentage (60%) was recorded when seeds were treated with 10% sulphuric acid for 10 minutes indicating enhanced coat permeability after acid scarification.

Sixtus *et al.* (2003) treated the seeds of *Ulex europaeus* L. with concentrated sulphuric acid for 0 (control), 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 min. The highest germination was observed in seeds treated with acid for 180 and 210 mın and showed 81% germination at the optimum incubation temperature of 15 ^{0}C.

Thus, the acid scarification was ineffective in some species but was beneficial in most of the species with seed-coat-imposed dormancy. The results recorded on the seed germination in *P. marsupium* are suggestive of the existence of the pericarp-imposed-dormancy that can be alleviated by scarification with either concentrated HCl or H_2SO_4.

5.3.3 *Santalum album* L.

5.3.3.1 Effect of concentrated HCl

The results on effect of scarification with concentrated HCl on seed germination in *Santalum album* are presented in Table V-4 and Fig V-2a-e.

With increase in the duration of acid scarification from 0 to 45 minutes, a gradual alleviation was observed in the germination pattern. However, the 15 min and 30 min treatments were only marginally effective as compared to the best control response.

A significant increase in germination pattern was observed in the seeds soaked for 45minutes in concentrated HCl as reflected in the rise in CPG T30 (25.33%, Fig V-2a) and mean CPG (16.44%, Fig V-2b) as compared to the best control response (CPG T30 = 16%, mean CPG = 9.11%) and seeds soaked in HCl for 15 and 30 min. The mean DGS was significantly boosted from 0.41% seeds per day in untreated seeds to 0.74% seeds per day in the seeds scarified for 45 min (Fig V-2d). The spread of the germination, however, remained unaffected as there was only marginal reduction in the MGT in the seeds scarified for 30 and 45 minutes (22.91 and 20.52 days respectively) as compared to the best control response (MGT = 22.92 days).

The FGP was only marginally improved (Fig V-2e) in the seeds scarified for 15, 30 and 45 minutes (21.33%, 25.67% and 26% respectively) as compared to the best control response (FGP = 21.33%). The seeds scarified for more than 45 min did not show any sign of germination.

The scarification with concentrated HCl apparently improved germination only in the early phases of trial as indicated by significant increase in the CPG T30 and mean DGS in the seeds treated with concentrated HCl for 45 min. However seeds scarified with HCl for different durations only marginally improved FGP and therefore

Plate V-2: Acid scarification of seeds and germination in *Santalum album* L.

a. Unscarified seeds
b. Seeds scarified with concentrated H_2SO_4
c. Seeds scarified with concentrated H_2SO_4 after washing
d. Seedlings in polythene bags

Plate V-2

V-4: Effect of scarification by concentrated HCl (11N) on seed germination in *Santalum album* L.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
10 Days	Control	1.33 ± 0.6	2.67 ± 1.2	1.33 ± 0.6	2.67 ± 1.2
	11 N HCl 15 min	1.00 ± 1.0	2.00 ± 2.0	1.00 ± 1.0	2.00 ± 2.0
	11 N HCl 30 min	0.33 ± 0.6	0.67 ± 1.2	0.33 ± 0.6	0.67 ± 1.2
	11 N HCl 45 min	1.67 ± 0.6	3.33 ± 1.2	1.67 ± 0.6	3.33 ± 1.2
20 Days	Control	3.00 ± 0.0	6.00 ± 0.0	4.33 ± 0.6	8.67 ± 1.2
	11 N HCl 15 min	3.67 ± 1.2	7.33 ± 2.3	4.67 ± 2.1	9.33 ± 4.2
	11 N HCl 30 min	6.67 ± 1.5	13.33 ± 3.1	7.00 ± 1.0	14.00 ± 2.0
	11 N HCl 45 min	8.67 ± 2.1	17.33 ± 4.2	10.33 ± 1.5	20.67 ± 3.1
30 Days	Control	3.67 ± 2.0	7.33 ± 4.0	8.00 ± 1.2	16.00 ± 2.3
	11 N HCl 15 min	3.67 ± 0.6	7.33 ± 1.2	8.33 ± 1.5	16.67 ± 1.0
	11 N HCl 30 min	3.67 ± 1.5	7.33 ± 3.1	10.67 ± 2.5	21.33 ± 5.0
	11 N HCl 45 min	2.33 ± 0.6	4.67 ± 1.2	12.67 ± 1.2	25.33 ± 2.3

The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

V-5: Effect of scarification by concentrated H_2SO_4 (36 N) on seed germination in *Santalum album* L.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
10 Days	Control	1.33 ± 0.6	2.67 ± 1.2	1.33 ± 0.6	2.67 ± 1.2
	36 N H_2SO_4 15 min	2.00 ± 0.0	4.00 ± 0.0	2.00 ± 0.0	4.00 ± 0.0
	36 N H_2SO_4 30 min	2.00 ± 0.0	4.0 ± 0.0	2.0 ± 0.0	4.00 ± 0.0
	36 N H_2SO_4 45 min	2.33 ± 0.6	4.67 ± 1.2	2.33 ± 0.6	4.67 ± 1.2
20 Days	Control	3.00 ± 0.0	6.00 ± 0.0	4.33 ± 0.6	8.67 ± 1.2
	36 N H_2SO_4 15 min	5.00 ± 0.0	10.00 ± 0.0	7.00 ± 0.0	14.00 ± 0.0
	36 N H_2SO_4 30 min	7.33 ± 0.6	14.67 ± 1.2	9.33 ± 0.6	18.67 ± 1.2
	36 N H_2SO_4 45 min	9.33 ± 2.1	18.67 ± 4.2	11.67 ± 1.5	23.33 ± 3.1
30 Days	Control	3.67 ± 2.0	7.33 ± 4.0	8.00 ± 1.2	16.00 ± 2.3
	36 N H_2SO_4 15 min	5.00 ± 1.0	10.00 ± 2.0	12.00 ± 1.0	24.00 ± 2.0
	36 N H_2SO_4 30 min	5.33 ± 0.6	10.67 ± 1.2	14.67 ± 0.6	29.33 ± 1.2
	36 N H_2SO_4 45 min	5.67 ± 0.6	11.33 ± 1.2	17.33 ± 1.2	34.67 ± 2.3

The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

scarification with concentrated HCl was not effective to promote germination in *S. album*.

5.3.3.2 Effect of concentrated H_2SO_4

The results recorded on effect of concentrated H_2SO_4 on seed germination in *S. album* (Table V-5 and Fig V-2a-e and Plate V-2) revealed that seeds scarified with concentrated H_2SO_4 showed significantly improved germination over the best control response. With increase in the duration of scarification from 0 to 45 min, a progressive improvement was noticed in the germination and the best germination was observed in the seeds scarified for 45 minutes. The scarification for more than 45 minutes was deleterious as it produced the weaker seedlings with burnt cotyledonary leaves and the seedlings exhibited very high rate of mortality (~ 85%).

Seed germination was significantly improved (Fig V-2a) in the seeds scarified for 15, 30 and 45 min as indicated in the CPG T30 that showed increment of 50%, 83% and 117% respectively over the best control response (CPG T15 = 16%). A similar trend was noticed in the mean CPG (Fig V-2b).

All the scarification treatments significantly enhanced the speed of germination as indicated by higher values of mean DGS (Fig V-2d) over the best control response. However, the germination spread was not significantly reduced by any of the scarification durations as revealed by more or less same MGT (Fig V-2c) in untreated seeds (MGT = 22.92 days) and seeds scarified for 15min (MGT = 22.54 days), 30 min (MGT = 22.57 days) and 45 min (MGT = 22.91 days).

The final output in terms of number of seedlings generated in each treatment, was only marginally improved in the seeds scarified for 15 min (FGP = 24%) as compared to the best control response (FP = 21.33%). Scarification for 30 and 45 min, on the other hand, produced

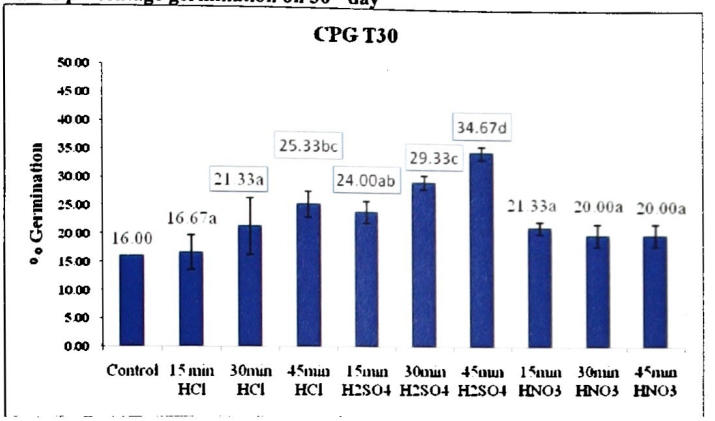
V- 6: Effect of scarification by concentrated HNO_3 (15 N) on seed germination in *Santalum album* L.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
10 Days	Control	1.33 ± 0.6	2.67 ± 1.2	1.33 ± 0.6	2.67 ± 1.2
	11 N HNO_3 15 min	2.00 ± 1.0	4.00 ± 2.0	2.00 ± 1.0	4.00 ± 2.0
	11 N HNO_3 30 min	1.67 ± 0.6	3.33 ± 1.2	1.67 ± 0.6	3.33 ± 1.2
	11 N HNO_3 45 min	1.00 ± 0.0	2.00 ± 0.0	1.00 ± 0.0	2.00 ± 0.0
20 Days	Control	3.00 ± 0.0	6.00 ± 0.0	4.33 ± 0.6	8.67 ± 1.2
	11 N HNO_3 15 min	2.00 ± 1.0	4.00 ± 2.0	4.00 ± 0.0	8.00 ± 0.0
	11 N HNO_3 30 min	1.33 ± 0.6	2.67 ± 1.2	3.00 ± 1.0	6.00 ± 2.0
	11 N HNO_3 45 min	1.00 ± 0.0	2.00 ± 0.0	2.00 ± 0.0	4.00 ± 0.0
30 Days	Control	3.67 ± 2.0	7.33 ± 4.0	8.00 ± 1.2	16.00 ± 2.3
	11 N HNO_3 15 min	6.67 ± 0.6	11.33 ± 1.2	10.67 ± 0.6	21.33 ± 1.2
	11 N HNO_3 30 min	7.00 ± 0.0	14.00 ± 0.0	10.00 ± 1.0	20.00 ± 2.0
	11 N HNO_3 45 min	8.00 ± 1.0	16.00 ± 2.0	10.00 ± 1.0	20.00 ± 2.0

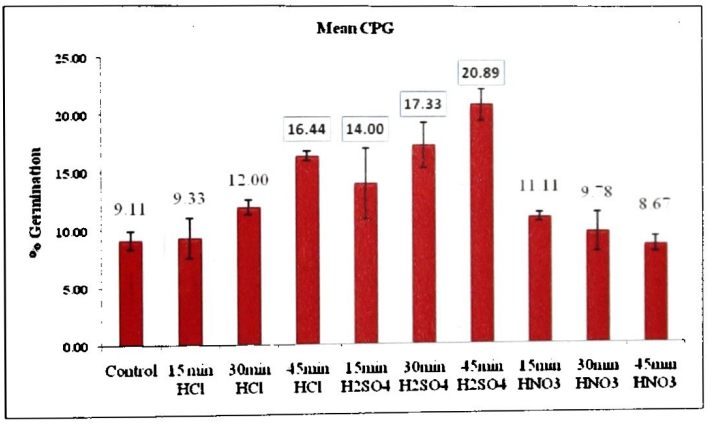
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

V-2 Effect of acid scarification on seed germination in *Santalum album* L.

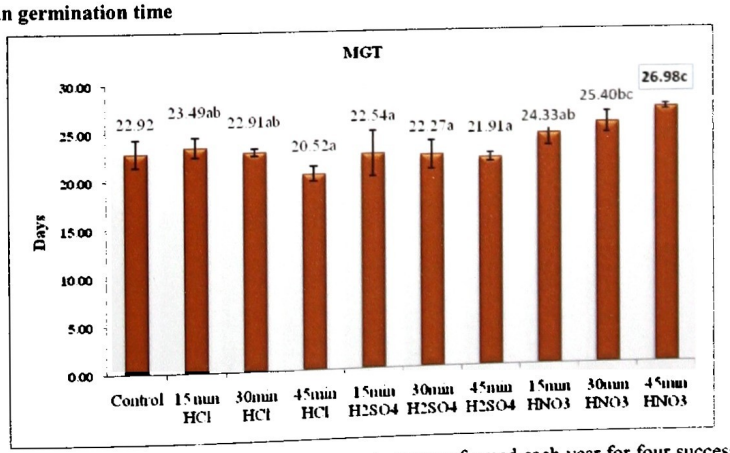
a) Cumulative percentage germination on 30th day



b) Mean Cumulative percentage germination



c) Mean germination time



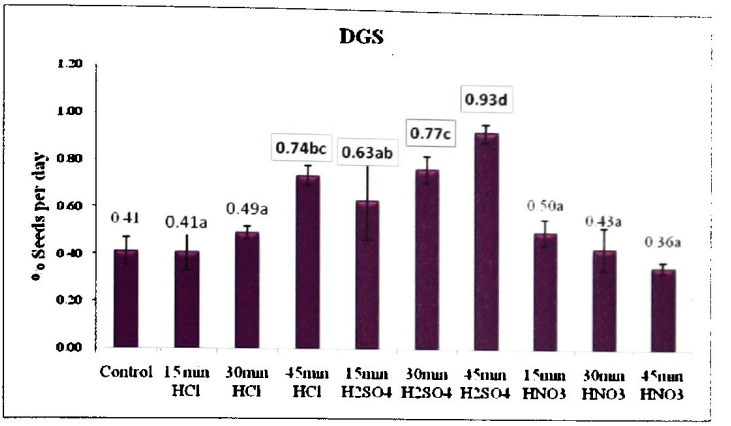
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.

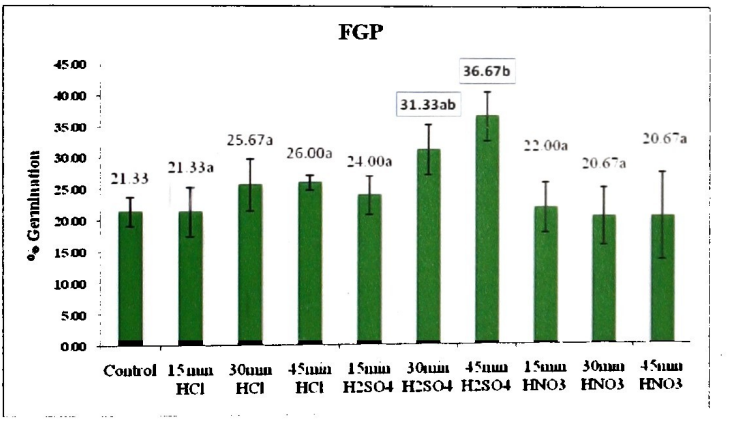
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

V-2 Effect of acid scarification on seed germination in *Santalum album* L.

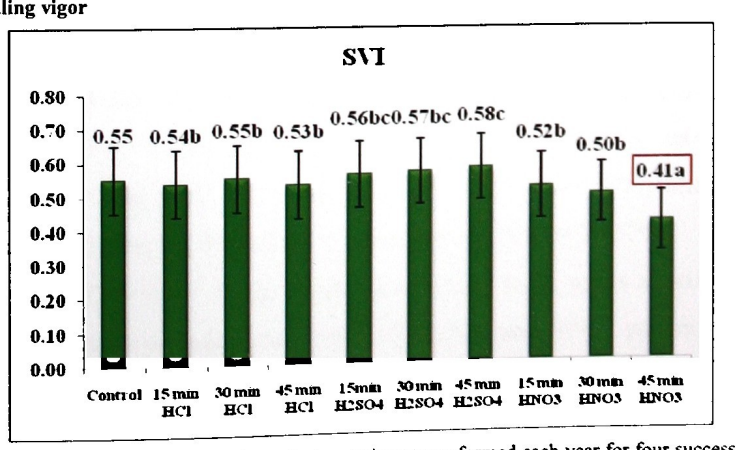
d) Mean daily germination speed



e) Final percentage germination on 60th Day



f) Seedling vigor



The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

significantly improved FGP (31.33% and 36.67% respectively) as compared to untreated seeds.

On the basis of these results, the seed scarification with concentrated H_2SO_4 for up to 45 min was beneficial for improving seed germination pattern in *S. album.*

5.3.3.3 Influence of concentrated HNO_3

The results on effect of scarification with concentrated HNO_3 on seed germination in *Santalum album* are presented in Table V-6 and Fig V-2a-e.

The scarification with concentrated HNO_3 was slightly effective to promote seed germination in *Santalum album.* In all the treatments (15, 30 and 45 minutes) the germination pattern remained almost similar as the CPG T30 values remained in the narrow range (Fig V-2a).

However, as compared to the best control response, higher MGT and lower mean DGS was recorded in all the treatments (15, 30 and 45 min) (Fig V-2c and V-2d). The scarification for 45 min significantly prolonged the spread of germination (MGT = 26.98 days), though only marginally lessened the speed (mean DGS = 0.36% seeds per day) as compared to control (MGT = 22.92 days and mean DGS = 0.41% seeds per day).

None of these treatments could improve the final percentage germination (Fig V-2e) over the best control response, as seen from indifference in the FGP in untreated seeds (FGP = 21.33%) and seeds scarified for 15 min (FGP = 22%), 30 and 45min (FGP = 20.67%).

The vigor in the seedling obtained from seeds scarified for different durations with concentrated HCl, H_2SO_4 and HNO_3 was not much different compared to seedlings obtained from non-scarified seeds (Fig V-2f). The seeds scarified with concentrated HNO_3 for 15 and 30 min, however, produced less vigorous seedlings compared to the best control

response and seedlings obtained from all the acid scarification methods. The vigor was significantly reduced compared to control seedlings in the seedlings obtained from seeds scarified with concentrated HNO_3 for 45 min.

5.4 Discussion

The fruit in *S. album* is a drupe where the true seed remains enclosed in a hard endocarp. If the seed is to germinate, a mechanical barrier is therefore one hurdle that is to be overcome. Acid scarification can improve seed germination in hard seeds by softening or scarifying the testa or pericarp (Ibanez and Passera, 1997).

Acid scarification treatments improve the germination by making seed coats more permeable to water and gases (Bonner *et al.*, 1974). Use of acid scarification dates back to early 1920s where the photo-induced germination was stimulated by acid scarification in the seeds of *Oenothera biennis* (Gardner, 1921). Higher germination rates (20 - 40%) have been recorded in the seeds of *Danthonia californica* following acid scarification (Laude, 1949).

In the present investigation, the seeds of *S. album* were acid scarified with concentrated HCl, H_2SO_4 and HNO_3 for 15, 30 and 45 min. Among these treatments, the treatment with concentrated HCl for 45 min stimulated CPG T30 to 25.33% from 16% in the best control response and the treatment of H_2SO_4 for 30 and 45 min significantly improved the FGP to 31.33% and 36.67% respectively over control (FGP = 21.33%). These results indicate that the germination pattern as well as final germination percentage can be enhanced by the acid scarification treatments in *Santalum album*.

The findings in the present investigation are similar to the earlier report of Nagaveni and Shrimathi (1981) suggesting scarification with concentrated H_2SO_4 was useful in enhancing the seed germination in

sandal. According to them, the acid scarification method reduced the time taken for germination in sandal. The treatment of concentrated H_2SO_4 for 50 and 60 min gave 80% germination in 70 days as compared to 35-40% germination in the control in the same period but there was no significant difference in the results obtained by treating the seeds with concentrated H_2SO_4 for 50 and 60 min.

The acid scarification was used to alleviate dormancy and increase the percentage germination in many other species. The germination in *Sapindus mukorossi* was stimulated to 77-82% within 21 days when seeds were scarified with concentrated hydrochloric acid (Brahamam *et al.*, 1996). Acid scarification for 6 or 12 h was the optimal pretreatment method for breaking the baobab seed coat inhibition for seed germination (Danthu *et al.*, 1995). Presoaking the seeds in concentrated acid was among the optimal treatments for breaking physiological or morpho-physiological dormancy and inducing germination in *Austrostipa compressa, Austrostipa macalpinei, Alyogyne huegelii, Actinotus leucocephalus* and *Grevillea scapigera* (Baker *et al.* (2005).

In *Harrisia fragrans,* sulphuric acid treatment increased the rate and percentage of germination (Fisher, 2002). Soaking the hard seeds of *Ipomoea obscura* in 97.7% H_2SO_4 for 40 min or longer produced maximum germination (Suwanketnikom and Julakasewee, 2004). The highest percentage germination occurred when seeds were soaked for 40 to 120 min. The fruits of *Talipariti elatum* (Sw.) Fryxell (Malvaceae), a highly esteemed tropical pioneer tree, are dehiscent capsules and their seeds have a poor and erratic germination unless pregerminative treatments such as thermal or acid scarification are applied (Álvarez, 1985).

The seeds of *Guazuma ulmiflia* presoaked in concentrated H_2SO_4 for 0 to 25 min increased the germination speed and the highest germination percentage (60%) was observed in the seeds scarified for 25 minutes (Juao Correia de Araujo Neto and Ivor Bergemann, 2000). Acid scarification has increased the rate of germination in hard, water-impermeable seeds of a number of palm species (Holmquist & Popenoe, 1967; Nagao *et al.*, 1980; Odetola, 1987).

Seed dormancy in *Chordospartium stevensonii* is imposed by water impermeable testa and without intentional seed scarification, germination is very poor. Acid scarification for 10-40 min in 18M H_2SO_4 permitted 100% germination within several days (Conner and Conner, 1988). The highest germination percentage (97–99%) in *Prosopis juliflora* was obtained from seeds that were treated sulphuric acid for 15–60 min (Shiferaw *et al.*, 2004). In *Berberis aristata* , acid scarification for 2,5 and 7 min resulted in remarkable improved germination to 96, 90 and 88% respectively compared to 40% germination in unscarified seeds (Thakur *et al.*, 2005).

The treatment of acid scarification, however, has been reported to be either ineffective or harmful in some genera.

Abbott and Van Heurck (1988) reported unimproved seed germination in *Persoonia elliptica* seeds acid scarified for as long as 3 h. Germination in *Cleome gynandra* after acid scarification did not improve total germination and the seeds that were treated with acid for 10 min had a similar germination percentage to the untreated control seeds. Acid scarification for 20 min drastically reduced germination while the seeds scarified for 30 min failed to germinate (Ochuodho *et al.*, 2005).

Similar to other species, in *S. album* also the acid scarification treatments thus resulted in preponement of germination and improvement in percentage germination. The probable reason behind alleviated germination pattern in *Santalum album* may be the dissolution of hard endocarp by strong acids, followed by improved imbibition that culminated in faster emergence of radicle.

Chapter VI

Chemical Stimulants And Germination

6.1 Introduction

The nitrogenous chemical compounds like potassium nitrate and thiourea are known to stimulate the seed germination process in many species. The stimulation may be due to triggering certain metabolic processes not linked to dormancy (Schmidt, 2000). Some of these compounds may interfere with physiological mechanisms associated with a particular type of dormancy. Seeds presoaked in these chemical stimulants may be relieved from dormancy by leaching of germination inhibitors thereby promoting seed germination. In some species their application may partly substitute temperature or light requirement (Schmidt, 2000).

The economically important species *Pterocarpus marsupium* and *Santalum album* under investigation have poor natural resurgence. The seed germination is not easy and is associated with delayed, non-uniform germination. The nitrogenous chemical stimulants, thiourea and potassium nitrate though are not routinely used by the nurserymen, are quite commonly used in seed science research. The influence of these chemical stimulants on seed germination pattern was tested and described in the present chapter.

6.2 Materials and methods

6.2.1 Effect of thiourea and potassium nitrate

The wing of diaspore in *P. marsupium* was cut by using a sharp scissors and such dewinged diaspores (termed as seeds) are used in the experiments on promotion of seed germination by the treatment of thiourea and potassium nitrate.

The chemicals thiourea, $CS(NH_2)_2$ and potassium nitrate (KNO_3) were procured from Sisco Research Laboratory, India and were of analytical grade.

Fifty randomly selected seeds of *P. marsupium* were allocated to each treatment. The seeds were soaked separately in

250ml solutions of 5, 10, 15, 20 and 25 mM Thiourea and 20, 40, 60, 80 and 100 mM KNO_3 for 24 and 36 h.

The untreated seeds (control) were soaked in distilled water for the same duration. After the treatment for the specified duration, solutions were decanted and the seeds were washed in running tap water for 2 h. The seeds were then dried and sown in cavity seeding trays and plastic bags containing moist garden soil as a medium.

The fruit in *Santalum album* is a drupe in which the pericarp consists of three layers viz., epicarp, mesocarp and endocarp. The epicarp is thin and membranous and mesocarp is fleshy while the endocarp is hard and stony. The epicarp and mesocarp were removed and the seeds enclosed in the hard endocarp were used for the experiments on seed germination.

The seeds of *S. album* were soaked separately in 25, 50, 75 and 100 mM solution of thiourea for 24 and 36 hours, while in case of KNO_3 the seeds were treated for 12 and 24 hours with 25, 50, 75 and 100 mM solution of KNO_3. After the specified treatments, the seeds were washed in running tap water for 2 h. The seeds were then dried and sown in cavity seeding trays and polythene bags containing moist garden soil as a medium.

6.3 Results

6.3.1 *Pterocarpus marsupium* Roxb.

6.3.1.1 Effect of thiourea [$CS(NH_2)_2$]

The results on the effect of presoaking in thiourea for 24 h on seed germination are presented in Table VI-1 and Fig VI-1a-f and Plate VI-1.

The seeds immersed for 24 hours in 0-25 mM thiourea showed gradual improvement in seed germination as seen from increased values of the CPG T15 (Fig VI-1a) as well as the mean CPG values (Fig VI-1b). The significant improvement by 92% over the best control response in CPG T15 (75.33%) was observed in seeds treated with 25 mM

VI-1: Effect of 24 h soaking in thiourea on seed germination in *Pterocarpus marsupium* Roxb.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
5 Days	Control	2.67 ± 0.6	5.33 ± 1.2	2.67 ± 0.6	5.33 ± 1.2
	5 mM Thiourea	3.67 ± 2.1	7.33 ± 4.2	3.67 ± 2.1	7.33 ± 4.1
	10 mM Thiourea	3.00 ± 0.0	6.00 ± 0.0	3.00 ± 0.0	6.00 ± 0.0
	15 mM Thiourea	3.67 ± 1.2	7.33 ± 2.3	3.67 ± 1.2	7.33 ± 2.3
	20 mM Thiourea	4.67 ± 2.1	9.33 ± 4.2	4.67 ± 2.1	9.33 ± 4.2
	25 mM Thiourea	4.67 ± 2.3	9.33 ± 4.6	4.67 ± 2.3	9.33 ± 4.6
10 Days	Control	8.00 ± 1.0	16.00 ± 2.0	10.67 ± 1.2	21.33 ± 2.3
	5 mM Thiourea	7.00 ± 3.0	14.00 ± 6.0	10.67 ± 2.1	21.33 ± 4.2
	10 mM Thiourea	10.67 ± 1.2	21.33 ± 2.3	13.67 ± 1.2	27.33 ± 2.3
	15 mM Thiourea	13.00 ± 2.7	26.00 ± 5.3	16.67 ± 1.5	33.33 ± 3.1
	20 mM Thiourea	18.00 ± 3.0	36.00 ± 6.0	22.67 ± 5.0	45.33 ± 10.0
	25 mM Thiourea	23.33 ± 3.8	46.67 ± 7.6	28.00 ± 6.1	56.00 ± 12.2
15 Days	Control	9.00 ± 4.4	18.00 ± 8.7	19.67 ± 5.5	39.33 ± 11.0
	5 mM Thiourea	11.00 ± 1.0	22.00 ± 2.0	21.67 ± 3.1	43.33 ± 6.1
	10 mM Thiourea	10.33 ± 1.5	20.67 ± 3.1	24.00 ± 2.7	48.00 ± 5.3
	15 mM Thiourea	12.33 ± 0.6	24.67 ± 1.2	29.00 ± 1.7	58.00 ± 3.5
	20 mM Thiourea	9.00 ± 2.0	18.00 ± 4.0	31.67 ± 6.1	63.33 ± 12.2
	25 mM Thiourea	9.67 ± 1.5	19.33± 3.0	37.67 ± 7.8	75.33 ± 15.1

The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

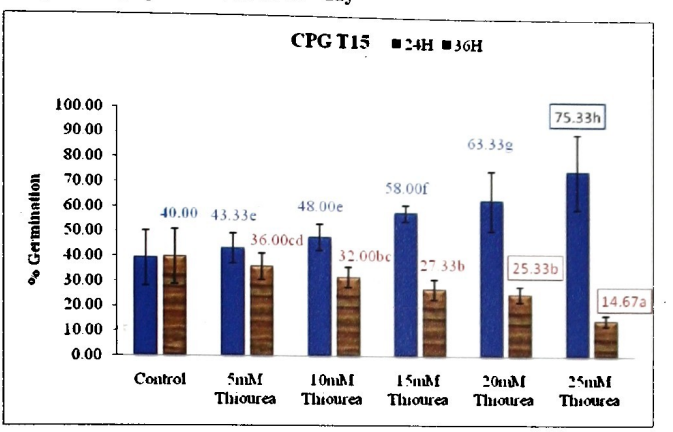
VI-2: Effect of 36 h soaking in thiourea on seed germination in *Pterocarpus marsupium* Roxb.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
5 Days	Control	2.33 ± 0.6	4.67 ± 1.2	2.33 ± 0.6	4.67 ± 1.2
	5 mM Thiourea	2.00 ± 1.0	4.00 ± 2.0	2.00 ± 1.0	4.00 ± 2.0
	10 mM Thiourea	1.00 ± 0.0	2.00 ± 0.0	1.00 ± 0.0	2.00 ± 0.0
	15 mM Thiourea	0.67 ± 0.6	1.33 ± 1.2	0.67 ± 0.6	1.33 ± 1.2
	20 mM Thiourea	0.33 ± 0.6	0.67 ± 1.2	0.33 ± 0.6	0.67 ± 1.2
	25 mM Thiourea	0.67 ± 1.2	1.33 ± 2.3	0.67 ± 1.2	1.33 ± 2.3
10 Days	Control	7.33 ± 1.0	14.67 ± 2.0	9.67 ± 1.2	19.33 ± 2.3
	5 mM Thiourea	6.67 ± 2.1	13.33 ± 4.2	8.67 ± 2.1	17.33 ± 4.2
	10 mM Thiourea	5.67 ± 1.2	11.33 ± 2.3	6.67 ± 1.2	13.33 ± 2.3
	15 mM Thiourea	4.33 ± 3.1	8.67 ± 6.1	5.00 ± 3.0	10.00 ± 6.0
	20 mM Thiourea	4.33 ± 2.9	8.67 ± 5.8	4.67 ± 3.2	9.33 ± 6.4
	25 mM Thiourea	1.67 ± 0.6	3.33 ± 1.2	2.33 ± 1.5	4.67 ± 3.1
15 Days	Control	9.33 ± 4.4	18.67 ± 8.7	20.00 ± 5.5	40.00 ± 11.0
	5 mM Thiourea	9.33 ± 0.6	18.67 ± 1.2	18.00 ± 2.6	36.00 ± 5.3
	10 mM Thiourea	9.33 ± 1.2	18.67 ± 2.3	16.00 ± 2.0	32.00 ± 4.0
	15 mM Thiourea	8.67 ± 2.1	17.33 ± 4.2	13.67 ± 2.1	27.33 ± 4.2
	20 mM Thiourea	8.00 ± 2.0	16.00 ± 4.0	12.67 ± 1.5	25.33 ± 3.1
	25 mM Thiourea	5.00 ± 1.0	10.00 ± 2.0	7.33 ± 1.2	14.67 ± 2.3

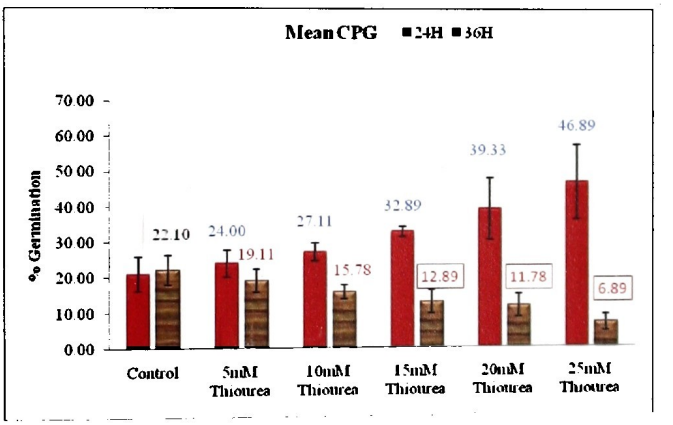
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

VI-1 Effect of thiourea on seed germination in *Pterocarpus marsupium* Roxb.

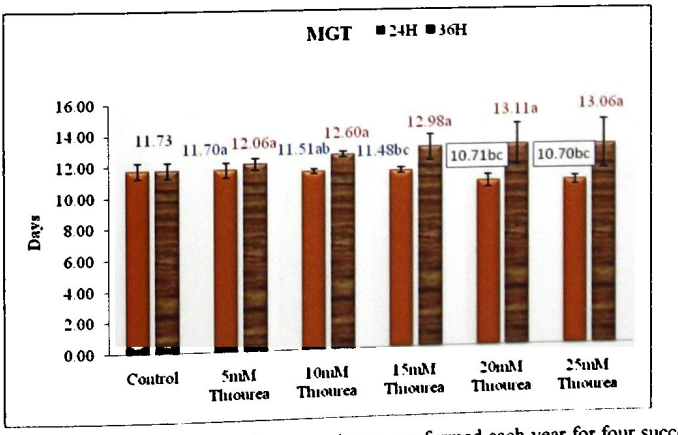
a) Cumulative percentage germination on 15th day



b) Mean Cumulative percentage germination



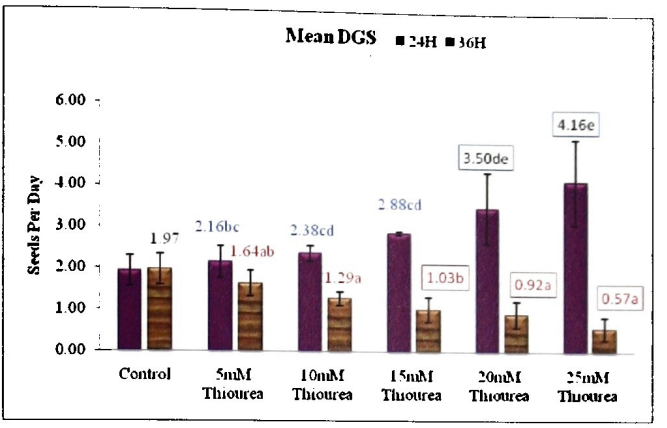
c) Mean germination time



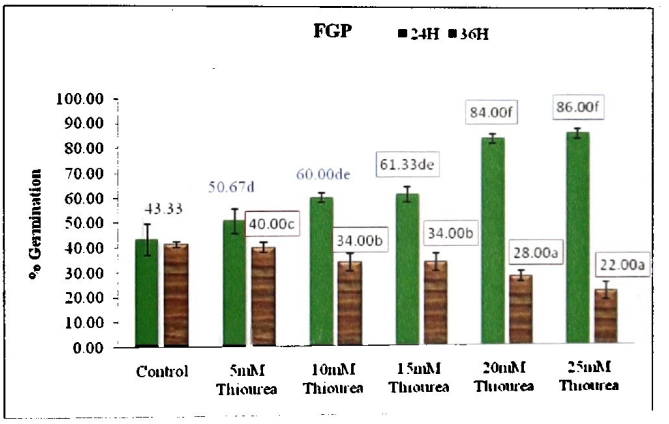
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

VI-1 Effect of thiourea on seed germination in *Pterocarpus marsupium* Roxb.

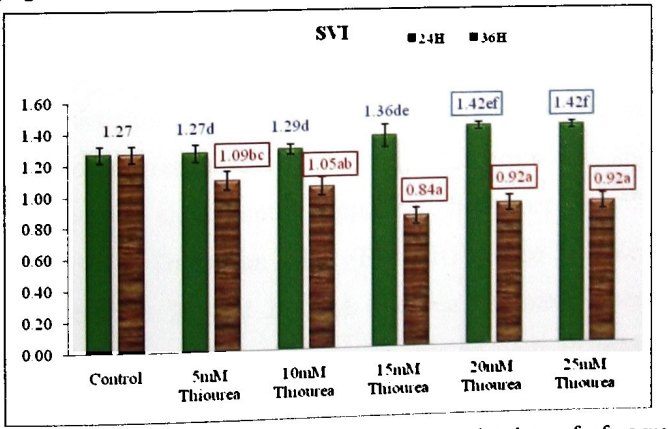
d) Mean daily germination speed



e) Final percentage germination on 30th Day



f) Seedling vigor



The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

thiourea. The mean CPG was more in all the treatments but was not significantly improved over the best control response.

The spread of the germination, however, remained almost unaltered as seen from marginal differences in the MGT (Fig VI-1c) observed for untreated (11.73 days) and treated seeds (maximum of 13.11 days in 20 mM treatment).

The speed of the germination (Fig VI-1d) was significantly improved in the seeds treated with 20 mM thiourea (mean DGS= 3.5% seeds per day) and 25 mM thiourea (mean DGS= 4.16% seeds per day) as compared to the best control response (mean DGS = 1.97% seeds per day).

The germination percentage (FGP, Fig VI-1e) also showed significant increments with increase in the concentration of thiourea. The untreated seeds showed only 43.33% germination as against 61.33%, 84% and 86% FGP in seeds treated with 15 mM, 20 mM and 25 mM thiourea respectively. The maximum increment was of 98% over the best control response in the seeds treated with 25 mM thiourea which was only marginally more than the increment observed in seeds treated with 20 mM thiourea (94% increment).

A longer exposure (36 h) of seeds to 5-25 mM thiourea was inhibitory to seed germination in *Pterocarpus marsupium* (Table Fig VI-2 and Fig VI-1a-f).

With increase in the concentration of thiourea, the cumulative percentage germination (CPG T15, Fig VI-1a) gradually decreased from 40% (the best control response) to 27.33% (15 mM thiourea) and 20 mM and 25 mM thiourea significantly suppressed it further to 25.33% and 14.67% respectively. The mean CPG (Fig VI-1b) also revealed similar pattern. In addition to 20 and 25 mM thiourea treatment (mean CPG= 11.78% and 6.78% respectively), the seeds treated with 15 mM thiourea also significantly suppressed mean CPG (12.89%).

Increase in the concentration of thiourea though resulted in longer spread of germination (higher MGT values, Fig VI-1c) as compared

Plate VI-1: Seedlings of *Pterocarpus marsupium* Roxb. and *Santalum album* L. from seeds treated with thiourea

A. *Pterocarpus marsupium* Roxb.

B. *Santalum album* L

Plate VI-1

A

B

to the best control response (MGT = 11.73 days). The maximum increase in MGT was of 1.38 days in the seeds treated with 20 mM thiourea. The speed of germination (mean DGS, Fig VI-1d), however, was significantly decreased from 1.97% seeds per day in control to 1.03, 0.92 and 0.57% seeds per day in the seeds treated with 15, 20 and 25 mM thiourea respectively.

A dramatic restraint in the seed germination after exposure to 5-25 mM thiourea for 36 h was observed in the FGP (Fig VI-1e). Except the treatment of 5 mM thiourea, all other treatments significantly suppressed the germination percentage and maximum of 51% decrease over the best control response (FGP = 43.33%) was observed in the seeds treated with 25 mM thiourea (FGP = 22%).

In the seeds treated with 5-15mM thiourea for 24 h the seedling vigor was equivalent to that of the seedlings obtained from the untreated seeds. The higher concentration of thiourea (20 and 25mM) used for the duration of 24h significantly enhanced the vigor of the seedlings. On the contrary, when the same concentrations were used for 36h duration, the seedling vigor was significantly reduced than the seedlings from untreated seeds (Fig VI-1f).

In conclusion, for better germination behavior in *P. marsupium,* treatment of the seeds with thiourea was beneficial and the optimum treatment was that of 25mM thiourea for 24 h.

6.3.1.2 Effect of potassium nitrate (KNO_3)

The treatment of seeds with 20-80 mM KNO_3 for 24h was beneficial for seed germination in *Pterocarpus marsupium,* while a higher treatment of 100 mM was slightly inhibitory compared to 80 mM KNO_3 treatment for 24 h (Table VI-3 and Fig VI-2a-f). The improved seed germination was reflected in all the parameters computed except MGT, which remained more or less similar to that of untreated seeds.

VI-3: Effect of 24 h soaking in KNO_3 on seed germination in *Pterocarpus marsupium* Roxb.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
5 Days	Control	1.00 ± 0.0	2.00 ± 0.0	1.00 ± 0.0	2.00 ± 0.0
	20 mM KNO_3	2.33 ± 0.6	4.67 ± 1.2	2.33 ± 0.6	4.67 ± 1.2
	40 mM KNO_3	2.33 ± 0.6	4.67 ± 1.2	2.33 ± 0.6	4.67 ± 1.2
	60 mM KNO_3	4.00 ± 1.0	8.00 ± 2.0	4.00 ± 1.0	8.00 ± 2.0
	80 mM KNO_3	6.33 ± 2.0	12.67 ± 4.2	6.33 ± 2.1	12.67 ± 4.2
	100 mM KNO_3	6.33 ± 3.8	12.67 ± 7.6	6.33 ± 3.8	12.67 ± 7.6
10 Days	Control	12.00 ± 2.0	24.00 ± 4.0	13.00 ± 2.0	26.00 ± 4.0
	20 mM KNO_3	13.33 ± 7.4	26.67 ± 14.8	15.67 ± 6.8	31.33± 13.6
	40 mM KNO_3	16.00 ± 2.7	32.00 ± 5.3	18.33 ± 2.1	36.67 ± 4.2
	60 mM KNO_3	15.33 ± 2.1	30.67 ± 4.2	19.33 ± 2.5	38.67 ± 5.0
	80 mM KNO_3	14.67 ± 2.5	29.33 ± 5.0	21.00 ± 2.7	42.00 ± 5.3
	100 mM KNO_3	14.00 ± 3.0	28.00 ± 6.0	20.33 ± 6.4	40.67 ± 12.9
15 Days	Control	5.00 ± 1.7	10.00 ± 3.5	18.00 ± 2.7	36.00 ± 5.3
	20 mM KNO_3	6.00 ± 6.6	12.00 ± 13.1	21.67 ± 4.0	43.33 ± 8.1
	40 mM KNO_3	6.33 ± 2.9	12.67 ± 5.8	24.67 ± 3.0	49.33 ± 6.1
	60 mM KNO_3	7.67 ± 1.2	15.33 ± 2.3	27.00 ± 3.6	54.00 ± 7.2
	80 mM KNO_3	10.00 ± 5.6	20.00 ± 11.1	31.00 ± 5.3	62.00 ± 10.6
	100 mM KNO_3	7.67 ± 1.5	15.33 ± 3.1	28.00 ± 5.2	56.00 ± 10.4

The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

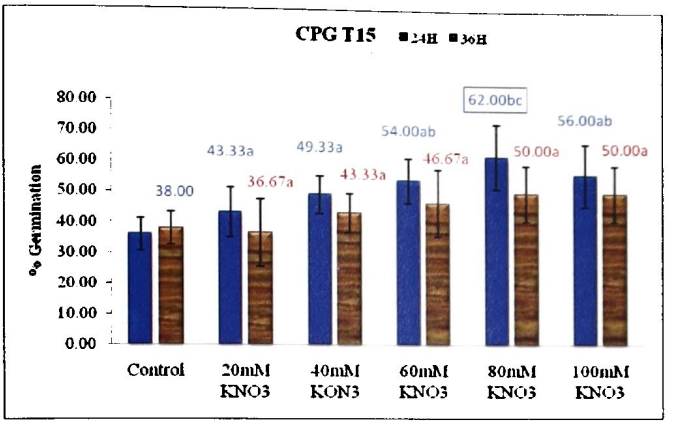
VI-4: Effect of 36 h soaking in KNO_3 on seed germination in *Pterocarpus marsupium* Roxb.

DAS	Treatment (for 36h)	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
5 Days	Control	1.00 ± 0.0	2.00 ± 0.0	1.00 ± 0.0	2.00 ± 0.0
	20 mM KNO_3	2.00 ± 0.0	4.00 ± 0.0	2.00 ± 0.0	4.00 ± 0.0
	40 mM KNO_3	2.33 ± 0.6	4.67 ± 1.1	2.33 ± 0.6	4.67 ± 1.2
	60 mM KNO_3	2.33 ± 0.6	4.67 ± 1.1	2.33 ± 0.6	4.67 ± 1.2
	80 mM KNO_3	2.33 ± 0.6	4.67 ± 1.1	2.33 ± 0.6	4.67 ± 1.2
	100 mM KNO_3	2.33 ± 0.6	4.67 ± 1.1	2.33 ± 0.6	4.67 ± 1.2
10 Days	Control	12.00 ± 2.0	24.00 ± 4.0	13.00 ± 2.0	26.00 ± 4.0
	20 mM KNO_3	9.33 ± 4.0	18.67 ± 8.1	11.33 ± 4.0	22.67 ± 8.1
	40 mM KNO_3	10.33 ± 2.1	20.67 ± 4.2	12.67 ± 1.5	25.33 ± 3.1
	60 mM KNO_3	11.67 ± 3.1	23.33 ± 6.1	14.00 ± 2.7	28.00 ± 5.3
	80 mM KNO_3	11.00 ± 3.0	22.00 ± 6.0	13.33 ± 3.5	26.67 ± 7.0
	100 mM KNO_3	11.00 ± 3.0	22.00 ± 6.0	13.33 ± 3.5	26.67 ± 7.0
15 Days	Control	6.00 ± 1.7	12.00 ± 3.5	19.00 ± 2.7	38.00 ± 5.3
	20 mM KNO_3	7.00 ± 5.3	14.00 ± 10.6	18.33 ± 5.5	36.67 ± 11.0
	40 mM KNO_3	9.00 ± 4.5	18.00 ± 9.1	21.67 ± 3.2	43.33 ± 6.4
	60 mM KNO_3	9.33 ± 5.0	18.67 ± 10.1	23.33 ± 5.5	46.67 ± 11.0
	80 mM KNO_3	11.67 ± 1.5	23.33 ± 3.1	25.00 ± 4.6	50.00 ± 9.2
	100 mM KNO_3	11.67 ± 1.5	23.33 ± 3.1	25.00 ± 4.6	50.00 ± 9.2

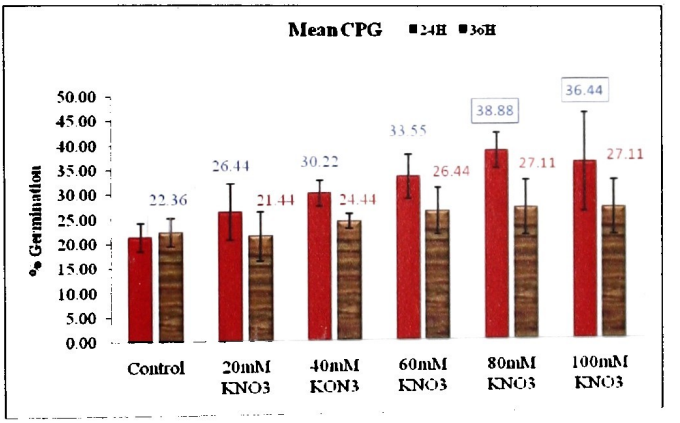
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

VI-2 Effect of KNO_3 on seed germination in *Pterocarpus marsupium* Roxb.

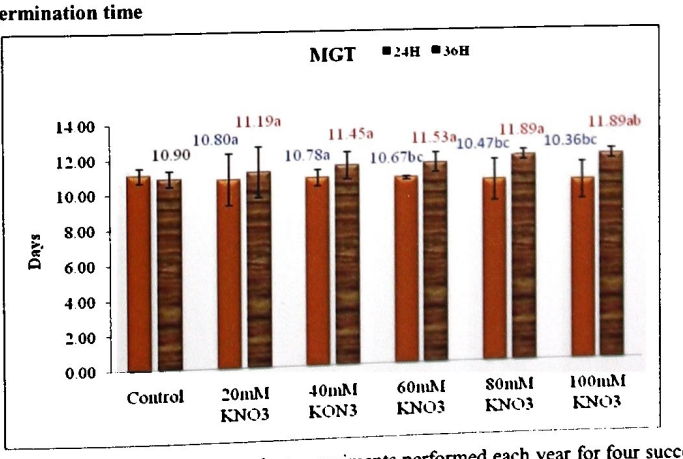
a) Cumulative percentage germination on 15th day



b) Mean Cumulative percentage germination



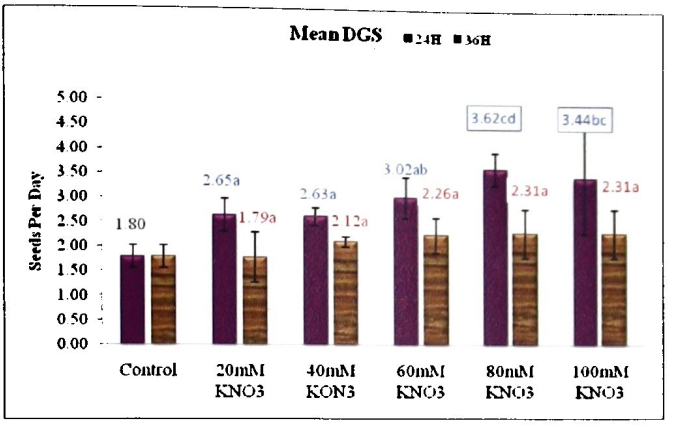
c) Mean germination time



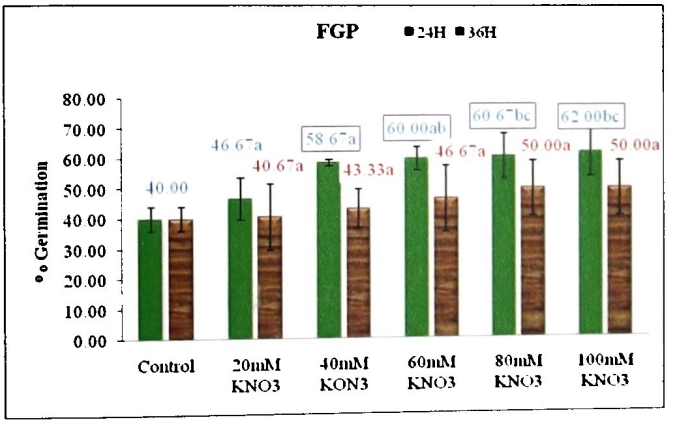
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

VI-2 Effect of KNO_3 on seed germination in *Pterocarpus marsupium* Roxb.

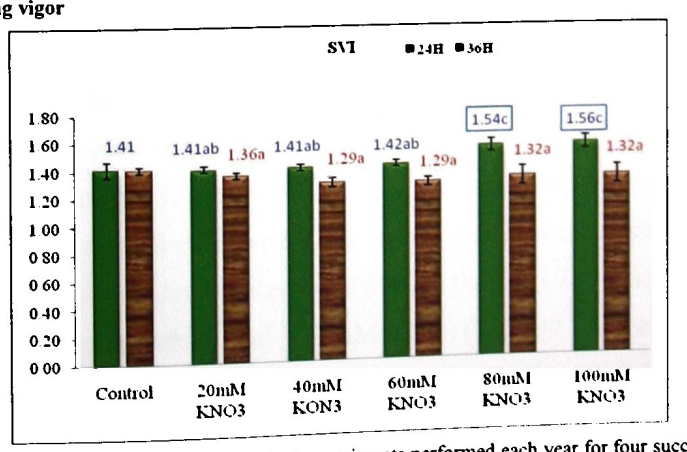
d) Mean daily germination speed



e) Final percentage germination on 30th Day



f) Seedling vigor



The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

The maximum boost in the CPG T15 was observed in the seeds treated with 80 mM KNO_3 (CPG T15 = 62%), which was significantly higher than the best control response (CPG T15 = 38%). A higher dose of 100 mM resulted in suppressed CPG T15 of 56% (Fig VI-2a).

The mean CPG (Fig VI-2b) also increased with the dose of KNO_3 and 80 and 100 mM KNO_3 gave significantly higher values of 38.88% and 36.44% respectively.

The spread of germination in terms of MGT (Fig VI-2c) was only slightly reduced in all the treatments (minimum 10.36 days in 100 mM KNO_3) from untreated seeds (MGT = 10.90 days). The treatment with 80 mM KNO_3 resulted in significantly fastest germination as revealed by highest mean DGS (3.62% seeds per day), whereas the untreated seeds germinated at the speed of 1.8% seeds per day (Fig VI-2d).

An important expectation after seed treatment by any method is elicited percentage germination. The seeds treated with 40-100 mM KNO_3 significantly improved percentage germination (Fig VI-2e). There was gradual increase in the FGP from 58.67% (40 mM KNO_3) to 62% (100 mM KNO_3).

In *P. marsupium,* a longer exposure (36 h) of seeds to the 20-100 mM KNO_3 though improved germination over the best control response (Table VI-4 and Fig VI-2a-f), the increase was statistically non significant and the values obtained for different parameters studied were less than those obtained in 24 h treatment of the same concentrations. Therefore, treatments of potassium nitrate at different concentrations given for 36 h were not much effective for enhancing the germination pattern.

The maximum increase of 39% in the CPG T15 was recorded in the seeds treated with 80 and 100 mM KNO_3 for 36 h. The germination was slightly delayed by 0.8 day in the seeds treated with 80 and 100 mM KNO_3. The maximum speed of germination (mean DGS) was 2.31% seeds

per day in the seeds treated with 80 and 100 mM KNO_3 as compared to 1.8% seeds per day in untreated seeds.

All the treatments could only marginally improve the germination percentage over control. The maximum percentage germination (FGP = 50%) was observed in the seeds treated with 80 and 100 mM KNO_3 as compared to 40% germination in the best control response.

Seedling vigor in the seedlings obtained from the seeds treated with 20-60 mM KNO_3 for 24 h was more or less similar to the vigor shown by the seedlings from the untreated seeds (Fig VI-2f). The treatments of 80 and 100mM KNO_3, however, produced slightly more vigorous seedlings. The application of 20-100 mM KNO_3 for 36 h resulted in decline in the vigor as compared to the seedlings from untreated seeds as well seeds treated with 20-100 mM KNO_3 for 24 h.

Thus, the seeds exposed to 20-100 mM KNO_3 for 36 h though showed improved seed germination in *Pterocarpus marsupium;* these treatments were less effective than the same treatments applied for 24 h.

6.3.2 *Santalum album* L.

6.3.2.1 Effect of thiourea [CS $(NH_2)_2$]

The results on the effect of presoaking in 25-100 mM thiourea for 24 h on seed germination are presented in Table VI-6, Fig VI-3a-f and Plate VI-1.

The pretreatment of seeds with thiourea was beneficial for improving seed germination. The direct effect was first observed on cumulative percentage germination (CPG T30, Fig VI-3a). With increase in the concentration of thiourea, there was linear increase in the CPG T30. The 25, 50 and 75 mM thiourea significantly improved CPG T30 and the highest value (33.33%) was observed in the seeds treated with 75 mM thiourea. A higher concentration of 100 mM was slightly inhibitory (CPG

VI-5: Effect of 24 h soaking in thiourea on seed germination in *Santalum album* L.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
10 Days	Control	1.00 ± 1.0	2.00 ± 2.0	1.00 ± 1.0	2.00 ± 2.0
	25 mM Thiourea	2.33 ± 0.6	4.67 ± 1.2	2.33 ± 0.6	4.67 ± 1.2
	50 mM Thiourea	2.67 ± 0.6	5.33 ± 1.2	2.67 ± 0.6	5.33 ± 1.2
	75 mM Thiourea	3.67 ± 0.6	7.33 ± 1.2	3.67 ± 0.6	7.33 ± 1.2
	100 mM Thiourea	3.00 ± 1.0	6.00 ± 2.0	3.00 ± 1.0	6.00 ± 2.0
20 Days	Control	2.00 ± 1.0	4.00 ± 2.0	3.00 ± 1.2	6.00 ± 2.3
	25 mM Thiourea	5.67 ± 1.5	11.33 ± 3.1	8.00 ± 1.7	16.00 ± 3.5
	50 mM Thiourea	7.33 ± 0.6	14.67 ± 1.2	10.00 ± 0.0	20.00 ± 0.0
	75 mM Thiourea	9.33 ± 0.6	18.67 ± 1.2	13.00 ± 0.0	26.00 ± 0.0
	100 mM Thiourea	8.67 ± 0.6	17.33 ± 1.2	11.67 ± 0.6	23.33 ± 1.2
30 Days	Control	6.00 ± 4.4	12.00 ± 8.7	9.00 ± 5.5	18.00 ± 11.0
	25 mM Thiourea	4.33 ± 2.1	8.67 ± 4.2	12.33 ± 2.3	24.67 ± 4.6
	50 mM Thiourea	4.33 ± 2.1	8.67 ± 4.2	14.33 ± 2.1	28.67 ± 4.2
	75 mM Thiourea	3.67 ± 1.5	7.33 ± 3.1	16.67 ± 1.5	33.33 ± 3.1
	100 mM Thiourea	4.00 ± 2.6	8.00 ± 5.3	15.67 ± 2.3	31.33 ± 4.6

The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

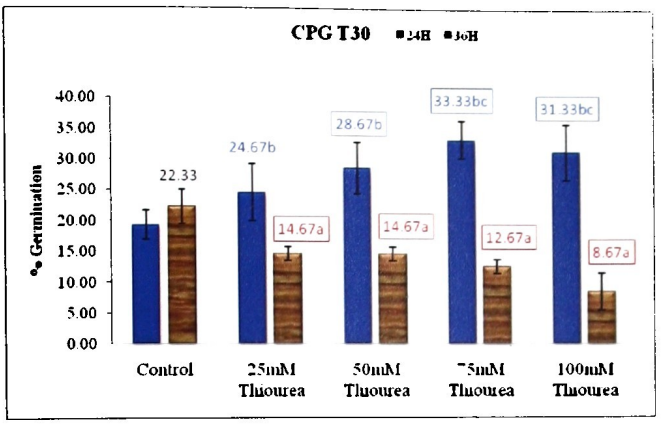
VI-6: Effect of 36 h soaking in thiourea on seed germination in *Santalum album* L.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
10 Days	Control	1.00 ± 1.0	2.00 ± 2.0	1.00 ± 1.0	2.00 ± 2.0
	25 mM Thiourea	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
	50 mM Thiourea	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
	75 mM Thiourea	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
	100 mM Thiourea	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
20 Days	Control	3.00 ± 1.0	6.00 ± 2.0	4.00 ± 1.2	8.00 ± 2.3
	25 mM Thiourea	0.67 ± 0.6	1.33 ± 1.2	0.67 ± 0.6	1.33 ± 1.2
	50 mM Thiourea	0.67 ± 0.0	1.33 ± 0.0	0.67 ± 0.0	1.33 ± 0.0
	75 mM Thiourea	0.33 ± 0.6	0.67 ± 1.2	0.33 ± 0.6	0.67 ± 1.2
	100 mM Thiourea	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
30 Days	Control	7.67 ± 4.4	14.33 ± 8.7	11.67 ± 5.5	22.33 ± 11.0
	25 mM Thiourea	6.67 ± 0.6	13.33 ± 1.2	7.33 ± 0.6	14.67 ± 1.2
	50 mM Thiourea	6.67 ± 1.5	13.33 ± 3.1	7.33 ± 1.5	14.67 ± 3.1
	75 mM Thiourea	6.00 ± 1.0	12.00 ± 2.0	6.33 ± 0.6	12.67 ± 1.2
	100 mM Thiourea	4.33 ± 1.5	8.67 ± 3.1	4.33 ± 1.5	8.67 ± 3.1

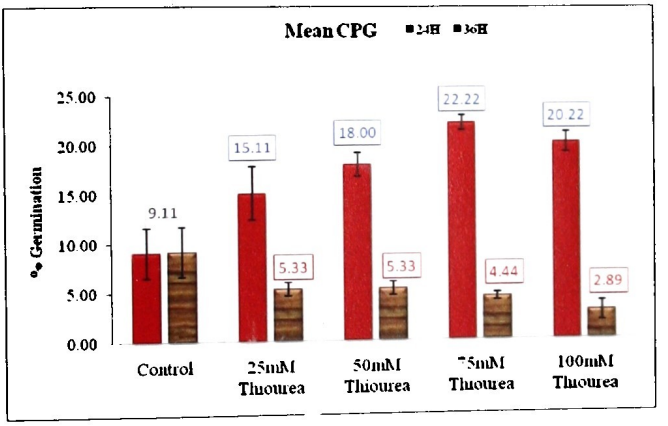
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

VI-3 Effect of thiourea on seed germination in *Santalum album* L.

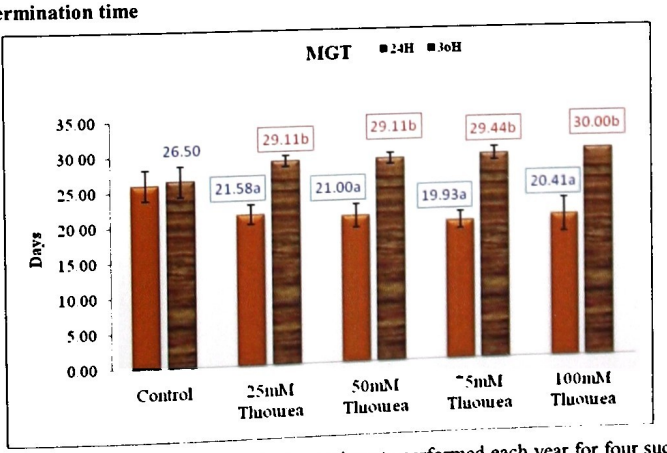
a) Cumulative percentage germination on 30th day



b) Mean Cumulative percentage germination



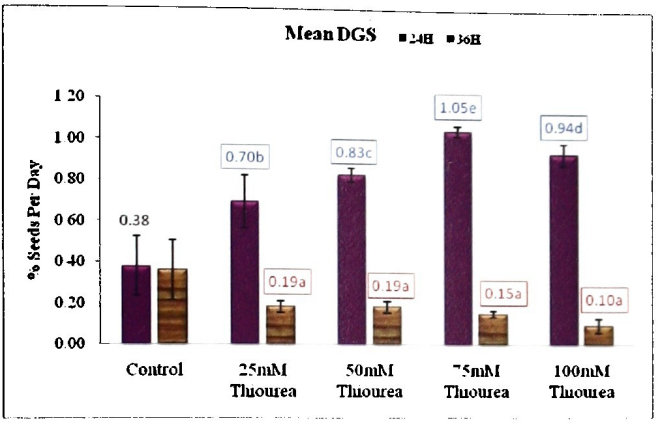
c) Mean germination time



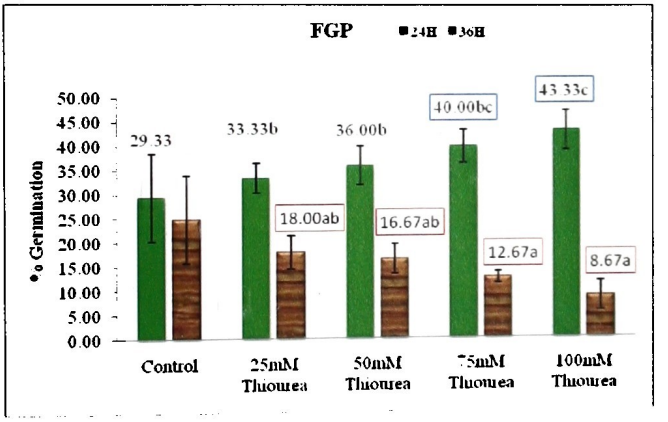
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

VI-3 Effect of thiourea on seed germination in *Santalum album* L.

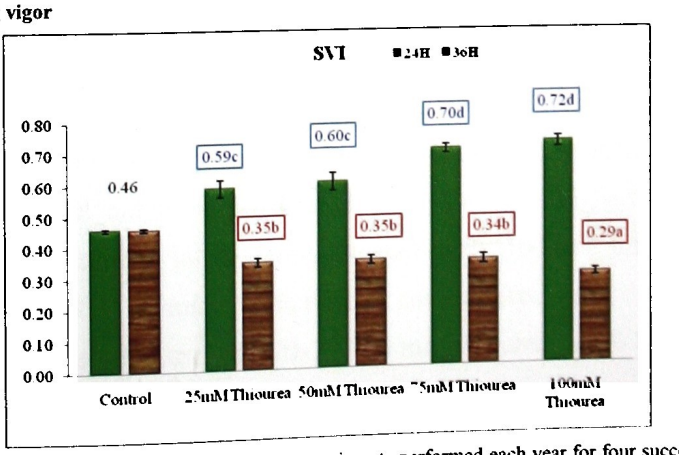
d) Mean daily germination speed



e) Final percentage germination on 30th Day



f) Seedling vigor



The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

T30 = 31.33%), though gave significantly improved germination over the best control response.

The mean CPG (Fig VI-3b), however, was significantly improved in all the treatments, with maximum increment of 147% over the best control response in the seeds treated with 75 mM thiourea.

The germination pattern was also improved in terms of spread of germination (MGT, Fig VI-3c) and all the treatments significantly lowered the mean time to complete germination. The lowest MGT was observed in the seeds treated with 75 mM thiourea (19.93 days) as compared to 26.50 days in the best control response.

The speed of seed germination in terms of mean DGS (Fig VI-3d) was also significantly improved in all the treatments. The maximum increment of 176% over the best control response was observed in the seeds treated with 75 mM thiourea. The higher dose of thiourea (100 mM) was inhibitory as it gave slightly lower mean DGS (0.94% seeds per day) as compared to 75 mM thiourea treatment (mean DGS = 1.05% seeds per day).

The germination percentages (FGP) increased gradually with increase in the concentration of thiourea. However, FGP was significantly improved only in the seeds treated with 75 and 100 mM thiourea given for 24 h (Fig VI-3e), indicating that the 25, and 50 mM thiourea treatment produced early and faster seed germination but failed to induce germination in the latter stages, while 75 and 100 mM thiourea treatment not only resulted in early germination but also induced germination in the seeds that would not have germinated otherwise. The highest FGP of 43.33% was recorded in the seeds treated with 100mM thiourea as compared to 29.33% germination in the best control response.

The seeds treated with 25-100 mM thiourea for 36 h showed significantly suppressed germination (Table VI-7 and Fig VI-3a-f). Restrained germination was reflected in all the pretreatments computed and the treatment of 100 mM thiourea for 36 h was the most inhibitory for seed

germination in *Santalum*. The CPG T30 significantly dropped from 22.33% in the best control response to 8.67% in the seeds treated with 100 mM thiourea.

The mean CPG also showed similar pattern. There was gradual decrease in mean CPG from 9.11% (Control) to 5.33% (25 and 50 mM thiourea), 4.44% (75mM thiourea) and 2.89% in the seeds treated with 100mM thiourea.

The inhibitory effect of 36 h treatment with 25-100 mM thiourea was reflected in significantly longer spread of germination (MGT) as compared to the best control response. With increase in concentration of thiourea, progressively more time was required to complete germination. The 25mM thiourea treatment resulted in 12% more MGT over the best control response, where maximum of 15% increase in MGT was observed in seeds treated with 100 mM thiourea.

The speed of germination (mean DGS) also was significantly suppressed in all the thiourea treatments given for 36 hours and the 100mM treatment was most inhibitory (mean DGS = 0.10% seeds per day) over the best control response (mean DGS = 0.38% seeds per day).

The FGP was significantly suppressed by all the treatments. The least FGP (8.67%) was observed in the seeds treated with 100mM thiourea, through 18% (25 mM thiourea), 16.67% (50 mM thiourea) and 12.67% (75 mM thiourea) as compared to untreated seeds (29.33%).

The presoaking of seeds in 25-100 mM thiourea for 24 h produced seedlings with markedly improved vigor. The seedlings obtained from seeds soaked in 100 mM thiourea for 24 h were the most vigorous with about 56% more vigor than the control seedlings (Fig VI-3f). There was delayed germination in the *Santalum album* seeds when the seeds were soaked for 36 h in the solutions of thiourea of the same concentrations. The seedlings obtained in these experiments had significantly less vigor compared to control seedlings as well as seedlings obtained from seeds treated with thiourea for 24 h.

6.3.2.2 Effect of potassium nitrate (KNO_3)

Table VI-8 and Fig VI-4a-f represent the results on seed germination in *S. album* after treatment of the seeds with 0-100 mM KNO_3 for 12 h.

The lower doses of KNO_3 (25 and 50 mM) only marginally improved the germination pattern as indicated by slightly higher CPG T30 (22% and 26%) as compared to the best control response (22%) (Fig VI-4a). Seeds treated with 75mM KNO_3, however, significantly improved the CPG T30 to 32.67% while a little higher dose of 100 mM was inhibitory to germination, indicated by lowered CPG T15 to 26%.

Effectiveness of 75 mM KNO_3 treatment was also observed in the significant increase in mean CPG (17.33%) over the best control response (8.89%). The 100 mM treatment, though inhibitory, significantly improved mean CPG to 13.56% (Fig VI-4b).

None of the KNO_3 treatments given for 12 h could significantly reduce the spread of germination (MGT) which remained in the range of 24.25 to 26.90 days as compared to 26.74 days in the untreated seeds (Fig VI-4c).

The speed of germination (mean DGS) increased with increase in the concentration of KNO_3. Significantly higher speed of germination was observed in the seeds treated with 75 and 100 mM KNO_3. Only 0.38 % seeds germinated per day in the best control response whereas in seeds treated with the 75 mM KNO_3, the MGT was 0.76% seeds per day (Fig VI-4d).

All the pretreatments could only marginally improve the FGP over the best control response and maximum increment of 57% was observed in the seeds treated with 75 mM KNO_3 (Fig VI-4e).

The sandal seeds treated with 25-100 mM KNO_3 for 24 h showed restrained germination as compared to untreated seeds (Table VI-9 and Fig VI-4a-f).

VI-7: Effect of 12 h soaking in KNO_3 on seed germination in *Santalum album* L.

DAS	Treatment (for 12h)	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
10 Days	Control	1.00 ± 0.0	2.00 ± 0.0	1.00 ± 0.0	2.00 ± 0.0
	25 mM KNO_3	1.00 ± 0.0	2.00 ± 0.0	1.00 ± 0.0	2.00 ± 0.0
	50 mM KNO_3	1.00 ± 0.0	2.00 ± 0.0	1.00 ± 0.0	2.00 ± 0.0
	75 mM KNO_3	2.33 ± 0.6	4.67 ± 1.2	2.33 ± 0.6	4.67 ± 1.2
	100 mM KNO_3	1.00 ± 1.0	2.00 ± 2.0	1.00 ± 1.0	2.00 ± 2.0
20 Days	Control	1.33 ± 1.5	2.67 ± 3.1	2.33 ± 1.5	4.67 ± 3.1
	25 mM KNO_3	1.33 ± 0.6	2.67 ± 1.2	2.33 ± 0.6	4.67 ± 1.2
	50 mM KNO_3	3.33 ± 0.6	6.67 ± 1.2	4.33 ± 0.6	8.67 ± 1.2
	75 mM KNO_3	5.00 ± 1.0	10.00 ± 2.0	7.33 ± 1.2	14.67 ± 2.3
	100 mM KNO_3	5.33 ± 0.6	10.67 ± 1.2	6.33 ± 0.6	12.67 ± 1.2
30 Days	Control	7.67 ± 1.2	15.33 ± 2.3	10.00 ± 1.7	20.00 ± 3.5
	25 mM KNO_3	8.67 ± 2.1	17.33 ± 4.2	11.00 ± 1.7	22.00 ± 3.5
	50 mM KNO_3	8.67 ± 3.2	17.33 ± 6.4	13.00 ± 2.6	26.00 ± 5.3
	75 mM KNO_3	9.00 ± 2.6	18.00 ± 5.3	16.33 ± 2.5	32.67 ± 5.0
	100 mM KNO_3	6.67 ± 2.5	13.33 ± 5.0	13.00 ± 3.0	26.00 ± 6.0

The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

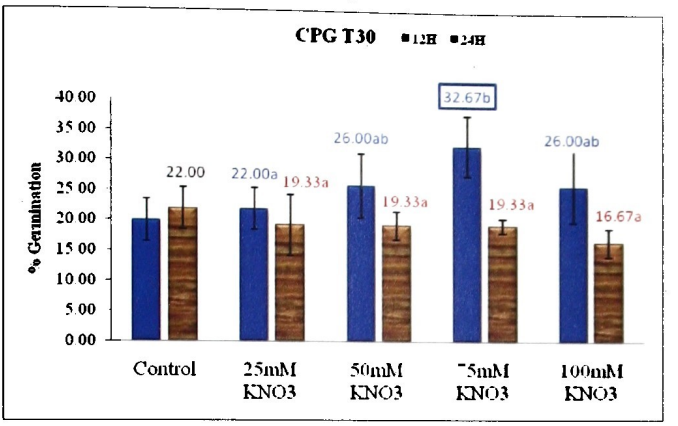
VI-8: Effect of 24 h soaking in KNO_3 on seed germination in *Santalum album* L.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
10 Days	Control	1.00 ± 0.0	2.00 ± 0.0	1.00 ± 0.0	2.00 ± 0.0
	25 mM KNO_3	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
	50 mM KNO_3	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
	75 mM KNO_3	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
	100 mM KNO_3	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
20 Days	Control	1.33 ± 1.5	2.67 ± 3.1	2.33 ± 1.5	4.67 ± 3.1
	25 mM KNO_3	1.33 ± 0.6	2.67 ± 1.2	1.33 ± 0.6	2.67 ± 1.2
	50 mM KNO_3	1.00 ± 1.0	2.00 ± 2.0	1.00 ± 1.0	2.00 ± 2.0
	75 mM KNO_3	1.00 ± 1.0	2.00 ± 2.0	1.00 ± 1.0	2.00 ± 2.0
	100 mM KNO_3	0.67 ± 0.6	1.33 ± 1.2	0.67 ± 0.6	1.33 ± 1.2
30 Days	Control	9.12 ± 1.2	18.33 ± 2.3	11.00 ± 1.7	22.00 ± 3.5
	25 mM KNO_3	8.33 ± 2.1	16.67 ± 4.2	9.67 ± 2.3	19.33 ± 4.6
	50 mM KNO_3	8.67 ± 1.5	17.33 ± 3.1	9.67 ± 1.2	19.33 ± 2.3
	75 mM KNO_3	8.67 ± 1.5	17.33 ± 3.1	9.67 ± 0.6	19.33 ± 1.2
	100 mM KNO_3	7.67 ± 0.6	15.33 ± 1.2	8.33 ± 1.2	16.67 ± 2.3

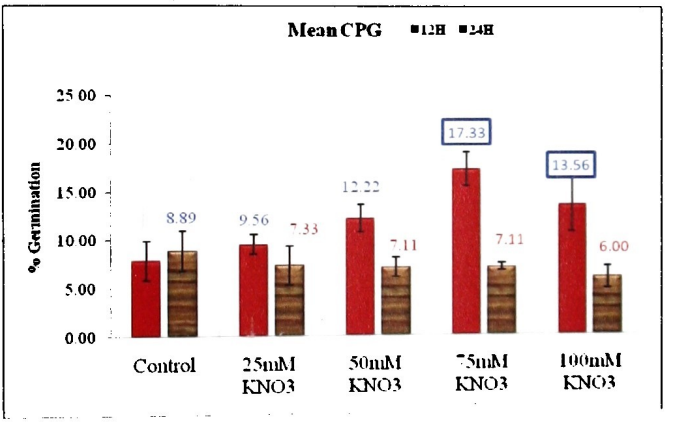
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

VI-4 Effect of KNO_3 on seed germination in *Santalum album* L.

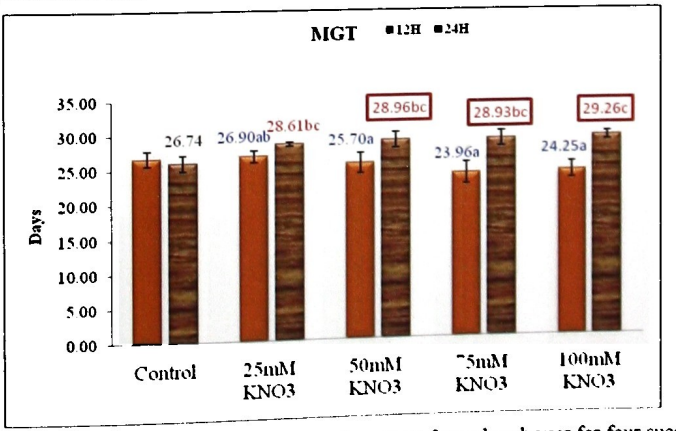
a) Cumulative percentage germination on 15th day



b) Mean Cumulative percentage germination



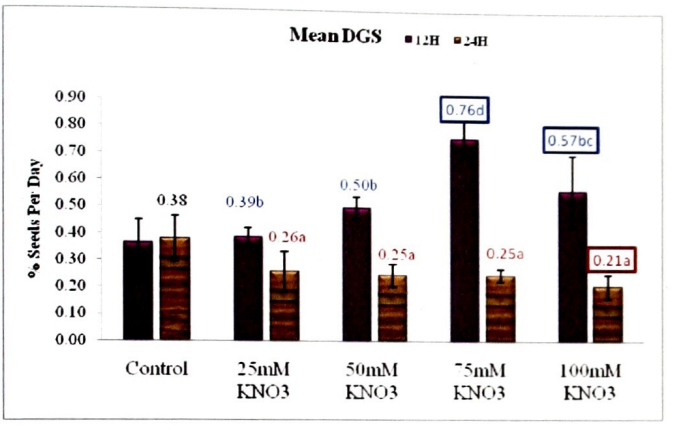
c) Mean germination time



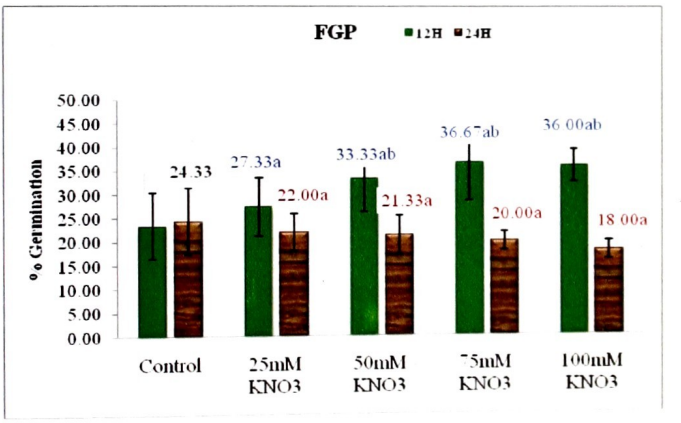
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

VI-4 Effect of KNO_3 on seed germination in *Santalum album* L.

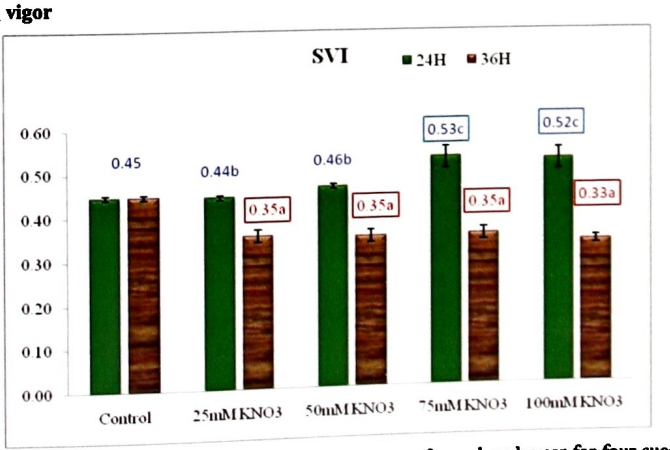
d) Mean daily germination speed



e) Final percentage germination on 30th Day



f) Seedling vigor



The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

The CPG T30 gradually decreased from 22% in untreated seeds to a minimum of 16.67% in the seeds treated with 100 mM KNO_3, which was the inhibitoriest treatment among all KNO_3 doses given for 24 h (Fig VI-4a). The mean CPG also exhibited the similar pattern with minimum of 6% mean CPG in the seeds treated with 100 mM KNO_3 as compared to 8.89% in control (Fig VI-4b).

The inhibitory effect of longer exposure to KNO_3 was clearly seen in the significantly longer MGT of 28.96, 28.93 and 29.26 days in the seeds treated with 50, 75 and 100 mM KNO_3 respectively, as compared to 26.74 days in the untreated seeds (Fig VI-4c).

Coupled with the higher MGT values, the speed of germination in terms of lesser mean DGS was observed with increase in the dose of KNO_3 (Fig VI-4d). The seeds exposed to 100mM KNO_3 for 24 h showed significantly slow speed of germination (0.21% seeds per day) as compared to 0.38% seeds per day in untreated seeds.

The inhibitory effect of longer exposure to KNO_3 was best reflected in the FGP (Fig VI-4e) which linearly decreased with increase in the concentration of KNO_3. The FGP dropped down to 18% in the seeds treated with 100 mM KNO_3 from 24.33% FGP in untreated seeds.

Seedling vigor remained more or less similar to that of control seedlings when the seeds were treated with 25 and 50 mM KNO_3 for 24 h. The treatments of 75 and 100 mM KNO_3, however, produced significantly vigorous seedlings compared to non treated seeds (Fig VI-4f). The longer exposure to KNO_3 did not improve the germination behavior in *Santalum album* and seedling vigor was dramatically reduced when seeds were exposed for 36 h to the same concentrations of KNO_3.

6.4 Discussion

6.4.1 Effect of thiourea

The seed dormancy has been alleviated in many species when the seeds imbibed solutions of any of several compounds, notably nitrates, nitrites, hydroxylamines, and thiourea. However, their effectiveness has long raised questions about how they act to break dormancies (Hendricks and Taylorson, 1975; Agrawal and Dadlani, 1995). Hartmann *et al.* (2002) postulated that the stimulating effect of thiourea on breaking dormancy might be mediated by deactivating the effect of inhibitors, e.g. ABA. Cetinbas and Koyuncu (2006) attributed the simulative effect of thiourea on seeds germination to a reduction of the preventive effect of seed coat and its cytokinin activity in overcoming inhibition. Thiourea is thought to counteract the effects of increased ABA and reduces the level of cytokinin in plant tissue exposed to drought induced by water stress, salinity, or high temperature (Kabar and Baltepe, 1989). According to Gul and Weber (1999), treating the seeds with thiourea is highly effective in alleviating the inhibition of germination by salinity or high temperature, through a physiological process.

In the present investigation, the seeds of *Pterocarpus marsupium* treated with 5-25 mM thiourea for 24 h showed improvement in the germination pattern. A significant increment of 92% over the best control response in CPG T15 (75.33%) was observed in seeds treated with 25 mM thiourea. The speed of the germination (Fig. 6.1d) was significantly improved over control in the seeds treated with 20 and 25 mM. The maximum increment in FGP was of 98% over the best control response in the seeds treated with 25 mM thiourea. A longer exposure (36 h) of seeds to 5-25 mM thiourea was inhibitory to seed germination in *Pterocarpus marsupium* which was reflected in decline in the values of all parameters computed except MGT which increased with increase in the concentration of thiourea.

The treatment of seeds of *Santalum album* with 25-100 mM thiourea for 24 h was beneficial for improving seed germination. All the treatments significantly reduced the mean time to complete germination. But the FGP was significantly improved only in the seeds treated with 75 and 100 mM thiourea given for 24 h (Fig. 6.3e).The seed treated with 25-75 mM thiourea for 36 h showed restrained germination and the treatment of 100 mM thiourea for 36 h was the most inhibitory for seed germination in *Santalum album.*

These results are similar to those reports suggesting the role of sulfur containing chemicals such as cysteine (Roberts and Smith 1977) and thiourea (Stokes 1965; Mayer and Poljakoff-Mayber, 1975) in stimulating seed germination. Thiourea, a sulfur containing molecule, has been proved effective in breaking photo-dormancy in a number of light sensitive seeds (Mayer and Poljakoff-Mayber 1982).

Maruyama *et al.* (1996) reported alleviated germination of cocklebur seeds (*Xanthium pennsylvanicum* Wallr.) by 30 mM allylthiourea (29.8%) and 30 mM thiourea (26.7%) as compared to 14.6% germination in untreated seeds. Thiourea and allylthiourea were equally effective for dry seeds, whereas for the pre-imbibed seeds, allylthiourea was much more effective than thiourea. The authors asserted that the action mechanism of thiourea and of the most active allylthiourea in germination control still remained undetermined.

Thompson and Kosar (1939) have shown that the dormant seeds of lettuce could be stimulated to germinate by treating the seeds with dilute solution of thiourea. Thompson (1940) have reported that lettuce seeds treated with 0.5% thiourea at 18 ^{0}C for 7 h not only stimulated seed germination (87.2% in treated as compared to 0.8% in untreated), but the seed could germinate even at the temperature of 33-35 ^{0}C, which is more than the normal range of temperatures required for lettuce seed germination.

Among several compounds studied, thiourea proved the most effective stimulant of germination in *Ziziphus mauritiana,* (Murthy and Reddy, 1989) where 24 h soaking in 1% thiourea solution enhanced total germination percentage from 41% (control) to 78% at 30°C.

Compounds like nitrate and thiourea are well-known to alleviate salinity induced dormancy in wild plants but the response depends on the plant species and climatic zone and very little information is available regarding their effects on tropical plants (Siddiqui, 2006). Treatment with thiourea has been shown to be highly effective in promoting germination when dormancy is related to salt stress (Esashi *et al.*, 1979; Khan & Ungar, 2001). Siddiqui *et al.* (2006) have demonstrated that increased salinity inhibited the germination of wheat seeds and thiourea alone or in combination with NaCl markedly enhanced germination velocities and showed significant alleviation of salinity-induced dormancy. The thiourea treatment improved the germination of *Prunus avium* seeds (Centinbas and Koyuncu, 2006) wherein the highest germination rates were observed with 120 days stratification + 10,000 ppm thiourea for seeds with coat and 100 days stratification + 10,000 ppm thiourea for seeds without coat. Thiourea promoted seed germination in *Salicornia rubra* (Khan *et al.*, 2002) under low saline conditions. At 300 mM NaCl treatment, application of thiourea substantially alleviated germination from 22% in the best control response to about 60%. Some germination was reported even at 900 mM NaCl in the presence of thiourea and potassium nitrate. The alleviating effect of thiourea on osmoinhibition gradually decreased with an increase in salinity.

In most of the earlier reports on the use of thiourea as a seed pretreatment, the seeds subjected to salinity stress were treated by thiourea and its positive influence on germination was demonstrated. In the present investigation, however, the seeds of *P. marsupium* as well as *S. album* not subjected to salinity stress showed improved germination after thiourea treatment. This indicate that thiourea has potential to alleviate seed

germination in seeds exposed to salt stress as well as in the seeds not subjected to salt stress.

Adams *et al.,* (1961) has used thiourea instead of cold stratification for inducing seed germination in buckbrush *Ceanothus*. Scott (1974) reported improved germination (35% within 5.5 weeks as compared to 2% in 5.7 weeks) after exposing the seeds of *Celmisia coriacea* to 10^{-4}M thiourea. In lettuce seed, the balance between endogenous inhibitors and promoters is changed by application of coumarin or thiourea, and these changes show some correlation with the germination response.

Comparative studies on the effect of different germination stimulants like thiourea and potassium nitrate have been carried out on seeds of *Ziziphus mauritiana* (Murthy and Reddy 1989), *Casuarina equisetifolia* (Maideen *et al.*,1990), and *Acacia nilotica* (Palani *et al.*1995). These compounds are not much used in practical seed propagation, but are widely used in seed research.

Change from dormant to germinating seeds is often accompanied by an increased functioning of the pentose-phosphate pathway of glucose use (Roberts, 1973) and inhibition of catalase (EC1.11.1.6; hydrogen-peroxide: hydrogen-peroxide oxidoreductase) activity favors the pentose-phosphate pathway (Hendricks and Taylorson, 1974). Hendricks and Taylorson (1975) have demonstrated irreversible inhibition of catalase activity in extracts from lettuce (*Lactuca sativa* L. cv. Grand Rapids) and pigweed (*Amaranthus albus* L.) seeds that had imbibed thiourea to a degree proportional to eventual germination. However, at the highest concentrations of thiourea used, the inhibitions were maintained or increased accompanied by proportionate decrease in germination values from the maximum germination values. This supports the results of the present investigation wherein the longer exposure and higher concentrations of thiourea not only affected the germination pattern but also reduced the final percentage of germination.

The earlier reports on use of thiourea in alleviating dormancy and stimulating seed germination in different species and the results of the present investigation on stimulated seed germination in *P. marsupium* and *Santalum album* suggest that thiourea is one of the effective chemical stimulants for seed germination.

6.4.2 Effect of potassium nitrate (KNO_3)

Use of KNO_3 has been an important seed treatment in seed-testing laboratories for many years without a good explanation for its action (Hartmann *et al.* 1997). Potassium nitrate (KNO_3) has been frequently recommended for inducing seed germination (ISTA, 1985; CFIA, 1997; AOSA, 2002).

Nitrogenous compounds in various forms, particularly nitrates (e.g. KNO_3), have been used to stimulate germination (Chaudhary *et al.*, 1996; McIntyre *et al.*, 1996).

In the present investigation, the treatment of seeds with up to 80 mM KNO_3 for 24 h enhanced seed germination in *Pterocarpus marsupium* (Table VI-3 and Fig VI-2a-f). The seeds of *Phalaris paradoxa* belonging to seven biotypes exhibited varying levels of innate dormancy and readily germinated in 20 mM KNO_3 (Taylor *et al.*, 2004).

The improved seed germination was reflected in all the parameters computed except MGT, which remained more or less similar to that of untreated seeds. A longer exposure (36 h) of seeds to the 20-100 mM KNO_3 improved the germination over untreated seeds (Table VI-4 and Fig VI-2a-f) but all these treatments were less effective as compared to 24 h treatment.

The lower doses of KNO_3 (25 and 50 mM) given for 12h only marginally improved the germination pattern in *Santalum album.* None of these treatments significantly reduced the spread of germination (MGT) while all the pretreatments could only marginally improve the FGP over the best control response. The seeds treated with 25-100 mM KNO_3

for 24 h showed restrained germination pattern as compared to untreated seeds (Table VI-9 and Fig VI-4a-f).

In *P. marsupium* 80mM KNO_3 treatment was the most effective in inducing germination while in *S. album,* 75mM KNO_3 treatment was superior over other treatments of KNO_3. These results are on the same line of the results reported in many other species like *Cucumis melo, Helianthus annuus, Angelica glauca, and Swertia angustifolia* (Bradford *et al.*, 1988; Nerson and Govers, 1986; Singh and Rao, 1993; Agrawal and Dadlani 1995; Butola and Badola, 2004; Bhatt *et al* 2005) wherein an improvement in the germination behavior in the seeds treated with different concentrations of potassium nitrate was observed.

Potassium nitrate has been demonstrated to enhance germination in crop species like *Cucumis melo* and *Helianthus annuus.* Seeds pretreated with KNO_3 showed markedly improved laboratory germination in muskmelon (*Cucumis melo* L.) seeds (Bradford *et al.*, 1988; Nerson and Govers, 1986). Singh and Rao (1993) reported that a 5 mM KNO_3 solution almost doubled the germination rate of cultivated sunflower (*Helianthus annuus* L.) seeds and postulated that KNO_3 may be influencing the formation of free radicals, which maintain and improve vigor.

Butola and Badola (2004) treated the seeds of *Angelica glauca,* a threatened medicinal herb, with 50, 100 and 150 mM KNO_3. The seeds treated with 150 mM KNO_3 took only 38.7 days to show first germinant, 48.9% mean percentage germination and 61.0 MGT as against for the best control response these values were 70.7, 24.4% and 94.6 respectively. The reason for this effectiveness of KNO_3 has long been suggested by Roberts and Smith (1977) who postulated that the action of KNO_3 is possibly through oxidized forms of nitrogen causing a shift in respiratory metabolism to the pentose phosphate pathway.

Cetinbas and Koyuncu (2006) demonstrated positive effects of 2500 – 10000 ppm KNO_3 on the germination of *Prunus avium* seeds with as well as without seed coat. Soaking in 7500 ppm and 10000 ppm

KNO_3 gave the most significant germination rates: 64.54% for seeds with coat and 74.24% for seeds without coat, respectively.

Palani *et al.* (1995) have reported improved germination percentage as well as vigor in acid pretreated *Acacia nilotica* seeds treated with different concentrations of KNO_3. At 1% concentration, germination increased from 37% (control) to 79%, and at 2% conc. it increased to 85%. In *Casuarina equisetifolia* germination increased from 46% in the control to 65% after soaking in 1.5% KNO_3 for 36 hours (Maideen *et al.*, 1990). Junttilla and Nilsen (1980) reported that germination of *P. arundinacea* (with light) at 27 ^{0}C was stimulated to 45% using 0.01 M KNO_3. On the other hand the treatment of seeds with potassium nitrate suppressed the germination in *Phalaris canariensis* (Matus-Ca´diz and Hucl, 2005).

Hendricks and Taylorson (1975) have demonstrated that 32 mM KNO_3 inhibited catalase activity of pigweed seeds by 8% and increased germination by 30% above the controls that had imbibed water. The inhibition activity might have promoted the pentose phosphate pathway thereby promoting seed germination. The results from the reports mentioned earlier and the results of the present investigation on *Pterocarpus marsupium* and *Santalum album* wherein the treatment of seeds with potassium nitrate has resulted improved seed germination pattern. In these cases also the potassium nitrate treatment might have suppressed catalase activity and induced the functioning of pentose phosphate pathway which culminated in enhanced seed germination.

Chapter VII

Effect of PGRs on Seed Germination

7.1 Introduction

The German botanist Julius von Sachs (1832–1897) proposed that plants produce, transport and perceive 'organ- forming substances' responsible for the formation and growth of different plant organs. Plant growth regulators (PGRs), formerly known as plant hormones or phytohormones, have multifaceted effects on plant development. These molecules represent a group of organic molecules that are produced by plant tissues and translocated to some other tissue where they influence many diverse developmental processes ranging from seed germination to root, shoot and flower formation (McCourt, 1999) at very low concentrations. These molecules influence the developmental processes by acting as chemical messengers for the communication among cells, tissues and organs of higher plants (Kucera et al., 2005). Plant hormones such as gibberellins (GAs) are found to play an important role in the germination process. Specific endogenous growth promoting and inhibiting compounds are directly involved in the control of seed development, dormancy, and germination (Hartman *et al.,* 1997a). Correlations of hormone concentrations with specific developmental stages, effect of applied hormones, and the relationship of hormones to metabolic activities are suggestive of involvement of hormones in these metabolic activities (Pedroza-Manrique *et al.,* 2005).

The germination of seed can be controlled by application of exogenous hormones at physiological concentrations. It is therefore possible that the natural control of germination involves interplay of hormones, both promoters and inhibitors (Khan, 1968). The present chapter describes the influence of different plant hormones on seed germination in *P. marsupium* and *S. album.*

7.2 Materials and methods

The source of the seeds, preparation of seeds and the selection of seeds for the experiment was followed as described in the Chapter III. Gibberellic acid (GA_3), 6-Benzylaminopurine (BA), Kinetin (Kin) and Indole-3-acetic acid (IAA) were used to study their effect on seed germination. All the plant growth regulators used in the present study were of analytical grade and procured from Sisco Research Laboratory (SRL), India.

7.2.1 Preparation of Stock solutions

7.2.1.1 Gibberellic acid (GA_3)

A 20 mM stock solution of GA_3 (molecular weight 346.4) was prepared by dissolving accurately weighed 3.5 g GA_3 powder in 10 ml of absolute alcohol and then raising the volume to 500 ml with distilled water in a volumetric flask.

7.2.1.2 6-Benzylaminopurine (BA)

A 5 mM stock solution of BA (molecular weight 225.3) was prepared by dissolving accurately weighed 560 mg BA powder in 5ml of 1N HCl and then raising the volume to 500 ml with distilled water in a volumetric flask.

7.2.1.3 Kinetin (Kin)

A 10 mM stock solution of Kinetin (molecular weight 215.2) was prepared by dissolving accurately weighed 1.075 g Kin powder in 5 ml of 1N HCl and then raising the volume to 500 ml with distilled water in a volumetric flask.

7.2.1.4 Indole-3-acetic acid (IAA)

A 10 mM stock solution of IAA (molecular weight 175.2) was prepared by dissolving accurately weighed 2.190 g IAA powder in 10 ml of absolute alcohol and then raising the volume to 500 ml with distilled water in a volumetric flask.

All the stock solutions were stored in refrigerator at 0 ^{0}C. The solutions of desired concentrations were prepared by diluting appropriate volume of stock solution with distilled water. The solutions of growth regulators at different concentrations were used for presoaking the seeds.

7.2.2 Seed presoaking treatments

Randomly selected 50 seeds were allocated to each seed treatment. The seeds were imbibed in 250 ml solution of each of the plant growth regulator for different durations as follows.

Seeds of *Pterocarpus marsupium* were soaked in 1, 2, 3, and 4 mM GA_3 solution for 12, 24 and 36 h. The seeds of *S. album* were soaked in 1, 2, 3, 4, and 5 mM GA_3 solution for 12, 24 and 36 h.

For treating the seeds of *P. marsupium* with BA, seeds were soaked in 0.25, 0.5, 0.75 and 1.0 mM BA solution for 12 and 24 h. The seeds of *S. album* were soaked in 0.5, 1.0, 1.5 and 2.0 mM BA solution for 6, 12, 18 and 24 h.

Seeds of *P. marsupium* were treated with kinetin by soaking in 1-5 mM kinetin solution for 12 and 24 h. The seeds of *S. album* were soaked in 0.5, 1.0, 1.5 and 2.0 mM kinetin for 6, 12, 18 and 24 h.

Seeds of *P. marsupium* were treated with 2.5, 5.0, 7.5 and 10 mM IAA solution for 6, 12, 18 and 24 h. The seeds of *S. album* were soaked in 0.5, 1.0, 1.5 and 2 mM IAA for 8, 16 and 24 h.

The seeds imbibed in distilled water for the same durations were maintained as control. For each of this experiment, the control was maintained separately but for statistical analysis, the treatment means were compared with the best control response. After the specified treatment, the

seeds were rinsed several times in running tap water and sown in polythene bags (4" × 6") and cavity seeding trays containing moist garden soil.

7.2.3 Amylase activity

α- amylase (EC 3.2.1.1) and β- amylase (EC 3.2.1.2) activities were studied in the seeds pretreated with different plant growth regulators for different durations. These enzymes were extracted by following the method of Duffus and Rosis (1973). For extraction of enzymes, 1 g seed was homogenized in 10 ml extraction buffer after 48 h of seed treatment.

The α- amylase was extracted in 12.7 μM calcium acetate buffer at pH 6.0 and the extract was subjected to 70 ^{0}C temperature for 10 min to inactivate β- amylase. After cooling the extract to 4 ^{0}C, it was centrifuged at 10000 ×g for 15 min. The assay mixture (2.0 ml) consisted of 1.2 ml of 12.7 mM starch (0.067% starch prepared in 0.06 M KH_2PO_4) and 0.2 ml enzyme preparation. The reaction mixture was incubated at 25 ^{0}C for 20 min and the intensity of colour developed with iodine was measured at 610 nm on spectrophotometer (Elico India Ltd., Model CL-27). The specific activity of the enzyme was calculated from the amount of starch hydrolyzed and was expressed as microgram of starch hydrolyzed per minute per milligram protein.

The β- amylase was extracted in 0.2 M sodium acetate buffer (pH 3.6) containing 0.1 mM EDTA as α- amylase activity is completely inhibited. The assay mixture (2.0 ml) consisted of 1.2 ml of 0.2 M sodium acetate buffer (pH 3.6) containing 0.1 mM EDTA, 0.6 ml starch solution (0.067% starch prepared in 0.06 M KH_2PO_4) and 0.2 ml enzyme preparation. The reaction mixture was incubated at 25 ^{0}C for 20 minutes and the intensity of colour developed with iodine was measured at 610 nm on spectrophotometer (Elico India Ltd., Model CL-27).

Soluble proteins were estimated by following method of Lowry *et al.* (1951). The Bovine Serum Albumin at the concentration of 10

mg per 100 ml 0.1 N NaOH was used as standard. The specific activity of α- amylase and β- amylase was expressed as microgram starch hydrolyzed per minute per milligram protein.

The results were expressed as mean ± standard deviation.

7.3 Results

7.3.1 *Pterocarpus marsupium* Roxb.

7.3.1.1 Effect of GA_3

The results on the pattern of seed germination after treatment of the seeds with GA_3 are presented in the Table VII-1, VII-2, VII-3 and Fig VII-1a-f and Plate VII-1.

The seed germination pattern was improved in the seeds treated with 1-4 mM GA_3 for 12 h. In this experiment, with increase in the concentration of GA_3 up to 4 mM, there was gradual enhancement in seed germination behavior. The improvement was reflected in the CPG T15 (Fig VII-1a), which increased from 40.67% in the best control response to 41.33% in the seeds treated with 1 mM GA and to 49.33% in the seeds treated with 4 mM GA_3. The seeds treated with 4 mM GA_3 showed a maximum of 21% increase in the mean CPG over the best control response (Fig VII-1b).

The MGT remained in the range of 11.72 – 12.31 days in the treated seeds, while the best control response showed MGT of 11.51 days (Fig VII-1c). This indicates that the spread of germination, however, was not influenced much by any of the GA_3 treatments given for 12 h. The speed of germination in terms of mean DGS was only marginally improved in the seeds treated with 1-4 mM GA_3. The highest value of mean DGS was 2.32% seeds per day observed in the seeds treated with 4 mM GA_3. The best response of untreated seeds showed mean DGS of 2.0% seeds per day (Fig VII-1d).

The FGP increased linearly with increase in the concentration of GA_3 up to 4 mM (Fig VII-1e). The 4 mM GA_3 treatment was the best

VII-1: Effect of 12 h soaking in GA_3 on seed germination in *Pterocarpus marsupium* Roxb.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
5 Days	Control	2.33 ± 0.6	4.67 ± 1.2	2.33 ± 0.6	4.67 ± 1.2
	1 mM GA_3	2.67 ± 0.6	5.33 ± 1.2	2.67 ± 0.6	5.33 ± 1.2
	2 mM GA_3	3.00 ± 0.0	6.00 ± 0.0	3.00 ± 0.0	6.00 ± 0.0
	3 mM GA_3	3.00 ± 1.0	6.00 ± 2.0	3.00 ± 1.0	6.00 ± 2.0
	4 mM GA_3	3.00 ± 1.0	6.00 ± 2.0	3.00 ± 1.0	6.00 ± 2.0
10 Days	Control	7.33 ± 1.0	14.67 ± 2.0	9.67 ± 1.2	19.33 ± 2.3
	1 mM GA_3	5.67 ± 1.5	11.33 ± 3.1	8.33 ± 1.2	16.67 ± 2.3
	2 mM GA_3	7.00 ± 1.0	14.00 ± 2.0	10.00 ± 1.0	20.00 ± 2.0
	3 mM GA_3	7.67 ± 1.2	15.33 ± 2.3	10.67 ± 2.1	21.33 ± 4.2
	4 mM GA_3	8.00 ± 1.7	16.00 ± 3.5	11.00 ± 2.6	22.00 ± 5.3
15 Days	Control	9.33 ± 4.4	18.67 ± 8.7	19.00 ± 5.5	38.33 ± 11.0
	1 mM GA_3	12.33 ± 2.1	24.67 ± 4.2	20.67 ± 3.2	41.33 ± 6.4
	2 mM GA_3	12.33 ± 2.5	24.67 ± 5.0	22.33 ± 1.5	44.67 ± 3.1
	3 mM GA_3	12.00 ± 1.0	24.00 ± 2.0	22.67 ± 1.2	45.33 ± 2.3
	4 mM GA_3	14.00 ± 2.6	28.00 ± 5.3	25.00 ± 1.0	50.00 ± 2.0

The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

VII-2: Effect of 24 h soaking in GA_3 on seed germination in *Pterocarpus marsupium* Roxb.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
5 Days	Control	2.67 ± 0.6	5.33 ± 1.2	2.67 ± 0.6	5.33 ± 1.2
	1 mM GA_3	5.00 ± 1.0	10.00 ± 2.0	5.00 ± 1.0	10.00 ± 2.0
	2 mM GA_3	5.00 ± 1.0	10.00 ± 2.0	5.00 ± 1.0	10.00 ± 2.0
	3 mM GA_3	8.00 ± 3.6	16.00 ± 7.2	8.00± 3.6	16.00 ± 7.2
	4 mM GA_3	8.33 ± 0.6	16.67 ± 1.2	8.33 ± 0.6	16.67 ± 1.2
10 Days	Control	8.00 ± 1.0	16.00 ± 2.0	10.67 ± 1.2	21.33 ± 2.3
	1 mM GA_3	11.33 ± 2.3	22.67 ± 4.6	16.33 ± 1.5	32.67 ± 3.1
	2 mM GA_3	11.67 ± 2.5	23.33 ± 5.0	16.67 ± 3.5	33.33 ± 7.0
	3 mM GA_3	14.67 ± 3.2	29.33 ± 6.4	22.67 ± 5.0	45.33 ± 10.1
	4 mM GA_3	16.00 ± 1.7	32.00 ± 3.5	24.33 ± 2.3	48.67 ± 4.6
15 Days	Control	9.00 ± 4.4	18.00 ± 8.7	19.67 ± 5.5	39.33 ± 11.0
	1 mM GA_3	10.00 ± 2.0	20.00 ± 4.0	26.33 ± 0.6	52.67 ± 1.2
	2 mM GA_3	9.33 ± 1.2	18.67 ± 2.3	26.00 ± 2.6	52.00 ± 5.3
	3 mM GA_3	11.00 ± 6.6	22.00 ± 13.1	33.67 ± 2.1	67.33 ± 4.2
	4 mM GA_3	10.67 ± 4.9	21.33 ± 9.9	35.00 ± 2.6	70.00 ± 5.2

The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

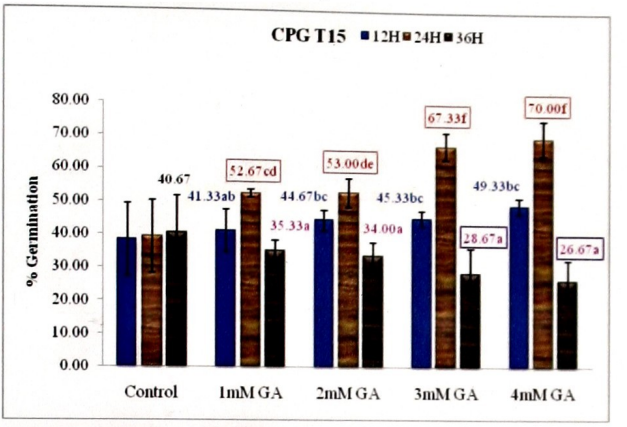
VII-3: Effect of 36 h soaking in GA_3 on seed germination in *Pterocarpus marsupium* Roxb.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
5 Days	Control	3.00 ± 0.0	6.00 ± 0.0	3.00 ± 0.0	6.00 ± 0.0
	1 mM GA_3	2.67 ± 0.6	5.33 ± 1.2	2.67 ± 0.6	5.33 ± 1.2
	2 mM GA_3	1.33 ± 0.6	2.67 ± 1.2	1.33 ± 0.6	2.67 ± 1.2
	3 mM GA_3	1.00 ± 1.0	2.00 ± 2.0	1.00 ± 1.0	2.00 ± 2.0
	4 mM GA_3	1.00 ± 1.0	2.00 ± 2.0	1.00 ± 1.0	2.00 ± 2.0
10 Days	Control	7.67 ± 1.0	15.33 ± 2.0	10.67 ± 1.2	21.33 ± 2.3
	1 mM GA_3	4.67 ± 1.2	9.33 ± 2.3	7.33 ± 0.6	14.67 ± 1.2
	2 mM GA_3	5.67 ± 1.2	11.33 ± 2.3	7.00 ± 1.7	14.00 ± 3.5
	3 mM GA_3	5.00 ± 3.0	10.00 ± 6.0	6.00 ± 3.6	12.00 ± 7.2
	4 mM GA_3	2.33 ± 2.1	4.67 ± 4.2	3.33 ± 1.5	6.67 ± 3.1
15 Days	Control	9.67 ± 4.4	19.33 ± 8.7	20.33 ± 5.5	40.67 ± 11.0
	1 mM GA_3	10.33 ± 1.5	20.67 ± 3.1	17.67 ± 1.5	35.33 ± 3.1
	2 mM GA_3	10.00 ± 2.6	20.00 ± 5.3	17.00 ± 2.0	34.00 ± 4.0
	3 mM GA_3	8.33 ± 2.3	16.67 ± 4.6	14.33 ± 3.8	28.67 ± 7.6
	4 mM GA_3	10.00 ± 1.7	20.00 ± 3.5	13.33 ± 3.1	26.67 ± 6.1

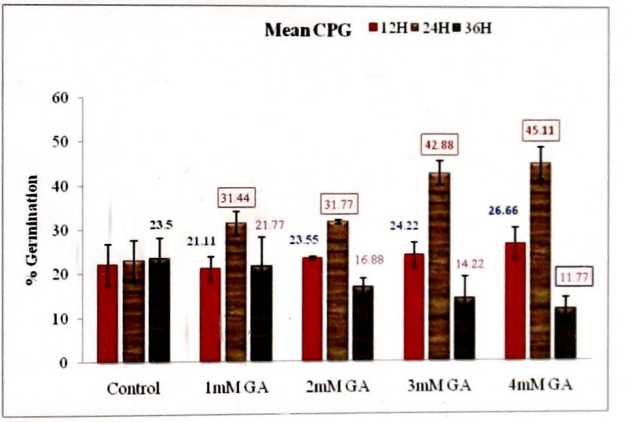
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

VII-1 Effect of GA on seed germination in *Pterocarpus marsupium* Roxb.

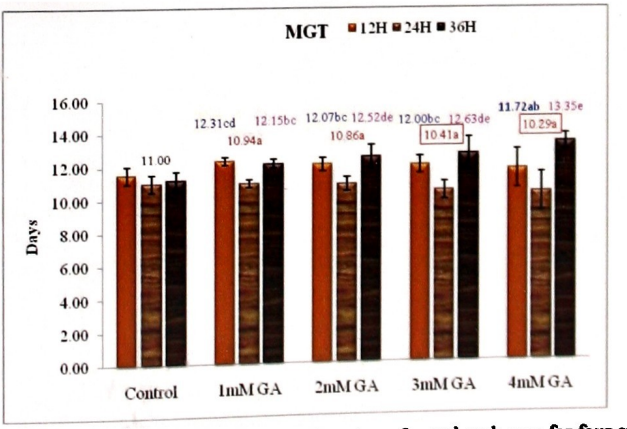
a) Cumulative percentage germination on 15th day



b) Mean Cumulative percentage germination



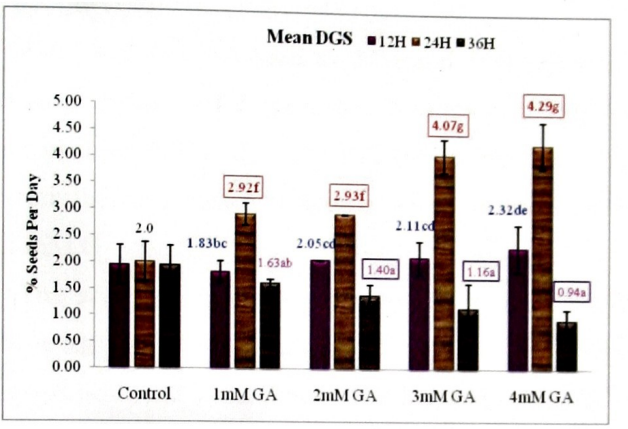
c) Mean germination time



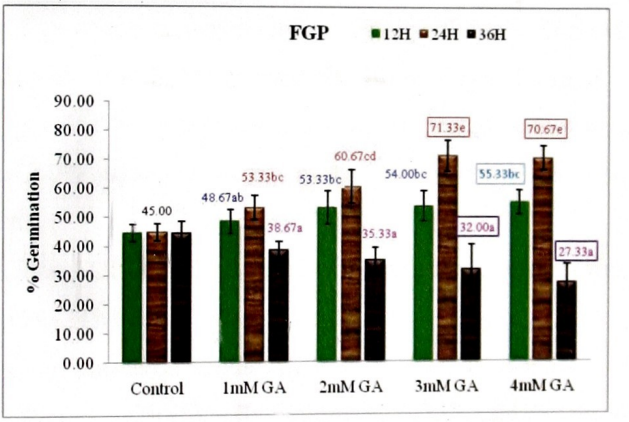
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

VII-1 Effect of GA on seed germination in *Pterocarpus marsupium* Roxb.

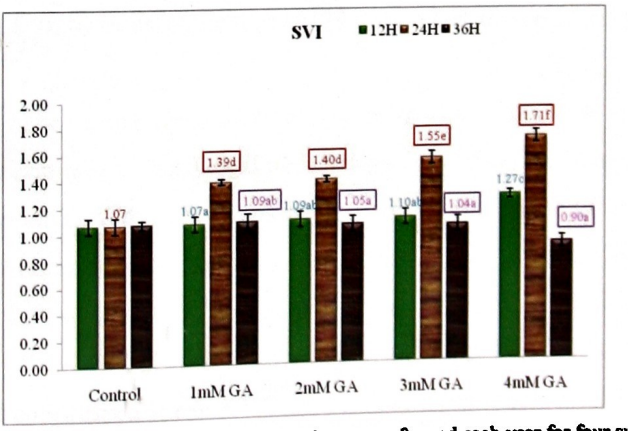
d) Mean daily germination speed



e) Final percentage germination on 30th Day



f) Seedling vigor



The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

among all the GA_3 treatments, which significantly improved FGP (55.33%) over the best control response (FGP = 45.0%).

The CPG T15 increased by 35% and 36% respectively over control in the seeds treated with 1 and 2 mM GA_3 for 24 h. (Fig VII-1a). A maximum increment of 78% in the CPG T15 and an increment of 105% in mean CPG over the best control response were observed in the seeds treated with 4 mM GA_3.

The seed germination was spread over a relatively shorter duration in the seeds treated with 3 mM and 4 mM GA_3. The shortest MGT of 10.29 days was observed in the seeds treated with 4 mM GA_3 as against 11.00 days in the untreated seeds (Fig VII-1c).

The speed of germination was significantly improved in the seeds treated with 1-4 mM GA_3 and the highest speed (mean DGS) of 4.29% seeds per day was observed in the seeds treated with 4 mM GA_3 as compared to 2.0% seeds per day in the best control response among untreated seeds.

The FGP was significantly improved in the seeds treated with 3 and 4 mM GA_3. The seeds treated with 3 mM GA_3 showed 60% increase in the FGP over untreated seeds (FGP = 45%). The seeds treated with 4 mM GA though significantly improved FGP, this treatment was marginally inhibitory compared to 3 mM GA_3 treatment as indicated by lower FGP of 70.67% as compared to 71.33% FGP in seeds treated with 3 mM GA_3.

The seeds treated with 1-4 mM GA_3 for 36 h exhibited suppressed germination behavior, which was magnified with increase in the concentration of GA_3. The seeds treated with 3 and 4 mM GA_3 showed significantly restrained germination as indicated by lowered values of CPG T15 (28.67 and 26.67% respectively) over the best control response (CPG T15 = 40.67%). The seeds treated with 4 mM GA_3 also showed significantly suppressed mean CPG of 11.71% as compared to 23.5% in the best control response.

The spread of germination (MGT) increased with increase in the concentration of GA_3, though the rise was not significant. The maximum MGT of 13.35 days was observed in the seeds treated with 4mM GA_3 as compared to 11.0 days in the untreated seeds.

Longer exposure to 1-4 mM GA_3 also affected the speed of germination and the mean DGS was significantly lowered from 2.0% seeds per day in untreated seeds to 0.94% seeds per day in the seeds treated with 4mM GA_3.

The FGP was also restrained by the 36 h exposure to 1-4 mM GA_3. With increase in the concentration of GA_3, there was gradual decrease in the FGP from 45% in the best control response to 38.67% (1 mM GA_3) and 35.66% (2 mM GA_3). A significant decrease in the FGP was observed in the seeds treated with 3 and 4mM GA_3 (32% and 27.33% respectively).

The seedling vigor remained more or less similar in the seedlings raised from the untreated seeds and the seeds treated with 1-4 mM GA_3 for 12 h (Fig VII-1f). However, soaking the seeds in the solutions of the same concentrations for 24 h significantly boosted the vigor. The longer exposure of 36 h in the 1-4 mM GA_3 solutions not only reduced the germination but also reduced the seedling vigor.

Thus, among the treatments of different concentrations of GA_3 for different durations, the 3 mM GA_3 treatment for 24 h was the best treatment to induce maximum seed germination in shorter duration in *P. marsupium.*

7.3.1.2 Effect of BA

The data presented in the Table VII-4 and VII-5 and Fig VII-2a-f showed that the seeds exposed to 0.25-1.0 mM BA for shorter duration of 12 h gave a better germination response wherein the lower

VII-4: Effect of 12 h soaking in BA on seed germination in *Pterocarpus marsupium* Roxb.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
5 Days	Control	2.33 ± 0.6	4.67 ± 1.2	2.33 ± 0.6	4.67 ± 1.2
	0.25 mM BA	12.33 ± 0.6	24.67 ± 1.2	12.33 ± 0.6	24.67 ± 1.2
	0.50 mM BA	9.00 ± 3.0	18.00 ± 6.0	9.00 ± 3.0	18.00 ± 6.0
	0.75 mM BA	5.67 ± 1.2	11.33 ± 2.3	5.67 ± 1.2	11.33 ± 2.3
	1.00 mM BA	3.67 ± 2.5	7.33 ± 5.0	3.67 ± 2.5	7.33 ± 5.0
10 Days	Control	7.33 ± 1.0	14.67 ± 2.0	9.67 ± 1.2	19.33 ± 2.3
	0.25 mM BA	6.67 ± 3.1	13.33 ± 6.1	19.00 ± 3.0	38.00 ± 6.0
	0.50 mM BA	11.33 ± 3.2	22.67 ± 6.4	20.33 ± 1.2	40.67 ± 2.3
	0.75 mM BA	7.33 ± 1.5	14.67 ± 3.1	13.00 ± 1.7	26.00 ± 3.5
	1.00 mM BA	9.33 ± 1.2	18.67 ± 2.3	13.00 ± 3.6	26.00 ± 7.2
15 Days	Control	9.33 ± 4.4	18.67 ± 8.7	19.00 ± 5.5	38.33 ± 11.0
	0.25 mM BA	7.67 ± 1.5	15.33 ± 3.1	26.67 ± 2.3	53.33 ± 4.6
	0.50 mM BA	5.00 ± 1.0	10.00 ± 2.0	25.33 ± 0.6	50.67 ± 1.2
	0.75 mM BA	8.67 ± 1.5	17.33 ± 3.1	21.67 ±3.2	43.33 ± 6.4
	1.00 mM BA	3.67 ± 1.5	7.33 ± 3.1	16.67 ± 3.1	33.33 ± 6.1

The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

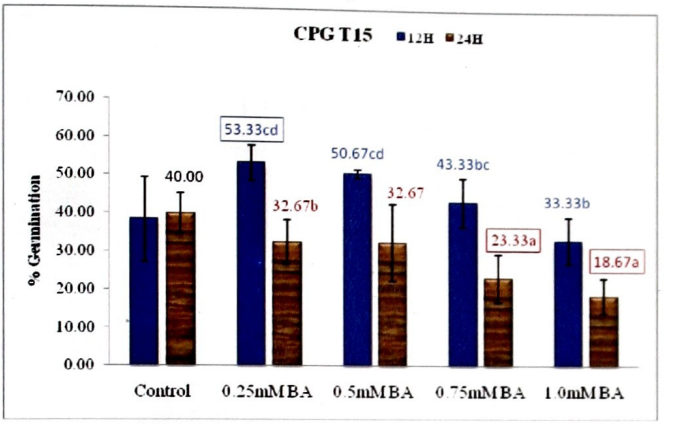
VII-5: Effect of 24 h soaking in BA on seed germination in *Pterocarpus marsupium* Roxb.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
5 Days	Control	3.00 ± 1.0	6.00 ± 2.0	3.00 ± 1.0	6.00 ± 2.0
	0.25 mM BA	1.33 ± 1.5	2.67 ±3.1	1.33 ± 1.5	2.67 ± 3.1
	0.50 mM BA	1.33 ± 0.6	2.67 ± 1.2	1.33 ± 0.6	2.67 ± 1.2
	0.75 mM BA	1.00 ± 0.0	2.00 ± 0.0	1.00 ± 0.0	2.00 ± 0.0
	1.00 mM BA	0.67 ± 0.6	1.33 ± 1.2	0.67 ± 0.6	1.33 ± 1.2
10 Days	Control	9.33 ± 4.5	18.67 ± 9.0	12.33 ± 5.0	24.67 ± 10.1
	0.25 mM BA	10.67 ± 1.2	21.33 ± 2.3	12.00 ± 1.7	24.00 ± 3.5
	0.50 mM BA	7.00 ± 2.0	14.00 ± 4.0	8.33 ± 2.1	16.67 ± 4.2
	0.75 mM BA	3.33 ± 1.2	6.67 ± 2.3	4.33 ± 1.2	8.67 ± 2.3
	1.00 mM BA	4.33 ± 2.5	8.67 ± 5.0	5.00 ± 2.6	10.00 ± 5.3
15 Days	Control	7.67 ± 4.9	15.33 ± 9.9	20.00 ± 2.6	40.00 ± 5.3
	0.25 mM BA	4.33 ± 1.2	8.67 ± 2.3	16.33 ± 2.9	32.67 ± 5.8
	0.50 mM BA	8.00 ± 6.1	16.00 ± 12.2	16.33 ± 5.0	32.67 ± 10.1
	0.75 mM BA	7.33 ± 2.1	14.67 ± 4.2	11.67 ± 3.2	23.33 ± 6.4
	1.00 mM BA	4.33 ± 0.6	8.67 ± 1.2	9.33 ± 2.3	18.67 ± 4.6

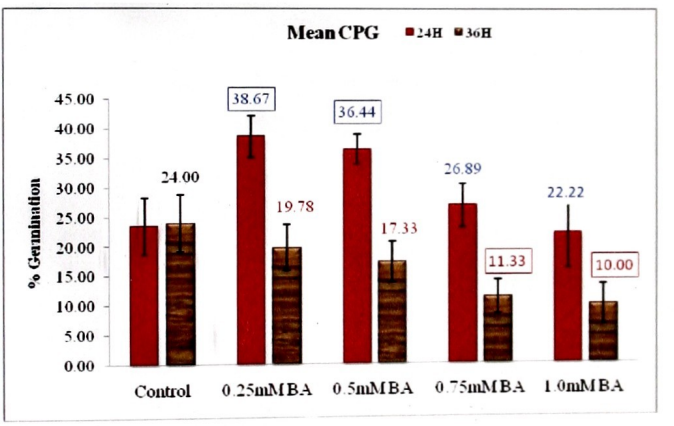
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

VII-2 Effect of BA on seed germination in *Pterocarpus marsupium* Roxb.

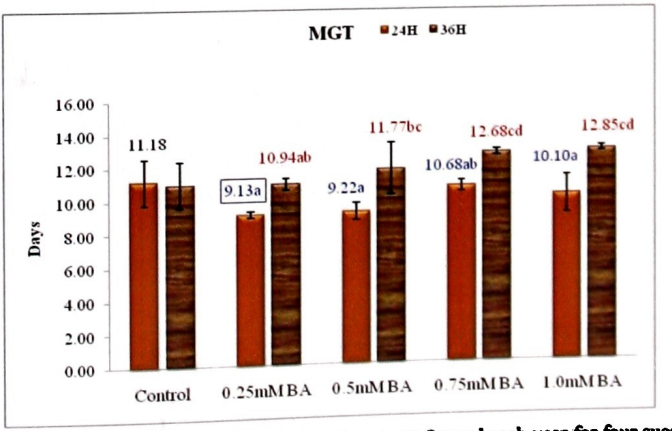
a) Cumulative percentage germination on 15th day



b) Mean Cumulative percentage germination



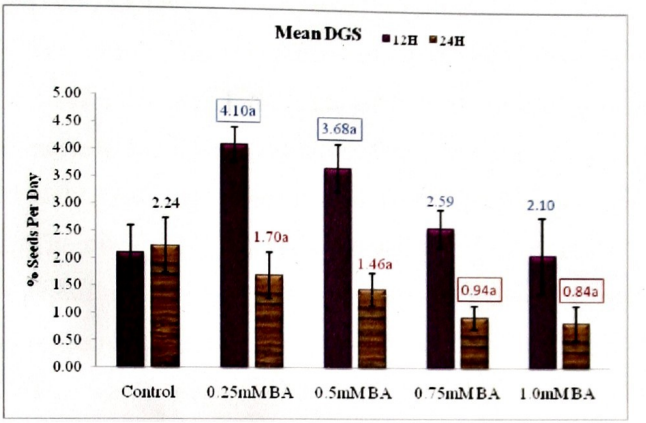
c) Mean germination time



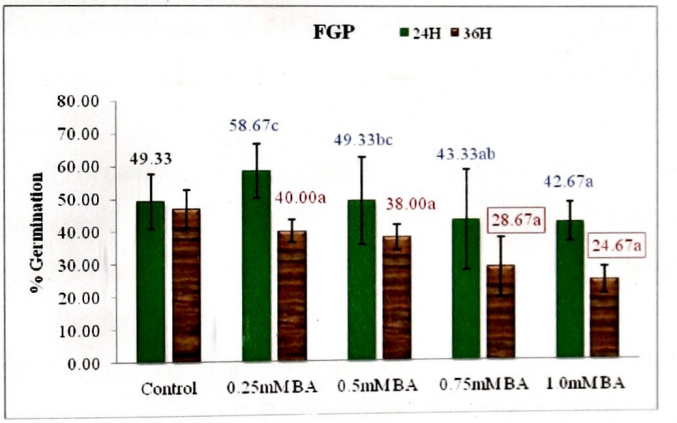
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

VII-2 Effect of BA on seed germination in *Pterocarpus marsupium* Roxb.

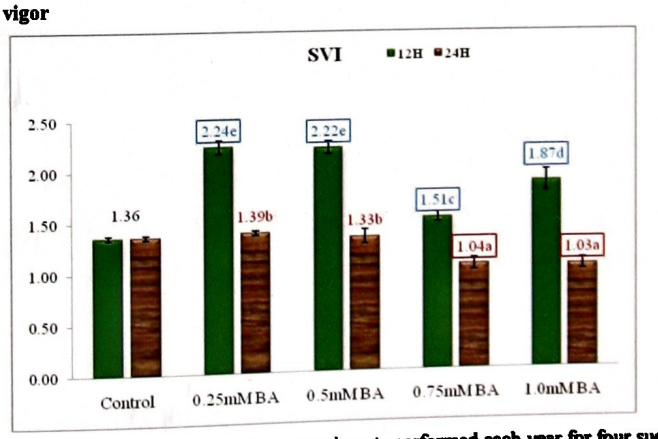
d) Mean daily germination speed



e) Final percentage germination on 30th Day



f) Seedling vigor



The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

concentrations (0.25 and 0.5 mM BA) were beneficial while higher concentrations (0.75 and 1.0 mM BA) were inhibitory.

The lowest concentration of 0.25 mM BA significantly improved the CPG T15 to 53.33% from 40% in the best control response and with the increase in the concentration; there was a gradual decrease in the CPG T15 to 33.33% in the seeds treated with 1.0 mM BA (Fig VII-2a).

The mean CPG showed a similar trend wherein the 0.25 and 0.5 mM BA significantly improved the mean CPG over the best control response and higher concentration suppressed the mean CPG (Fig VII-2b).

The seeds treated with 0.25 mM BA showed improved germination in terms of shorter MGT of 9.13 days as compared to 11.18 days in the untreated seeds. All the higher treatments resulted in spread of germination over a longer period as compared to the best control response (Fig VII-2c).

The treatments of 0.25 and 0.5 mM BA also significantly enhanced the speed of germination (mean DGS) to 4.10 and 3.68% seeds per day over 2.24% seeds per day in the untreated seeds (Fig VII-2d).

The inhibitory effect of BA was also reflected in the FGP. The only treatment to boost the FGP was 0.25 mM BA that gave 58.67% germination as compared to 49.33% germination in the untreated seeds. All the other treatments suppressed the germination, though not significantly, as indicated by lower values of FGP over the best control response (Fig VII-2e).

The seed germination was restrained after exposing the seeds to 0.25- 1.0 mM BA for 24 h and the effects became pronounced with the increase in the concentration of BA. There was a gradual decrease in the CPG T15 (Fig VII-2a) and mean CPG (Fig VII-2b) with increase in the concentration of BA. The untreated seeds showed highest CPG T15 of 40% which deceased to 32.67% in the seeds treated with 0.25 and 0.5 mM BA. The seeds treated with 0.75 and 1.0 mM BA showed significant decrease in

the CPG T15 (23.33 and 18.67% respectively). A similar trend was exhibited by mean CPG values.

The germination was spread over a longer period in the seeds treated with BA, except the 0.25 mM BA treatment which marginally decreased MGT (Fig VII-2c). A higher MGT was coupled with slower speed of germination (mean DGS, Fig VII-2d). The 0.75 and 1.0 mM BA treatments significantly lowered the mean DGS to 0.94% and 0.84% seeds per day respectively from 2.24% seeds per day in the untreated seeds.

The inhibitory effect of all the BA treatments given for 24 h was also reflected in FGP, which gradually decreased with increase in the concentration of BA. The 0.75 and 1.0 mM BA significantly lowered the FGP to 28.67 and 24.67% respectively from 49.33% in the untreated seeds (Fig VII-2e).

All the concentrations of BA (0.25-1.0 mM) used to soak the seeds for 12 h significantly enhanced the seedling vigor over the best control response (Fig VII-2f) whereas the seedlings obtained thorough the seeds treated for 24 h with the BA solutions of the same concentrations resulted in reduced seedling vigor. Among these treatments, 0.75 and 1.0 mM BA treatments significantly lowered the seedling vigor.

Thus, for improving seed germination in *P. marsupium*, the treatment of presoaking the seeds for 12 h in 0.25 mM BA solution was optimum and higher concentrations of BA and longer duration treatments were inhibitory for seed germination.

7.3.1.3 Influence of Kin

The results on influence of 1-5 mM Kin on the germination response of seeds are recorded in Table VII-6 and VII-7 and Fig VII-3a-f.

The seeds presoaked in 1 mM and 2 mM Kin for 12 h did not show much variation in the CPG T15 and mean CPG as compared to the best control response (Fig VII-3a and VII-3b). The seeds treated with 3 mM Kin showed enhanced germination as indicated by higher CPG T15

VII-6: Effect of 12 h soaking in Kin on seed germination in *Pterocarpus marsupium* Roxb.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
5 Days	Control	4.00 ± 1.0	8.00 ± 2.0	4.00 ± 1.0	8.00 ± 2.0
	1 mM Kin	4.33 ± 1.5	8.67 ± 3.1	4.33 ± 1.5	8.67 ± 3.1
	2 mM Kin	4.67 ± 1.2	9.33 ± 2.3	4.67 ± 1.2	9.33 ± 2.3
	3 mM Kin	4.67 ± 1.2	9.33 ± 2.3	4.67 ± 1.2	9.33 ± 2.3
	4 mM Kin	4.00 ± 2.0	8.00 ± 4.0	4.00 ± 2.0	8.00 ± 4.0
	5 mM Kin	4.00 ± 2.0	8.00 ± 4.0	4.00 ± 2.0	8.00 ± 4.0
10 Days	Control	6.00 ±2.0	12.00 ± 4.0	10.00 ± 2.6	20.00 ± 5.3
	1 mM Kin	12.00 ± 1.0	24.00 ± 2.0	16.33 ± 2.3	32.67 ± 4.6
	2 mM Kin	12.67 ± 1.2	25.33 ± 2.3	17.33 ± 1.2	34.67 ± 2.3
	3 mM Kin	17.00 ± 1.7	34.00 ± 3.5	21.67 ± 2.5	43.33 ± 5.0
	4 mM Kin	10.67 ± 0.6	21.33 ± 1.2	14.67 ± 2.5	29.33 ± 5.0
	5 mM Kin	8.67 ± 2.5	17.33 ± 5.0	12.67 ± 4.0	25.33 ± 8.1
15 Days	Control	11.67 ± 1.2	23.33 ± 2.3	21.67 ± 3.2	43.33 ± 6.4
	1 mM Kin	5.33 ± 1.5	10.67 ± 3.1	21.67 ± 2.5	43.33 ± 5.0
	2 mM Kin	5.33 ± 2.9	10.67 ± 5.8	22.67 ± 2.5	45.33 ± 5.0
	3 mM Kin	4.67 ± 3.8	9.33 ± 7.6	26.33 ± 2.1	52.67 ± 4.2
	4 mM Kin	6.67 ± 2.3	13.33 ± 4.6	21.33 ± 4.7	42.67 ± 9.5
	5 mM Kin	6.67 ± 1.5	13.33 ± 3.1	19.33 ± 5.5	38.67 ± 11.0

The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

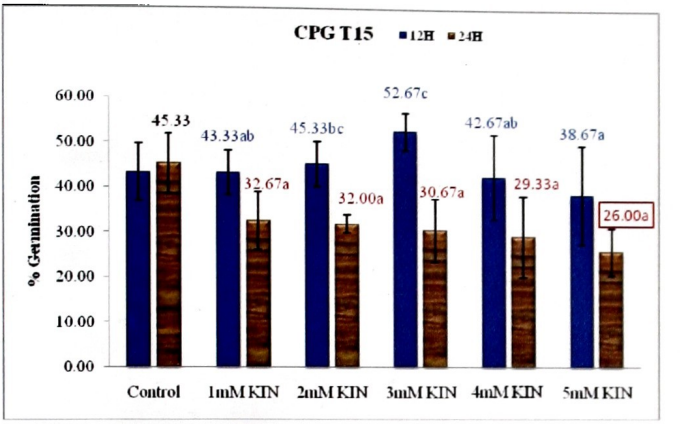
VII-7: Effect of 24 h soaking in Kin on seed germination in *Pterocarpus marsupium* Roxb.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
5 Days	Control	3.67 ± 1.0	7.33 ± 2.0	3.67 ± 1.0	7.33 ± 2.0
	1 mM Kin	1.67 ± 0.6	3.33 ± 1.2	1.67 ± 0.6	3.33 ± 1.2
	2 mM Kin	0.67 ± 1.2	1.33 ± 2.3	0.67 ± 1.2	1.33 ± 2.3
	3 mM Kin	0.67 ± 1.2	1.33 ± 2.3	0.67 ± 1.2	1.33 ± 2.3
	4 mM Kin	0.67 ± 1.2	1.33 ± 2.3	0.67 ± 1.2	1.33 ± 2.3
	5 mM Kin	0.33 ± 0.6	0.67 ± 1.2	0.33 ± 0.6	0.67 ± 1.2
10 Days	Control	7.33 ±2.0	14.67 ± 4.0	11.00 ± 2.6	22.00 ± 5.3
	1 mM Kin	11.00 ± 2.6	22.00 ± 5.3	12.67 ± 3.1	25.33 ± 6.1
	2 mM Kin	6.00 ± 1.0	12.00 ± 2.0	6.67 ± 0.6	13.33 ± 1.2
	3 mM Kin	5.33 ± 2.5	10.67 ± 5.0	6.00 ± 3.6	12.00 ± 7.2
	4 mM Kin	4.67 ± 3.5	9.33 ± 7.0	5.33 ± 4.5	10.67 ± 9.0
	5 mM Kin	4.33 ± 2.5	8.67 ± 5.0	4.67 ± 3.1	9.33 ± 6.1
15 Days	Control	11.67 ± 1.2	23.33 ± 2.3	22.67 ± 3.2	45.33 ± 6.4
	1 mM Kin	3.67 ± 1.5	7.33 ± 3.1	16.33 ± 3.2	32.67 ± 6.4
	2 mM Kin	9.33 ± 1.2	18.67 ± 2.3	16.00 ± 1.0	32.00 ± 2.0
	3 mM Kin	9.33 ± 0.6	18.67 ± 1.2	15.33 ± 3.5	30.67 ± 7.0
	4 mM Kin	9.33 ± 0.6	18.67 ± 1.2	14.67 ± 4.5	29.33 ± 9.0
	5 mM Kin	8.33 ± 0.6	16.67 ± 1.2	13.00 ± 2.6	26.00 ± 5.3

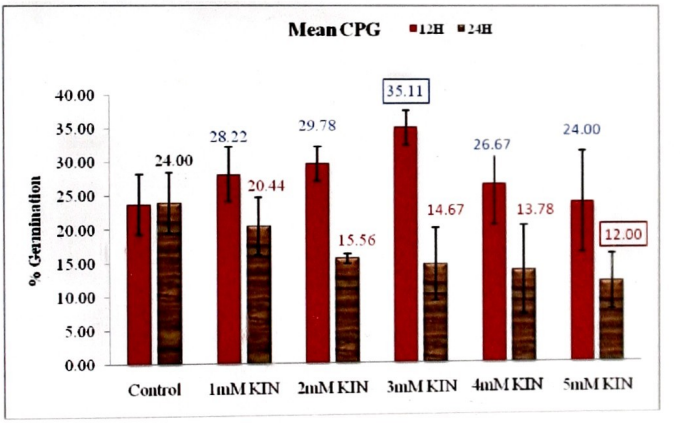
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

VII-3 Effect of Kin on seed germination in *Pterocarpus marsupium* Roxb.

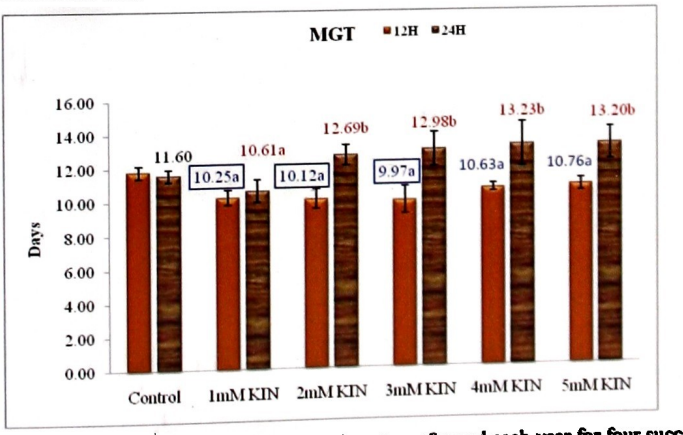
a) Cumulative percentage germination on 15th day



b) Mean Cumulative percentage germination



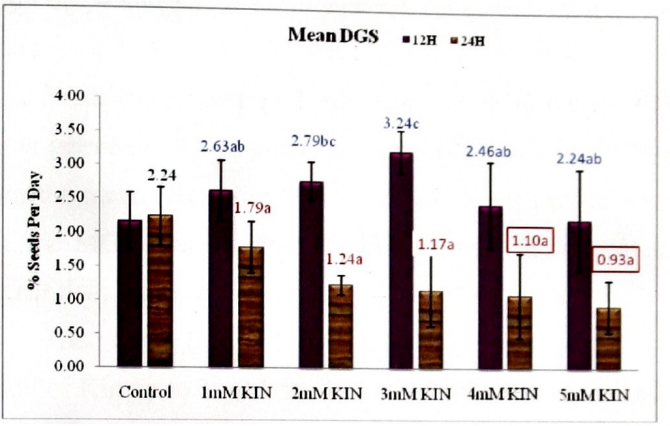
c) Mean germination time



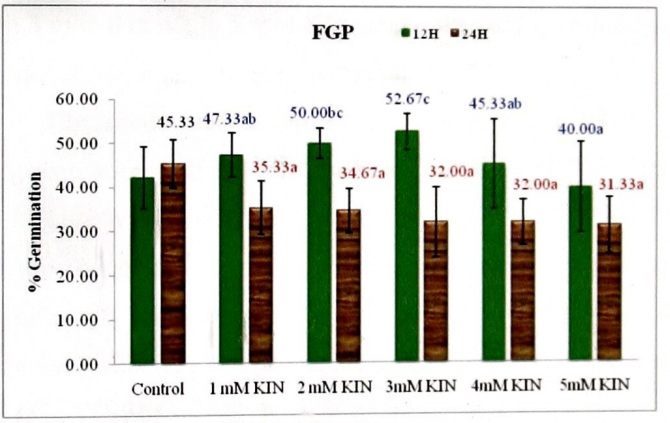
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

VII-3 Effect of Kin on seed germination in *Pterocarpus marsupium* Roxb.

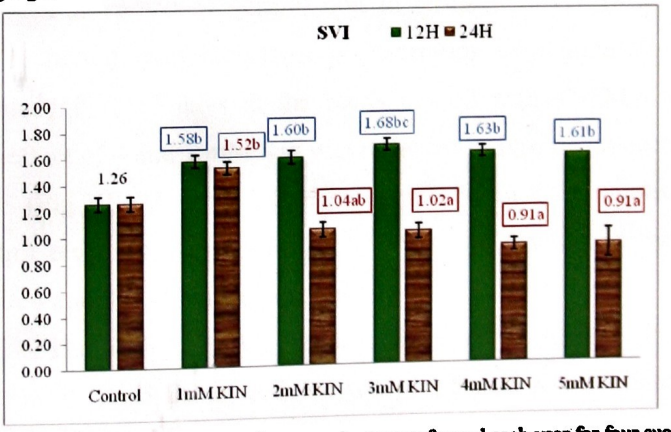
d) Mean daily germination speed



e) Final percentage germination on 30th Day



f) Seedling vigor



The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

(52.67%) and significantly improved mean CPG (35.11%). The higher concentration of 4 and 5 mM Kin restrained the germination by lowering CPG T15 and mean CPG.

Seeds treated with 1-3 mM Kin though restrained CPG T15 showed faster germination by significantly lowering MGT (Fig VII-3c) and by elevating the mean DGS (Fig VII-3d). In the seeds treated with 4 and 5 mM Kin, the MGT was lower in comparison with the untreated seeds, but was higher than the seeds treated with 3 mM Kin.

The FGP (Fig VII-3e) was improved with increasing concentration of Kin up to 3 mM, while 4 and 5 mM Kin restrained it. The highest germination percentage of 52.67% was obtained in the seeds treated with 3 mM Kin while 5 mM Kin gave only 40% germination which was lower than the best control response (FGP = 45.33%).

The seeds treated with 1-5 mM Kin for 24 h showed restrained germination pattern which was reflected in all the parameters computed. There was gradual decrease in the CPG T15 (Fig VII-3a) with increase in the concentration of Kin and the seeds treated with 5 mM Kin showed significantly suppressed CPG T15 (26%) as compared to the best control response (CPG T15 = 45.33%). A similar trend was observed in the mean CPG (Fig VII-3b).

The spread of germination in terms of MGT (Fig VII-3c) gradually increased with increasing concentration of Kin, reaching the highest value of 13.23 days in the seeds treated with 4 mM Kin. The inhibitory effect of 4 and 5 mM Kin was reflected in significant decrease in mean DGS (Fig VII-3d) from 2.16% seeds per day in the untreated seeds (best control response) to 1.10% and 0.93% seeds per day in the seeds treated with 4 and 5 mM Kin respectively.

The FGP (Fig VII-3e) also declined with increase in the concentration of Kin and the seeds treated with 5 mM Kin exhibited least FGP of 31.33% as compared to 45.33% in the untreated seeds.

Among the different concentrations of Kin used for enhanced germination, the seeds treated with 1-5 mM Kin for 12 h improved the germination pattern and also significantly enhanced the seedling vigor over control seedlings (Fig VII-3f). The seeds exposed for 24 h to the solutions of Kin of the same concentrations resulted not only in inferior germination pattern but also produced seedlings with less vigor than the seedlings obtained from untreated seeds as well as seedlings obtained from seeds treated with 1-5 mM Kin for 12 h.

In conclusion, the seed germination in *P. marsupium* was influenced by the treatments of Kin and the germination was best alleviated by soaking the seeds for 12 h in 3 mM Kin solution.

7.3.1.4 Effect of IAA

The results on the seed germination in the seeds treated with 2.5-10.0 mM IAA are presented in the Table VII-8 and VII-9 and Fig VII-4a-f. The seed germination was improved in the seeds treated with 2.5 and 5.0 mM IAA for 8h while the higher concentrations of IAA (7.5 and 10.0 mM) suppressed the germination pattern. This was reflected in all the parameters computed.

The CPG T15 and mean CPG in the seeds treated with 5.0 mM IAA (Fig VII-4a and b) reached the maximum values of 44.67% and 27.11% respectively. In the best control response, the values of CPG T15 and mean CPG were 41.33% and 24.67% respectively. This indicated that the germination was improved with the treatment of low concentration of IAA. Higher concentration of IAA (7.5 and 10.0 mM) significantly suppressed the germination by lowering the CPG T15 to minimum of 31.33% in the seeds treated with 10.0 mM IAA. A similar pattern was observed in the mean CPG, which was significantly lowered (15.56%) in the seeds treated with 10.0 mM IAA.

VII-8: Effect of 8 h soaking in IAA on seed germination in *Pterocarpus marsupium* Roxb.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
5 Days	Control	1.33 ± 0.58	2.67 ± 1.5	1.33 ± 0.58	2.67 ± 1.5
	2.5 mM IAA	5.33 ± 0.6	10.67 ± 1.2	5.33 ± 0.6	10.67 ± 1.2
	5.0 mM IAA	5.33 ± 0.6	10.67 ± 1.2	5.33 ± 0.6	10.67 ± 1.2
	7.5 mM IAA	1.67 ± 1.2	3.33 ± 2.3	1.67 ± 1.2	3.33 ± 2.3
	10.0 mM IAA	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
10 Days	Control	5.67 ± 1.5	11.33 ± 3.0	7.00 ± 2.6	14.00 ± 4.2
	2.5 mM IAA	5.33 ± 1.5	10.67 ± 3.1	10.67 ± 2.1	21.33 ± 4.2
	5.0 mM IAA	7.67 ± 1.2	15.33 ± 2.3	13.00 ± 1.0	26.00 ± 2.0
	7.5 mM IAA	7.67 ± 1.2	15.33 ± 2.3	9.33 ± 1.2	18.67 ± 2.3
	10.0 mM IAA	7.67 ± 1.2	15.33 ± 2.3	7.67 ± 1.2	15.33 ± 2.3
15 Days	Control	11.00 ± 1.0	22.00 ± 2.0	18.67 ± 3.6	36.33 ± 6.2
	2.5 mM IAA	11.67 ± 1.5	23.33 ± 3.1	22.33 ± 0.6	44.67 ± 1.2
	5.0 mM IAA	9.33 ± 2.1	18.67 ± 4.2	22.33 ± 1.2	44.67 ± 2.3
	7.5 mM IAA	9.33 ± 2.1	18.67 ± 4.2	18.67 ± 2.1	37.33 ± 4.2
	10.0 mM IAA	8.00 ± 1.0	16.00 ± 2.0	15.67 ± 0.6	31.33 ± 1.2

The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

VII-9: Effect of 12 h soaking in IAA on seed germination in *Pterocarpus marsupium* Roxb.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
5 Days	Control	2.67 ± 1.5	5.33 ± 3.1	2.67 ± 1.5	5.33 ± 3.1
	2.5 mM IAA	2.67 ± 0.6	5.33 ± 1.2	2.67 ± 0.6	5.33 ± 1.2
	5.0 mM IAA	2.00 ± 1.0	4.00 ± 2.0	2.00 ± 1.0	4.00 ± 2.0
	7.5mM IAA	1.00 ± 1.0	2.00 ± 2.0	1.00 ± 1.0	2.00 ± 2.0
	10.0 mM IAA	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
10 Days	Control	7.00 ± 1.0	14.00 ± 2.0	9.67 ± 0.6	19.33 ± 1.2
	2.5 mM IAA	5.67 ± 0.6	11.33 ± 1.2	8.33 ± 1.2	16.67 ± 2.3
	5.0 mM IAA	5.00 ± 1.7	10.00 ± 3.5	7.00 ± 1.0	14.00 ± 2.0
	7.5mM IAA	3.33 ± 0.6	6.67 ± 1.2	4.33 ± 0.6	8.67 ± 1.2
	10.0 mM IAA	1.00 ± 1.0	2.00 ± 2.0	1.00 ± 1.0	2.00 ± 2.0
15 Days	Control	11.00 ± 1.0	22.00 ± 2.0	20.67 ± 0.6	41.33 ± 1.2
	2.5 mM IAA	11.67 ± 0.6	23.33 ± 1.2	20.00 ± 1.7	40.00 ± 3.5
	5.0 mM IAA	10.00 ± 1.7	20.00 ± 3.5	17.00 ± 2.6	34.00 ± 5.3
	7.5mM IAA	12.00 ± 1.0	24.00 ± 2.0	16.33 ± 0.6	32.67 ± 1.2
	10.0 mM IAA	14.33 ± 2.1	28.67 ± 4.2	15.33 ± 2.9	30.67 ± 5.8

The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

VII-9b: Effect of GA_3 on amylase activity in seeds of *P. marsupium* Roxb.

Duration of treatment	Treatment	α- amylase (µg starch hydrolyzed min^{-1} mg^{-1})	β- amylase (µg starch hydrolyzed min^{-1} mg^{-1})
12 h	Control	11.67 ± 1.5	29.33 ± 3.1
	1 mM GA_3	13.72 ± 1.6	34.33 ± 1.2*
	2 mM GA_3	13.97 ± 1.4*	34.00 ± 2.0*
	3 mM GA_3	14.50 ± 1.6*	36.00 ± 2.0*
	4 mM GA_3	14.13 ± 2.0*	33.00 ± 0.0*
24 h	Control	12.11 ± 2.7	28.27 ± 2.1
	1 mM GA_3	14.34 ± 0.4*	44.33 ± 2.2*
	2 mM GA_3	16.33 ± 1.7*	45.56 ± 2.0*
	3 mM GA_3	17.56 ± 1.2*	47.12 ± 2.0*
	4 mM GA_3	16.21 ± 1.4*	45.22 ± 0.0*
36 h	Control	12.34 ± 2.7	28.12 ± 2.1
	1 mM GA_3	12.34 ± 0.4	29.56 ± 2.0
	2 mM GA_3	12.33 ± 1.7	29.12 ± 3.0
	3 mM GA_3	11.56 ± 1.2	28.34 ± 3.2
	4 mM GA_3	10.21 ± 1.4*	26.78 ± 2.4

Values represent mean ± SD of three independent experiments performed on Seeds of 2007 seed lot.
* = differ significantly from control as per Dunett's test at p = 0.05

VII-9c: Effect of cytokinins on amylase activity in seeds of *P. marsupium* Roxb.

Duration of treatment	Treatment	α- amylase (μg starch hydrolyzed min^{-1} mg^{-1})	β- amylase (μg starch hydrolyzed min^{-1} mg^{-1})
12 h	Control	12.11 ± 1.2	28.27 ± 2.3
	0.25 mM BA	13.34 ± 1.2	30.11 ± 2.3
	0.50 mM BA	13.64 ± 1.2	30.13 ± 2.5
	0.75 mM BA	13.30 ± 1.4	30.45 ± 2.8
	1.00 mM BA	13.29 ± 1.3	30.19 ± 2.8
24 h	Control	13.11 ± 1.2	28.89 ± 2.1
	0.25 mM BA	13.46 ± 1.2	30.13 ± 2.5
	0.50 mM BA	13.50 ± 1.2	30.45 ± 2.2
	0.75 mM BA	13.77 ± 1.4	30.89 ± 2.1
	1.00 mM BA	13.29 ± 1.3	30.39 ± 2.1
12 h	Control	12.44 ± 2.2	29.24 ± 2.8
	1 mM Kin	13.34 ± 1.2	30.11 ± 2.3
	2 mM Kin	13.64 ± 1.2	30.13 ± 2.5
	3 mM Kin	13.30 ± 1.4	30.45 ± 2.8
	4 mM Kin	13.29 ± 1.3	30.19 ± 2.8
	5 mM Kin	13.15 ± 1.2	28.27 ± 2.3
24 h	Control	12.98 ± 2.2	29.56 ± 2.8
	1 mM Kin	13.12 ± 2.1	29.11 ± 2.5
	2 mM Kin	13.15 ± 2.5	29.13 ± 2.5
	3 mM Kin	13.17 ± 2.6	30.11 ± 2.5
	4 mM Kin	13.11 ± 2.1	30.49 ± 2.5
	5 mM Kin	13.10 ± 2.2	29.17 ± 2.8

Values represent mean ± SD of three independent experiments performed on Seeds of 2007 seed lot.
* = differ significantly from control as per Dunett's test at p = 0.05

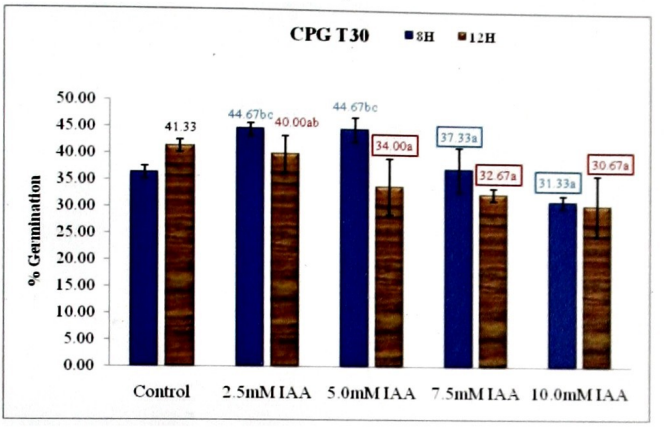
VII-9d: Effect of IAA on amylase activity in seeds of *P. marsupium* Roxb.

Duration of treatment	Treatment	α- amylase (µg starch hydrolyzed min^{-1} mg^{-1})	β- amylase (µg starch hydrolyzed min^{-1} mg^{-1})
8 h	Control	11.23 ± 2.5	26.12 ± 2.8
	2.5 mM IAA	10.93 ± 2.5	25.50 ± 2.7
	5.0 mM IAA	10.73 ± 2.8	24.12 ± 1.8
	7.5 mM IAA	10.23 ± 2.5	24.17 ± 1.9
	10.0 mM IAA	10.13 ± 2.5	24.10 ± 2.1*
12 h	Control	12.44 ± 2.2	29.24 ± 2.8
	2.5 mM IAA	11.44 ± 2.2	28.14 ± 2.8
	5.0 mM IAA	11.44 ± 2.2	26.87 ± 2.8
	7.5 mM IAA	10.44 ± 2.2	26.24 ± 2.8
	10.0 mM IAA	9.67 ± 2.2*	25.11 ± 2.8*

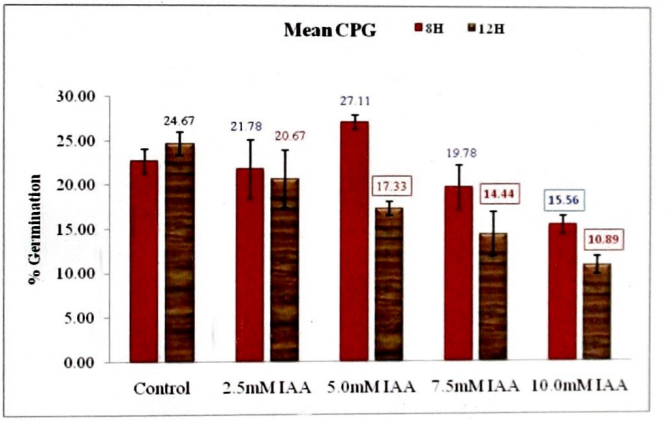
Values represent mean ± SD of three independent experiments performed on Seeds of 2007 seed lot.
* = differ significantly from control as per Dunett's test at p = 0.05

VII-4 Effect of IAA on seed germination in *Pterocarpus marsupium* Roxb.

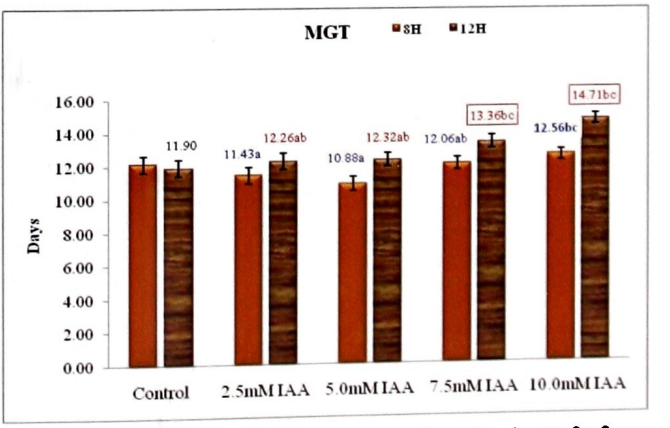
a) Cumulative percentage germination on 15th day



b) Mean Cumulative percentage germination



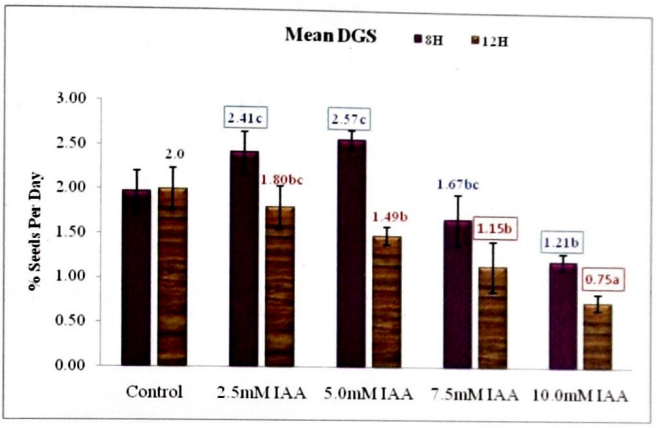
c) Mean germination time



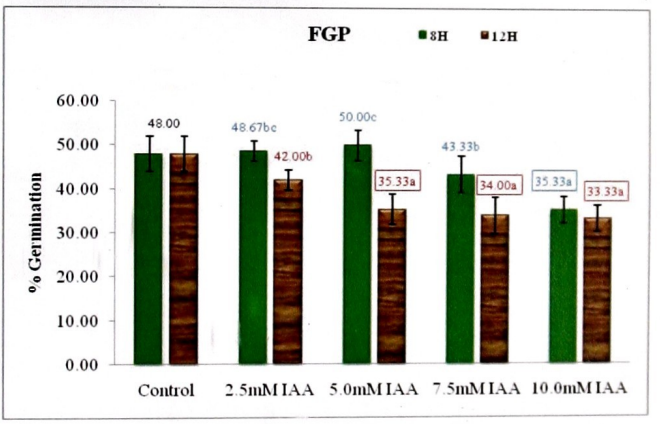
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

VII-4 Effect of IAA on seed germination in *Pterocarpus marsupium* Roxb.

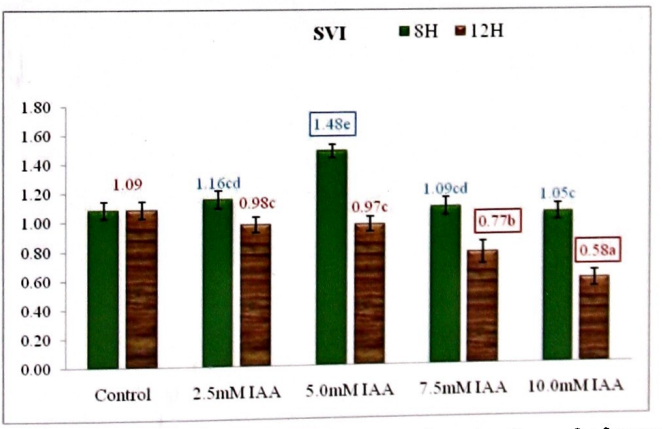
d) Mean daily germination speed



e) Final percentage germination on 30th Day



f) Seedling vigor



The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

The spread of germination (MGT) decreased in the seeds treated with 2.5 and 5.0 mM IAA and the higher concentrations resulted in higher MGT (Fig VII-4c). Likewise, the speed of germination (mean DGS) was improved up to 2.57% seeds per day in the seeds treated with 5.0 mM IAA as compared to 2.0% seeds per day in the untreated seeds (Fig VII-4d).

The seeds treated with 5.0 mM IAA exhibited marginally improved FGP of 50% as compared to 48% FGP in the untreated seeds. The treatment of 10.0 mM IAA resulted in suppressing the FGP significantly to 35.33% as compared to other treatments of IAA and the untreated seeds (Fig VII-4e).

The seed germination was restrained dramatically when the seeds were treated with 5.0-10.0 mM IAA for 12 h. Among these treatments, the treatment of lowest concentration of IAA (2.5 mM) was only marginally inhibitory.

There was a significant reduction in the CPG T15 to as low as 30.67% in the seeds treated with 10.0 mM IAA (Fig VII-4a). A similar trend was observed in the mean CPG which was significantly reduced in the seeds treated with 5.0-10.0 mM IAA, exhibiting lowest mean CPG of 10.89% in the seeds treated with 10.0 mM IAA (Fig VII-4b).

With increase in the concentration of IAA from 2.5 mM to 10.0 mM, there was gradual increase in the MGT and a significantly higher MGT of 14.71 days was observed in the seeds treated with 10.0 mM IAA as compared to 12.15 days in the untreated seeds (Fig VII-4c). The speed of germination (mean DGS) also declined progressively from 2.0% seeds per day in the untreated seeds to significantly lowered 1.15% and 0.75% seeds per day in the seeds treated with 7.5 mM and 10.0 mM IAA respectively (Fig VII-4d).

All the treatments of IAA for 12 h resulted in the suppressed germination as indicated by decrease in the FGP in all the treatments as compared to untreated seeds. The treatment of 10 mM IAA was the most

Plate VII-1: Seedlings of *Pterocarpus marsupium* Roxb. and *Santalum album* L. from seeds treated with GA_3

A. *Pterocarpus marsupium* Roxb.

B. *Santalum album* L

Plate VII-1

A

B

severe that significantly suppressed germination to 33.33% as compared to 48% in the untreated seeds. The seeds treated with 5.0 and 7.5 mM IAA also reduced the FGP significantly (Fig VII-4e).

The seedling vigor in the control seedlings was 1.09. There was rise in the seedling vigor in the seedlings obtained from the seeds treated with 2.5 and 5.0 mM IAA for 8 h. At higher concentration of IAA, however, there was gradual decline in the vigor (Fig VII-4f). The treatment of 5.0 mM IAA significantly improved the vigor. The seedling vigor was significantly reduced when the seeds were treated with 7.5 and 10.0 mM IAA for 12 h.

In general, the treatments of various concentrations of IAA given to the seeds of *Pterocarpus marsupium* suppressed the seed germination pattern as compared to the untreated seeds.

7.3.1.5 Activity of α- amylase

7.3.1.5.1 Effect of GA_3

The amylase activity in the seeds imbibed in different concentrations of GA_3, BA, Kin and IAA for different durations is presented in table VII-9b.

It was observed that the specific activity of α-amylase was 12.11 μg starch hydrolyzed min^{-1} mg^{-1} protein in the seeds imbibed for 24 h in distilled water without growth regulators. The seeds imbibed in GA_3 showed stimulation of α-amylase activity over the control. The activity of α-amylase increased linearly with increase in the GA_3 concentration up to 3 mM GA. Incorporation of higher concentration of GA_3 was inhibitory. The maximum α-amylase activity (17.56 μg starch hydrolyzed $min^{-1}mg^{-1}$ protein) was noticed in the seeds treated with 3 mM GA_3 for 24 h.

The seeds soaked in different concentrations of GA_3 solutions for 12 h also showed similar trend in the activity of α-amylase and maximum activity of 14.50 μg starch hydrolyzed min^{-1} $mg^{-1}$was

noticed in seeds treated with 3 mM GA_3. However, the seeds soaked in GA_3 solutions for 36 h showed slightly reduced activities as compared to seeds treated with the GA_3 solutions for 24 h.

Thus, the treatment of 3 mM GA for 24 h was most effective for stimulating the α-amylase activity in the seeds of *P. marsupium.* The stimulation of α-amylase activity might be the reason behind superior germination pattern in the seeds treated with 3 mM GA_3 for 24 h

7.3.1.5.2 Effect of BA and Kin

The results depicted in the table VII-9c on the influence of BA and Kin on α-amylase activity indicate that the treatment of BA and Kin slightly induced the activity of α-amylase. However, the activity of this enzyme in the treated seeds was not significantly higher than that observed in the seeds soaked in distilled water. The treatment of higher concentration of Kin and BA was inhibitory to α-amylase activity and germination of seeds.

7.3.1.5.3 Effect of IAA

The results depicted in the table VII-9d on the influence of IAA on α-amylase activity indicate that the exogenous application of IAA was inhibitory to the amylase activity. With increase in the concentration of IAA, there was gradual decrease in the α-amylase activity. The untreated seeds soaked for 12 h in distilled water showed the specific activity of 12.44 μg starch hydrolyzed min^{-1} mg^{-1} protein whereas the seeds imbibed in the 10 mM IAA for 12 h showed significantly reduced activity of the magnitude 9.67 μg starch hydrolyzed min^{-1} mg^{-1} protein.

7.3.1.6 Activity of β- amylase

7.3.1.6.1 Effect of GA_3

The results recorded in table VII-9b showed that the β-amylase activity also revealed trend similar to the α-amylase activity with different concentrations and durations of GA_3 used to treat the seeds. However, the activity of β-amylase was higher than the activity of α-amylase. The maximum activity of 47.12 μg starch hydrolyzed min^{-1} mg^{-1} protein was observed in the seeds imbibed with 3 mM GA_3 for 24 h. This might be the reason behind the maximum seed germination in the seeds treated with 3 mM GA_3 for 24 h.

7.3.1.6.2 Effect of BA and Kin

The β-amylase activity (Table VII-9c) in the seeds treated with different concentrations of BA and Kin for different durations was higher than that observed in the untreated control seeds. In case of BA, the maximum activity was observed in the seeds treated with 0.75 mM BA for 24 h, whereas, for Kin, it was observed in the seeds treated with 4 mM Kin for 24 h. Though there was induction in the amylase activity in the treated seeds, the increment was statistically insignificant.

7.3.1.6.3 Effect of IAA

Similar to the α-amylase activity, the exogenous application of IAA resulted in suppression of the β-amylase activity and with increase in the concentration of IAA and duration of soaking; there was gradual decrease in the β-amylase activity (Table VII-9d).

7.3.2 *Santalum album* L.

7.3.2.1 Effect of GA

The presoaking of the seeds in 1-5mM GA_3 for 12 h and 24 h substantially improved seed germination pattern in *S. album* (Table

VII-10: Effect of 12 h soaking in GA_3 on seed germination in *Santalum album* L.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
10 Days	Control	1.33 ± 0.6	2.67 ± 1.2	1.33 ± 0.6	2.67 ± 1.2
	1 mM GA_3	2.00 ± 1.0	4.00 ± 2.0	2.00 ± 1.0	4.00 ± 2.0
	2 mM GA_3	2.67 ± 0.6	5.33 ± 1.2	2.67 ± 0.6	5.33 ± 1.2
	3 mM GA_3	3.33 ± 0.6	6.67 ± 1.2	3.33 ± 0.6	6.67 ± 1.2
	4 mM GA_3	2.67 ± 0.6	5.33 ± 1.2	2.67 ± 0.6	5.33 ± 1.2
	5 mM GA_3	3.33 ± 0.6	6.67 ± 1.2	3.33 ± 0.6	6.67 ± 1.2
20 Days	Control	3.67 ± 0.5	6.33 ± 1.5	5.00 ± 0.0	10.00 ± 0.0
	1 mM GA_3	4.33 ± 1.5	8.67 ± 3.1	6.33 ± 2.5	12.67 ± 5.0
	2 mM GA_3	6.33 ± 0.6	12.67 ± 1.2	9.00 ± 1.0	18.00 ± 2.0
	3 mM GA_3	6.33 ± 1.2	12.67 ± 2.3	9.67 ± 1.5	19.33 ± 3.1
	4 mM GA_3	7.67 ± 1.5	15.33 ± 3.1	10.33 ± 1.5	20.67 ± 3.1
	5 mM GA_3	9.00 ± 0.0	18.00 ± 0.0	12.33 ± 0.6	24.67 ± 1.2
30 Days	Control	8.33 ± 2.1	16.67 ± 4.2	13.33 ± 2.3	26.67 ± 4.6
	1 mM GA_3	9.33 ± 1.2	18.67 ± 2.3	15.67 ± 1.5	31.33 ± 3.1
	2 mM GA_3	6.67 ± 0.6	13.33 ± 1.2	15.67 ± 1.5	31.33 ± 3.1
	3 mM GA_3	6.67 ± 2.1	13.33 ± 4.2	16.33 ± 0.6	32.67 ± 1.2
	4 mM GA_3	6.00 ± 1.7	12.00 ± 3.5	16.33 ± 2.5	32.67 ± 5.0
	5 mM GA_3	7.33 ± 0.6	14.67 ± 1.2	19.67 ± 1.2	39.33 ± 2.3

The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

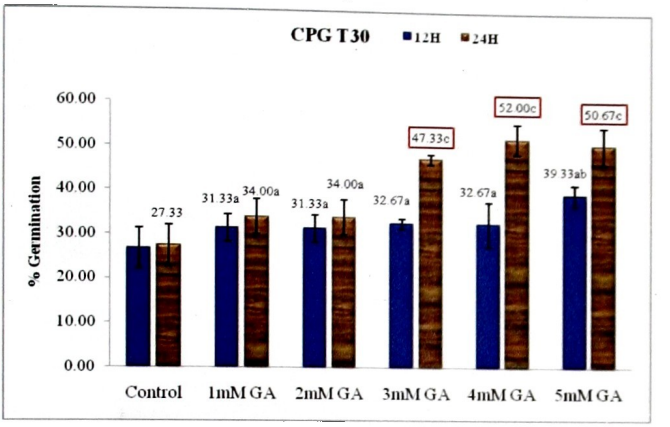
VII-11: Effect of 24 h soaking in GA_3 on seed germination in *Santalum album* L.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
10 Days	Control	1.33 ± 0.6	2.67 ± 1.2	1.33 ± 0.6	2.67 ± 1.2
	1 mM GA_3	2.33 ± 0.6	4.67 ± 1.2	2.33 ± 0.6	4.67 ± 1.2
	2 mM GA_3	3.00 ± 0.0	6.00 ± 0.0	3.00 ± 0.0	6.00 ± 0.0
	3 mM GA_3	5.33 ± 0.6	10.67 ± 1.2	5.33 ± 0.6	10.67 ± 1.2
	4 mM GA_3	5.00 ± 0.0	10.00 ± 0.0	5.00 ± 0.0	10.00 ± 0.0
	5 mM GA_3	6.67 ± 1.5	13.33 ± 3.1	6.67 ± 1.5	13.33 ± 3.1
20 Days	Control	3.00 ± 0.0	6.00 ± 0.0	4.33 ± 0.6	8.67 ± 1.2
	1 mM GA_3	5.33 ± 1.5	10.67 ± 3.1	7.67 ± 2.1	15.33 ± 4.2
	2 mM GA_3	7.33 ± 1.5	14.67 ± 3.1	10.33 ± 1.5	20.67 ± 3.1
	3 mM GA_3	9.33 ± 3.8	18.67 ± 7.6	14.67 ± 4.2	29.33 ± 8.3
	4 mM GA_3	13.33 ± 0.6	26.67 ± 1.2	18.33 ± 0.6	36.67 ± 1.2
	5 mM GA_3	14.00 ± 0.0	28.00 ± 0.0	20.67 ± 1.5	41.33 ± 3.1
30 Days	Control	9.33 ± 2.1	18.67 ± 4.2	13.67 ± 2.3	27.33 ± 4.6
	1 mM GA_3	9.33 ± 0.6	18.67 ± 1.2	17.00 ± 2.0	34.00 ± 4.0
	2 mM GA_3	6.67 ± 0.6	13.33 ± 1.2	17.00 ± 2.0	34.00 ± 4.0
	3 mM GA_3	9.00 ± 3.6	18.00 ± 7.2	23.67 ± 0.6	47.33 ± 1.2
	4 mM GA_3	7.67 ± 1.5	15.33 ± 3.1	26.00 ± 1.7	52.00 ± 3.5
	5 mM GA_3	4.67 ± 0.6	9.33 ± 1.2	25.33 ± 2.1	50.67 ± 4.2

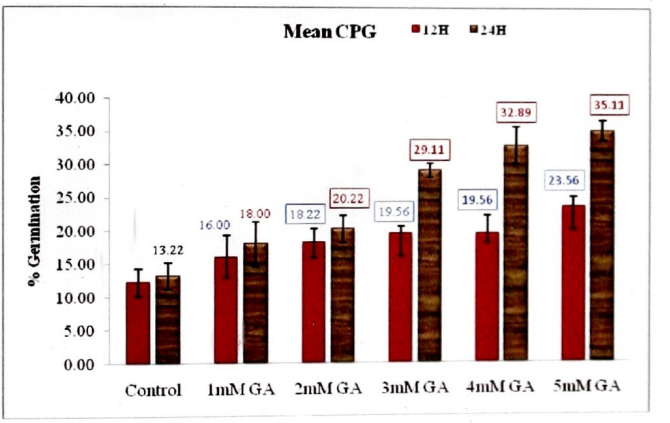
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

VII-5 Effect of GA_3 on seed germination in *Santalum album* L.

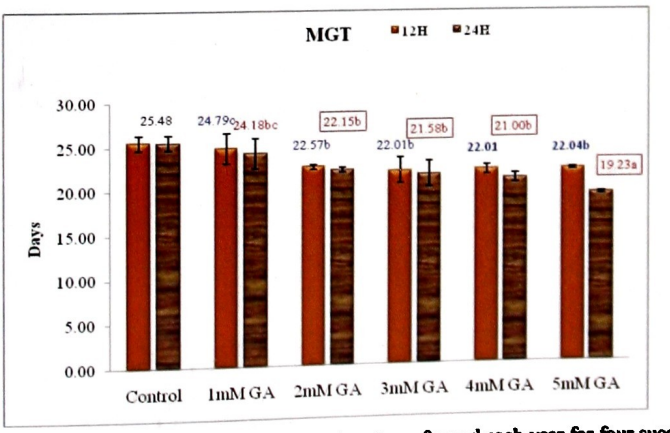
a) Cumulative percentage germination on 30th day



b) Mean Cumulative percentage germination



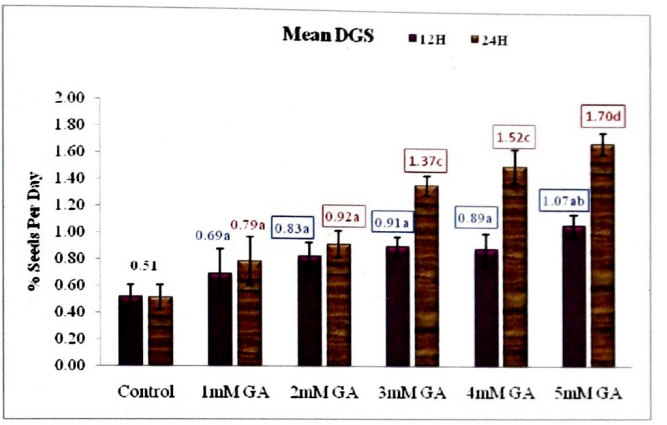
c) Mean germination time



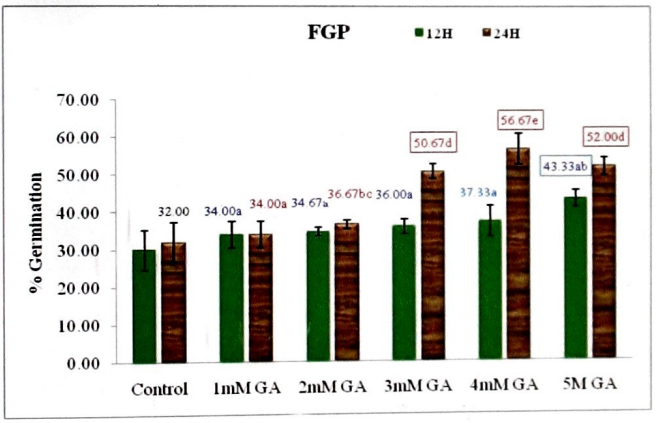
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

VII-5 Effect of GA_3 on seed germination in *Santalum album* L.

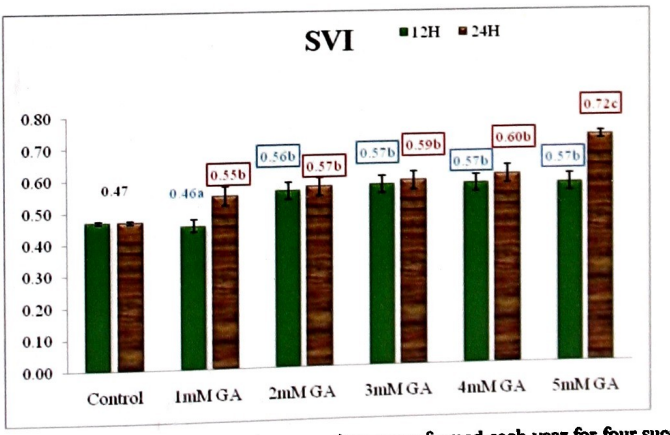
d) Mean daily germination speed



e) Final percentage germination on 30th Day



f) Seedling vigor



The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

VII-10, VII-11 and Fig VII-5a-f and Plate VII-1). The seeds treated with 1-5 mM GA_3 showed more germination as compared to the best control response. This was reflected in the higher CPG T30 which was significantly enhanced to 39.33% in the seeds treated with 5 mM GA_3, as compared to 27.33% in the untreated seeds (Fig VII-5a). The treatments of 2-5 mM GA_3 given for 12 h significantly improved mean CPG (Fig VII-5b). The highest mean CPG (23.56%) was observed in the seeds treated with 5 mM GA_3.

There was gradual decline in the MGT with increase in the concentration of GA_3. The lowest MGT (22.01 days) was observed in the seeds treated with 3 and 4 mM GA_3 as compared to untreated seeds where MGT was 25.48 days (Fig VII-5c). The mean DGS was more or less similar to that of untreated seeds. However, the 2-5 mM GA_3 treatment significantly enhanced the mean DGS and the maximum increment of about 100% was noticed in the seeds treated with 5mM GA_3 for 12 h (Fig VII-5d).

The FGP in the untreated seeds was 32% whereas in the 1-4 mM GA_3 treated seeds; it remained in the range of 34-38%. The FGP was significantly improved in the seeds treated with 5mM GA_3 (Fig VII-5e, 43.33%).

The germination was considerably increased in the seeds treated with 1-5 mM GA_3 for 24 h. There was boost in the CPG T30 in the seeds treated with 3, 4 and 5 mM GA_3 (Fig VII-5a). These treatments significantly improved the CPG T30 to 67.33%, 70% and 50.67% respectively. The 5mM GA_3 was slightly inhibitory as compared to 1-4 mM GA_3 treatments. The mean CPG also revealed the same trend and there was increase in the mean CPG with increase in the concentration of GA_3 and maximum mean CPG (35.11%) was observed in the seed treated with 5 mM GA_3 (Fig VII-5b).

A significant decline in the spread of germination was observed in the seeds treated with 2-5 mM GA_3 (Fig VII-5c). The treatment

of 5 mM GA_3 was best that showed about 33% reduction in MGT. Likewise, the speed of germination was also significantly improved in the seeds treated with 2-5 mM GA_3. The maximum mean DGS of 1.7% seeds per day was obtained in the seeds treated with 5 mM GA_3 (Fig VII-5d).

The FGP was significantly improved in the seeds treated with 3, 4 and 5 mM GA_3 (Fig VII-5e). Maximum FGP (56.67%) was observed in the seeds treated with 4 mM GA_3. The seeds treated with 1 and 2 mM GA_3 showed slightly better FGP (34% and 34.67% respectively) than untreated seeds (FGP = 32%).

The seed germination pattern was improved in the seeds treated with 1-5 mM GA_3 for 12 h and the resultant seedlings were significantly more vigorous than the control seedlings (Fig VII-5). Seeds treated with 5 mM GA_3 produced seedlings with about 20% more vigor than the seedlings obtained from untreated seeds. In the seeds treated with 1-5 mM GA_3 for 24 h, the vigor was further boosted and about 53% increase in the vigor was observed in the seedlings obtained from seeds treated with 5 mM GA_3 for 24 h.

In conclusion, the seeds presoaked in 1-5 mM GA_3 for 12 and 24 h showed better germination behavior. Among these treatments, the treatment of 4 and 5 mM GA_3 were the most effective to enhance seed germination in *Santalum album.*

7.3.2.2 Influence of BA

The results on the effect of presoaking the seeds in 0.5 – 2.0 mM BA for 12 and 24 h are presented in the Table VII-12 and VII-13 respectively and Fig VII-6a-f.

Seeds presoaked in the 0.5 – 2.0 mM BA for 12 h showed improved germination pattern over the untreated seeds. The CPG T30 and mean CPG increased progressively with increase in the concentration of BA up to 1.5 mM (Fig VII-6a and b). The treatment of 2.0 mM BA restrained the germination. The maximum CPG T30 of 37.33% and mean

VII-12: Effect of 12 h soaking in BA on seed germination in *Santalum album* L.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
10 Days	Control	2.67 ± 0.6	5.33 ± 1.2	2.67 ± 0.6	5.33 ± 1.2
	0.5 mM BA	2.67 ± 0.6	5.33 ± 1.2	2.67 ± 0.6	5.33 ± 1.2
	1.0 mM BA	3.33 ± 0.6	6.67 ± 1.2	3.33 ± 0.6	6.67 ± 1.2
	1.5 mM BA	3.33 ± 0.6	6.67 ± 1.2	3.33 ± 0.6	6.67 ± 1.2
	2.0 mM BA	4.33 ± 0.6	8.67 ± 1.2	4.33 ± 0.6	8.67 ± 1.2
20 Days	Control	5.33 ± 1.5	10.67 ± 3.1	8.00 ± 2.0	16.00 ± 4.0
	0.5 mM BA	6.67 ± 1.5	13.33 ± 3.1	9.33 ± 2.1	18.67 ± 4.2
	1.0 mM BA	8.00 ± 2.0	16.00 ± 4.0	11.33 ± 2.1	22.67 ± 4.2
	1.5 mM BA	8.67 ± 0.6	17.33 ± 1.2	12.00 ± 1.0	24.00 ± 2.0
	2.0 mM BA	8.00 ± 0.0	16.00 ± 0.0	12.33 ± 0.6	24.67 ± 1.2
30 Days	Control	9.00 ± 1.0	18.00 ± 2.0	17.00 ± 2.6	34.00 ± 5.3
	0.5 mM BA	7.67 ± 0.6	15.33 ± 1.2	17.00 ± 2.0	34.00 ± 4.0
	1.0 mM BA	7.00 ± 1.0	14.00 ± 2.0	18.33 ± 1.2	36.67 ± 2.3
	1.5 mM BA	6.67 ± 1.2	13.33 ± 2.3	18.67 ± 1.5	37.33 ± 3.1
	2.0 mM BA	3.67 ± 1.2	7.33 ± 2.3	16.00 ± 1.0	32.00 ± 2.0

The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

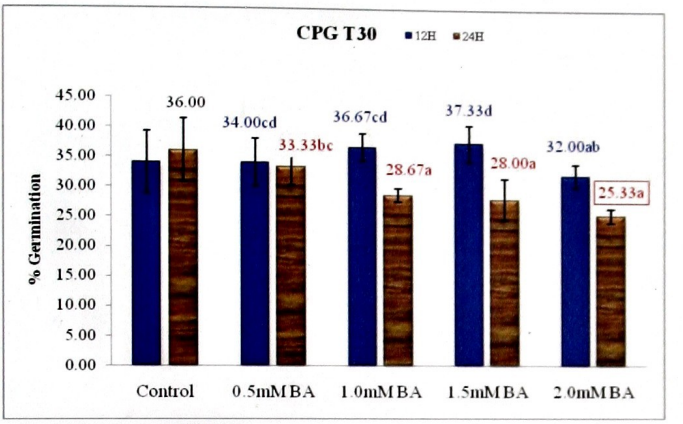
VII-13: Effect of 24 h soaking in BA on seed germination in *Santalum album* L.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
10 Days	Control	2.33 ± 0.6	4.67 ± 1.2	2.33 ± 0.6	4.67 ± 1.2
	0.5 mM BA	2.00 ± 1.0	4.00 ± 2.0	2.00 ± 1.0	4.00 ± 2.0
	1.0 mM BA	1.00 ± 0.0	2.00 ± 0.0	1.00 ± 0.0	2.00 ± 0.0
	1.5 mM BA	0.33 ± 0.6	0.67 ± 1.2	0.33 ± 0.6	0.67 ± 1.2
	2.0 mM BA	0.33 ± 0.6	0.67 ± 1.2	0.33 ± 0.6	0.67 ± 1.2
20 Days	Control	6.67 ± 1.5	13.33 ± 3.1	8.00 ± 2.0	16.00 ± 4.0
	0.5 mM BA	6.33 ± 1.5	12.67 ± 3.1	8.33 ± 2.1	16.67 ± 4.2
	1.0 mM BA	4.67 ± 0.6	9.33 ± 1.2	5.67 ± 0.6	11.33 ± 1.2
	1.5 mM BA	5.67 ± 1.5	11.33 ± 3.1	6.00 ± 1.0	12.00 ± 2.0
	2.0 mM BA	4.67 ± 0.6	9.33 ± 1.2	5.00 ± 1.0	10.00 ± 2.0
30 Days	Control	9.00 ± 1.0	18.00 ± 2.0	18.00 ± 2.6	36.00 ± 5.3
	0.5 mM BA	8.33 ± 1.2	16.67 ± 2.3	16.67 ± 1.5	33.33 ± 3.1
	1.0 mM BA	8.67 ± 0.6	17.33 ± 1.2	14.33 ± 0.6	28.67 ± 1.2
	1.5 mM BA	8.00 ± 1.0	16.00 ± 2.0	4.00 ± 1.7	28.00 ± 3.5
	2.0 mM BA	7.67 ± 1.2	15.33 ± 2.3	12.67 ± 0.6	25.33 ± 1.2

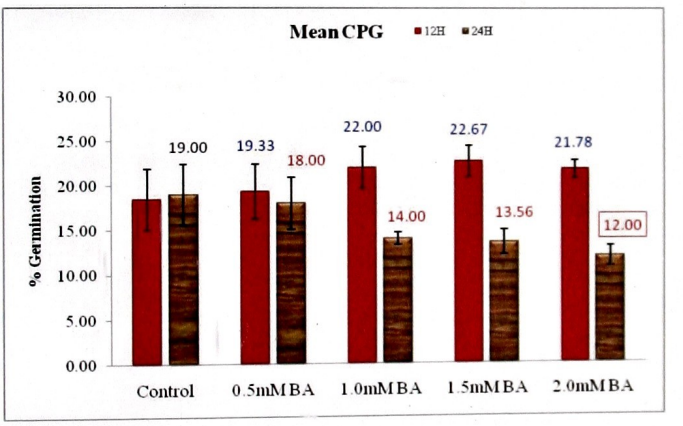
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

VII-6 Effect of BA on seed germination in *Santalum album* L.

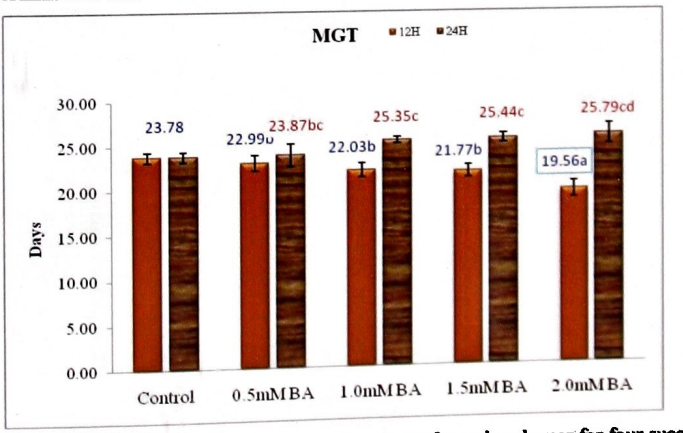
a) Cumulative percentage germination on 15th day



b) Mean Cumulative percentage germination



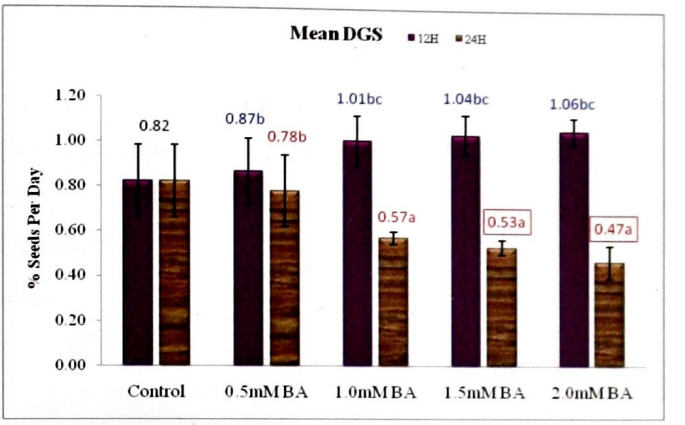
c) Mean germination time



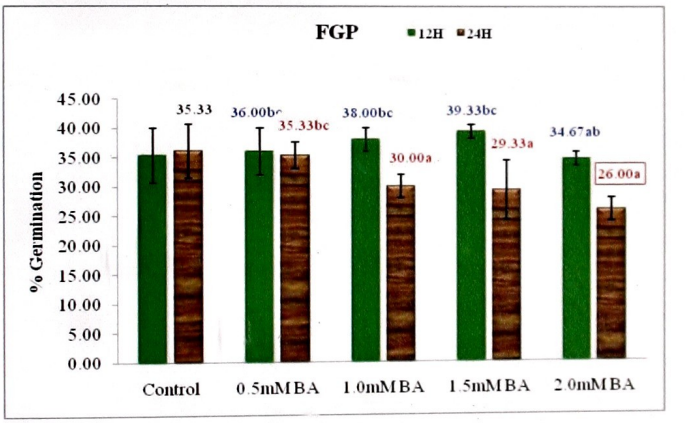
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

VII-6 Effect of BA on seed germination in *Santalum album* L.

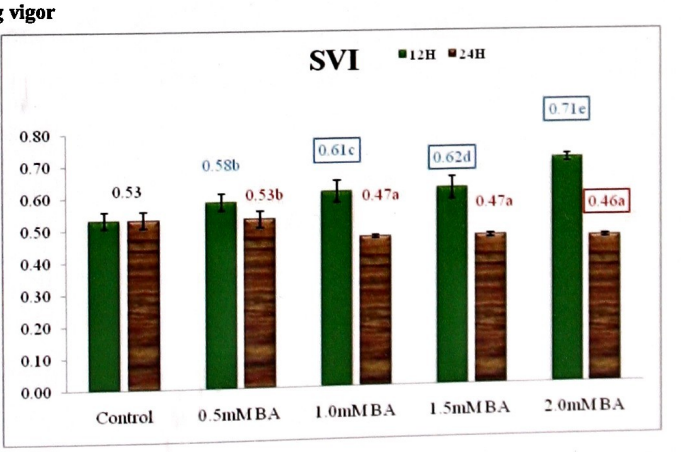
d) Mean daily germination speed



e) Final percentage germination on 30th Day



f) Seedling vigor



The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

CPG of 22.67% were recorded in the seeds treated with 1.5 mM BA for 12 h.

The spread of germination reduced gradually with increase in the concentration of BA. A significantly less spread (19.56 days) was observed in the seeds treated with 2.0 mM BA as compared to untreated seeds (MGT = 23.78 days , Fig VII-6c). Decrease in the MGT was coupled with non-significant increase in mean DGS from 0.82% seeds per day in the untreated seeds to 1.06% seeds per day in the seeds treated with 2.0 mM BA (Fig VII-6d).

The promoting effect of BA treatment was also reflected in the FGP which increased with increase in the concentration of BA up to 1.5 mM. The treatment of 1.5 mM BA showed 39.33% FGP as compared to 35.33% FGP in untreated seeds (Fig VII-6d). Seeds treated with 2.0 mM BA for 12 h, however, showed restrained germination.

The seeds treated with 0.5 – 2.0 mM BA for 24 h showed restrained germination pattern (Table VII-13). The inhibitory effect increased with increase in BA concentration.

There was gradual decline in CPG T30 up to significantly lower 25.33% in the seeds treated with 2.0 mM BA for 24 h as compared to 36% in the untreated seeds (Fig VII-6a). The mean CPG also exhibited the same trend and the seeds treated with 2.0 mM BA showed significantly lower mean CPG of 12% (Fig VII-6b).

The deleterious effect of 24 h BA treatments was reflected in the form of progressively increasing MGT from 23.78 days in the untreated seeds to 25.79 days in the seeds treated with 2.0 mM BA. This delayed germination was coupled to slow speed of germination, wherein a significant drop by 60% was observed in mean DGS in the seeds treated with 2.0 mM BA.

The final germination percentage (FGP) also decreased with increase in the concentration of BA and lowest percentage of 26% was

recorded in the seeds treated with 2.0 mM BA as compared to 35.33% FGP in the untreated seeds.

Among the different concentrations of BA used to prime the seeds of *Santalum album* for different durations, only the higher concentrations (1.0, 1.5 and 2.0 mM) used for shorter duration could significantly alter the seedling vigor compared to control seedlings. The seeds treated with 1.5 and 2.0 mM BA for 12 h produced the most vigorous seedlings with about 36% more vigor compared to those obtained from untreated seeds (Fig VII-6f).

On the basis of above results on the effect of presoaking of *Santalum album* seeds in BA solutions of different concentrations, the germination was substantially improved in the seeds presoaked in 1.5 mM and 2.0 mM BA for 12h.

7.3.2.3 Effect of Kin

The seed germination pattern in the seeds presoaked in 0.5 – 2.0 mM Kin for 12 h and 24 h is presented in the Table VII-14 and Table VII-15 respectively and Fig VII-7a-f.

The treatments of Kin for 12 h slightly improved the germination pattern in *S. album* and among the different concentrations used, 1.0 mM Kin was better for enhancing germination while higher concentrations inhibited the seed germination.

The seeds treated with 1.0 mM Kin showed highest CPG T30 of 32% and mean CPG of 18.67% (Fig VII-7a and b). The spread of the germination increased with increase in the concentration of Kin and seeds treated with 2.0 mM Kin showed highest MGT of 28.31 days as compared to 24.28 days in the untreated seeds (Fig VII-7c). The speed of germination also decreased with increase in the concentration of Kin. The lowest speed of 0.27% seeds per day was recorded in the seeds treated with 2.0 mM Kin as compared to 0.68% seeds per day in the untreated seeds (Fig VII-7d).

VII-14: Effect of 12 h soaking in Kin on seed germination in *Santalum album* L.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
10 Days	Control	1.33 ± 1.5	2.67 ± 3.1	1.33 ± 1.5	2.67 ± 3.1
	0.5 mM Kin	1.33 ± 1.5	2.67 ± 3.1	1.33 ± 1.5	2.67 ± 3.1
	1.0 mM Kin	2.33 ± 0.6	4.67 ± 1.2	2.33 ± 0.6	4.67 ± 1.2
	1.5 mM Kin	1.67 ± 0.6	3.33 ± 1.2	1.67 ± 0.6	3.33 ± 1.2
	2.0 mM Kin	1.00 ± 1.0	2.00 ± 2.0	1.00 ± 1.0	2.00 ± 2.0
20 Days	Control	5.33 ± 1.5	10.67 ± 3.1	6.67 ± 2.3	13.33 ± 4.6
	0.5 mM Kin	6.67 ± 0.6	13.33 ± 1.2	8.00 ± 1.0	16.00 ± 2.0
	1.0 mM Kin	7.33 ± 0.6	14.67 ± 1.2	9.67 ± 0.6	19.33 ± 1.2
	1.5 mM Kin	6.33 ± 1.2	12.67 ± 2.3	8.00 ± 1.7	16.00 ± 3.5
	2.0 mM Kin	5.00 ± 0.0	10.00 ± 0.0	6.00 ± 1.0	12.00 ± 2.0
30 Days	Control	7.67 ± 1.2	15.33 ± 2.3	14.33 ± 3.1	28.67 ± 6.1
	0.5 mM Kin	7.33 ± 1.5	14.67 ± 3.1	15.33 ± 2.1	30.67 ± 4.2
	1.0 mM Kin	6.33 ± 0.6	12.67 ± 1.2	16.00 ± 1.0	32.00 ± 2.0
	1.5 mM Kin	7.67 ± 1.5	15.33 ± 3.1	15.67 ± 1.2	31.33 ± 2.3
	2.0 mM Kin	5.33 ± 0.6	10.67 ± 1.2	11.33 ± 1.2	22.67 ± 2.3

The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

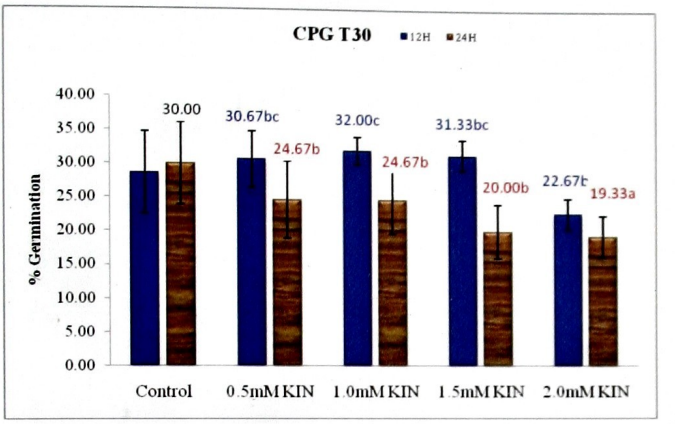
VII-15: Effect of 24 h soaking in Kin on seed germination in *Santalum album* L.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
10 Days	Control	2.00 ± 1.0	4.00 ± 2.0	2.00 ± 1.0	4.00 ± 2.0
	0.5 mM Kin	1.67 ± 1.2	3.33 ± 2.3	1.67 ± 1.2	3.33 ± 2.3
	1.0 mM Kin	0.67 ± 0.6	1.33 ± 1.2	0.67 ± 0.6	1.33 ± 1.2
	1.5 mM Kin	0.33 ± 0.6	0.67 ± 1.2	0.33 ± 0.6	0.67 ± 1.2
	2.0 mM Kin	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
20 Days	Control	6.33 ± 1.5	12.67 ± 3.1	8.33 ± 2.3	16.67 ± 4.6
	0.5 mM Kin	5.00 ± 2.0	10.00 ± 4.0	6.67 ± 2.3	13.33 ± 4.6
	1.0 mM Kin	3.67 ± 1.5	7.33 ± 3.1	4.33 ± 2.1	8.67 ± 4.2
	1.5 mM Kin	3.67 ± 1.5	7.33 ± 3.1	4.00 ± 2.0	8.00 ± 4.0
	2.0 mM Kin	1.67 ± 0.6	3.33 ± 1.2	1.67 ± 0.6	3.33 ± 1.2
30 Days	Control	7.33 ± 1.2	14.67 ± 2.3	15.67 ± 2.3	30.33 ± 6.1
	0.5 mM Kin	5.67 ± 0.6	11.33 ± 1.2	12.33 ± 2.9	24.67 ± 5.8
	1.0 mM Kin	8.00 ± 1.0	16.00 ± 2.0	12.33 ± 2.5	24.67 ± 5.0
	1.5 mM Kin	6.00 ± 0.0	12.00 ± 0.0	10.00 ± 2.0	20.00 ± 4.0
	2.0 mM Kin	8.00 ± 1.0	16.00 ± 2.0	9.67 ± 1.5	19.33 ± 3.1

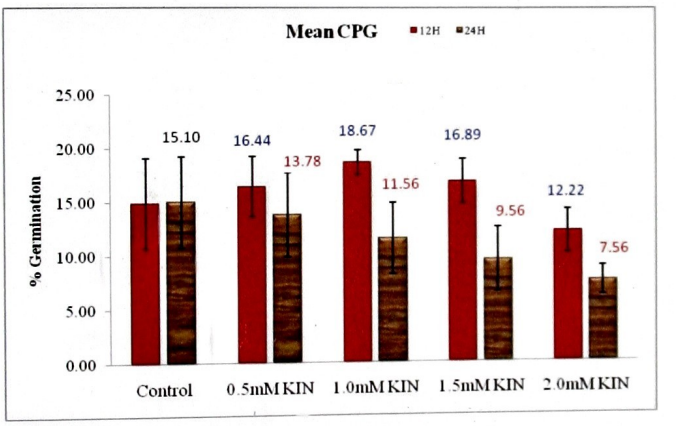
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

VII-7 Effect of Kin on seed germination in *Santalum album* L.

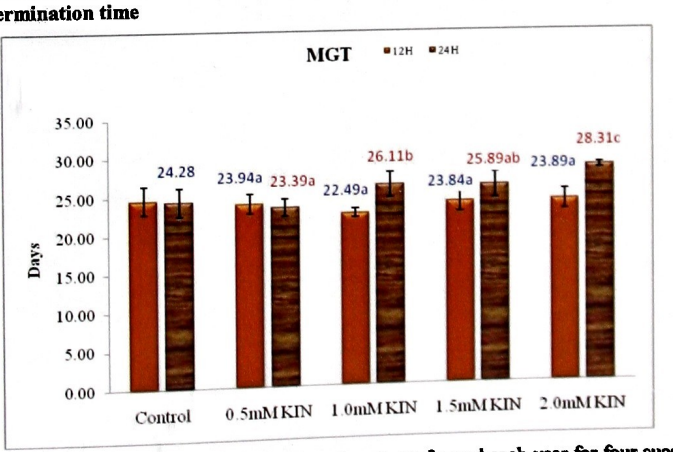
a) Cumulative percentage germination on 30th day



b) Mean Cumulative percentage germination



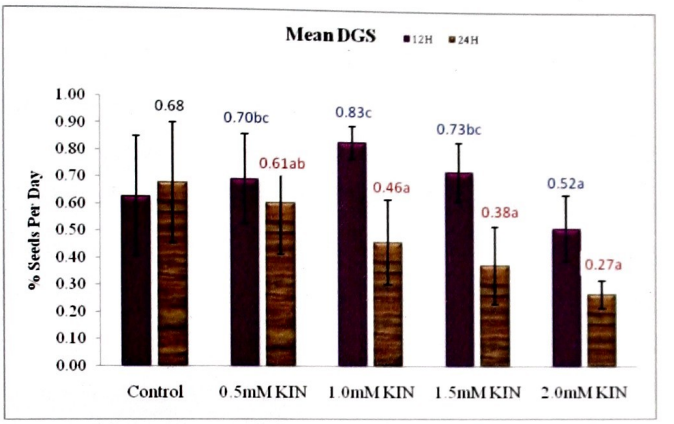
c) Mean germination time



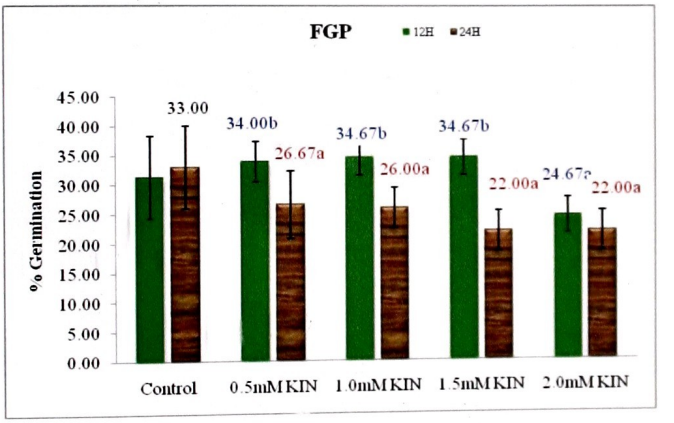
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

VII-7 Effect of Kin on seed germination in *Santalum album* L.

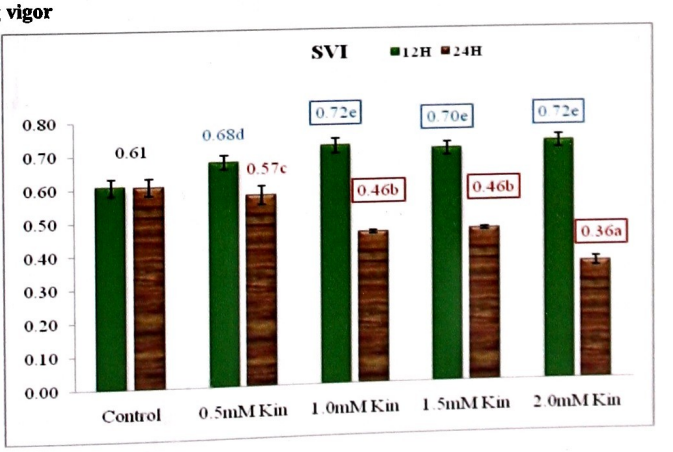
d) Mean daily germination speed



e) Final percentage germination on 30th Day



f) Seedling vigor



The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

The restrained germination was also reflected in the FGP which decreased with increase in the concentration of Kin. The seeds treated with 2.0 mM Kin showed least FGP of 22% as compared to 33% in the untreated seeds.

The seeds exposed to 0.5mM – 2.0 mM Kin for longer duration (24 h) hindered germination in *Santalum album.* With increase in the concentration of Kin, there was gradual decline in the germination. The treatment of 2.0 mM Kin was the most detrimental for germination. Seeds from this treatment showed 33%, 100% and 29% decline in the values of CPG T30, mean DGS and FGP respectively from the untreated seeds.

The treatment of seeds with 1.0, 1.5 and 2.0 mM Kin for 12 h significantly boosted the seedling vigor, but when the same treatments were given for 24 h, a dramatic decline in the vigor was observed (Fig VII-7f). The seeding vigor was almost same in the seedlings obtained from seeds treated with 1.0, 1.5 and 2.0 mM Kin for 12. The seedling obtained from the seeds treated with 2.0 mM Kin for 24 h produced seedlings with about 60% less vigor than the control seedlings.

In summary, the *Santalum album* seeds treated with 1.0 and 1.5 mM Kin for 12 h substantially improved the seed germination behavior. The lower levels of Kin applied for the same duration were not much effective, whereas the higher levels as well as longer exposure to 0.5-2.0 mM Kin hindered the seed germination.

7.3.2.4 Effect of IAA

The seed germination pattern in the seeds presoaked in 0.5 – 2.0 mM IAA for 12 h and 24 h is presented in the Table VII-16 and Table VII-17 respectively and Fig VII-8a-f. The seed germination pattern was improved when the seeds were presoaked in the 0.5 and 1.0 mM IAA for 12 h. This was reflected in the significantly improved CPG T30 (Fig VII-8a) and mean CPG (Fig VII-8b) in the seeds treated with 1.0 mM IAA. However, none of the levels of IAA used for 12 h soaking could

VII-16: Effect of 12 h soaking in IAA on seed germination in *Santalum album* L.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
10 Days	Control	1.67 ± 0.6	3.33 ± 1.2	1.67 ± 0.6	3.33 ± 1.2
	0.5 mM IAA	2.00 ± 1.0	4.00 ± 2.0	2.00 ± 1.0	4.00 ± 2.0
	1.0 mM IAA	2.67 ± 0.6	5.33 ± 1.2	2.67 ± 0.6	5.33 ± 1.2
	1.5 mM IAA	2.33 ± 1.2	4.67 ± 2.3	2.33 ± 1.2	4.67 ± 2.3
	2.0 mM IAA	1.00 ± 0.0	2.00 ± 0.0	1.00 ± 0.0	2.00 ± 0.0
20 Days	Control	3.33 ± 0.6	6.67 ± 1.2	5.00 ± 1.0	10.00 ± 2.0
	0.5 mM IAA	5.00 ± 1.0	10.00 ± 2.0	7.00 ± 1.7	14.00 ± 3.5
	1.0 mM IAA	6.33 ± 0.6	12.67 ± 1.2	9.00 ± 1.0	18.00 ± 2.0
	1.5 mM IAA	4.00 ± 1.7	8.00 ± 3.5	6.33 ± 2.9	12.67 ± 5.8
	2.0 mM IAA	5.00 ± 1.7	10.00 ± 3.5	6.00 ± 1.7	12.00 ± 3.5
30 Days	Control	9.00 ± 2.6	18.00 ± 5.3	14.00 ± 3.5	28.00 ± 6.9
	0.5 mM IAA	11.67 ± 1.5	23.33 ± 3.1	18.67 ± 3.1	37.33 ± 6.1
	1.0 mM IAA	11.67 ± 1.5	23.33 ± 3.1	20.67 ± 1.2	41.33 ± 2.3
	1.5 mM IAA	9.33 ±1.5	18.67 ± 3.1	15.67 ± 1.5	31.33 ± 3.1
	2.0 mM IAA	6.67 ± 0.6	13.33 ± 1.2	12.67 ± 1.2	25.33 ± 2.3

The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

VII-17: Effect of 24 h soaking in IAA on seed germination in *Santalum album* L.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
10 Days	Control	2.00 ± 1.0	4.00 ± 2.0	2.00 ± 1.0	4.00 ± 2.0
	0.5 mM IAA	2.00 ± 1.0	4.00 ± 2.0	2.00 ± 1.0	4.00 ± 2.0
	1.0 mM IAA	1.33 ± 0.6	2.67 ± 1.2	1.33 ± 0.6	2.67 ± 1.2
	1.5 mM IAA	1.00 ± 1.0	2.00 ± 2.0	1.00 ± 1.0	2.00 ± 2.0
	2.0 mM IAA	0.33 ± 0.6	0.67 ± 1.2	0.33 ± 0.6	0.67 ± 1.2
20 Days	Control	6.33 ± 1.5	12.67 ± 3.1	8.33 ± 2.3	16.67 ± 4.6
	0.5 mM IAA	5.00 ± 1.0	10.00 ± 2.0	7.00 ± 1.7	14.00 ± 3.5
	1.0 mM IAA	6.33 ± 0.6	12.67 ± 1.2	7.67 ± 1.2	15.33 ± 2.3
	1.5 mM IAA	3.67 ± 1.5	7.33 ± 3.1	4.67 ± 2.5	9.33 ± 5.0
	2.0 mM IAA	4.67 ± 0.6	9.33 ± 1.2	5.00 ± 1.0	10.00 ± 2.0
30 Days	Control	8.33 ± 1.2	16.67 ± 2.3	16.67 ± 2.3	33.33 ± 6.1
	0.5 mM IAA	9.00 ± 2.0	18.00 ± 4.0	16.00 ± 3.6	32.00 ± 7.2
	1.0 mM IAA	8.00 ± 2.0	16.00 ± 4.0	15.67 ± 1.2	31.33 ± 2.3
	1.5 mM IAA	6.67 ± 0.6	13.33 ± 1.2	11.33 ± 3.1	22.67 ± 6.1
	2.0 mM IAA	6.00 ± 0.0	12.00 ± 0.0	11.00 ± 1.0	22.00 ± 2.0

The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

VII-17b Effect of GA_3 on amylase activity in seeds of *S. album* L.

Duration of treatment	Treatment	α- amylase (µg starch hydrolyzed min^{-1} mg^{-1})	β- amylase (µg starch hydrolyzed min^{-1} mg^{-1})
12 h	Control	21.54 ± 2.1	44.10 ± 3.3
	1 mM GA_3	22.54 ± 2.2	45.67 ± 3.5
	2 mM GA_3	22.89 ± 2.3	45.98 ± 3.5
	3 mM GA_3	23.11 ± 2.3*	46.34 ± 3.1
	4 mM GA_3	24.00 ± 2.0*	47.78 ± 3.1*
	5 mM GA_3	23.74 ± 2.0*	47.23 ± 3.2*
24 h	Control	21.59 ± 3.1	44.15 ± 2.3
	1 mM GA_3	23.12 ± 2.2	46.56 ± 3.1
	2 mM GA_3	23.23 ± 2.2	46.43 ± 2.5
	3 mM GA_3	25.56 ± 2.2*	48.34 ± 4.1*
	4 mM GA_3	26.23 ± 2.2*	48.67 ± 4.1*
	5 mM GA_3	25.89 ± 2.5*	47.16 ± 1.2*

Values represent mean ± SD of three independent experiments performed on Seeds of 2007 seed lot.
* = differ significantly from control as per Dunett's test at p = 0.05

VII-17c Effect of cytokinins on amylase activity in seeds of *S. album* L.

Duration of treatment	Treatment	α- amylase (µg starch hydrolyzed min^{-1} mg^{-1})	β- amylase (µg starch hydrolyzed min^{-1} mg^{-1})
12 h	Control	21.54 ± 2.1	44.10 ± 3.2
	0.5 mM BA	22.12 ± 2.0	45.13 ± 3.6
	1.0 mM BA	22.34 ± 2.4	45.19 ± 3.1
	1.5 mM BA	22.44 ± 2.1	45.23 ± 3.1
	2.0 mM BA	22.14 ± 2.3	45.15 ± 3.7
24 h	Control	21.54 ± 2.1	44.10 ± 3.2
	0.5 mM BA	22.34 ± 1.5	45.56 ± 3.6
	1.0 mM BA	22.56 ± 2.8	45.69 ± 3.1
	1.5 mM BA	22.56 ± 2.2	45.74 ± 3.1
	2.0 mM BA	22.57 ± 2.6	45.75 ± 3.7
12h	Control	21.54 ± 2.1	44.10 ± 3.2
	0.5 mM Kin	22.44 ± 2.0	45.62 ± 3.1
	1.0 mM Kin	22.67 ± 2.9	45.79 ± 3.5
	1.5 mM Kin	22.79 ± 3.5	45.78 ± 3.5
	2.0 mM Kin	22.64 ± 3.6	45.72 ± 3.3
24 h	Control	21.54 ± 2.0	44.10 ± 2.2
	0.5 mM Kin	22.64 ± 2.0	45.89 ± 3.6
	1.0 mM Kin	22.78 ± 2.0	45.91 ± 3.2
	1.5 mM Kin	22.69 ± 2.5	46.11 ± 3.8
	2.0 mM Kin	21.64 ± 2.1	45.12 ± 3.7

Values represent mean ± SD of three independent experiments performed on Seeds of 2007 seed lot.
* = differ significantly from control as per Dunett's test at p = 0.05

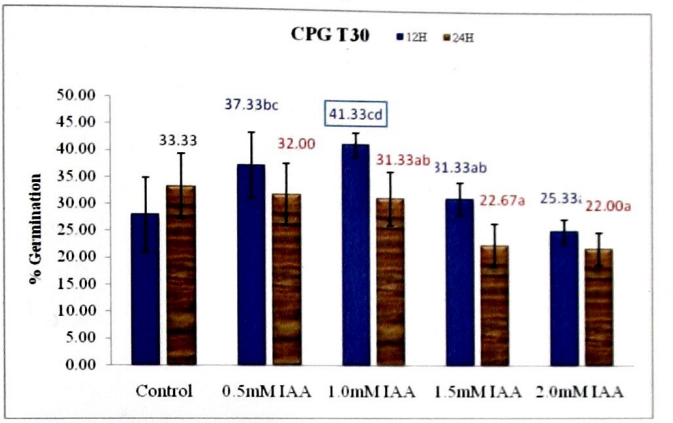
VII-17d Effect of IAA on amylase activity in seeds of *S. album* L.

Duration of treatment	Treatment	α- amylase (µg starch hydrolyzed min^{-1} mg^{-1})	β- amylase (µg starch hydrolyzed min^{-1} mg^{-1})
12 h	Control	21.54 ± 2.1	44.10 ± 3.3
	0.5 mM IAA	21.34 ± 4.1	43.99 ± 3.7
	1.0 mM IAA	21.24 ± 3.2	43.40 ± 3.2
	1.5 mM IAA	20.84 ± 4.7	43.11 ± 3.9
	2.0 mM IAA	19.64 ± 3.1*	43.00 ± 3.0
24h	Control	21.74 ± 2.1	44.90 ± 3.3
	0.5 mM IAA	21.10 ± 4.3	43.00 ± 3.5
	1.0 mM IAA	20.14 ± 4.2	43.10 ± 3.5
	1.5 mM IAA	19.74 ± 4.2*	41.11 ± 3.2*
	2.0 mM IAA	19.27 ± 4.1*	40.00 ± 3.1*

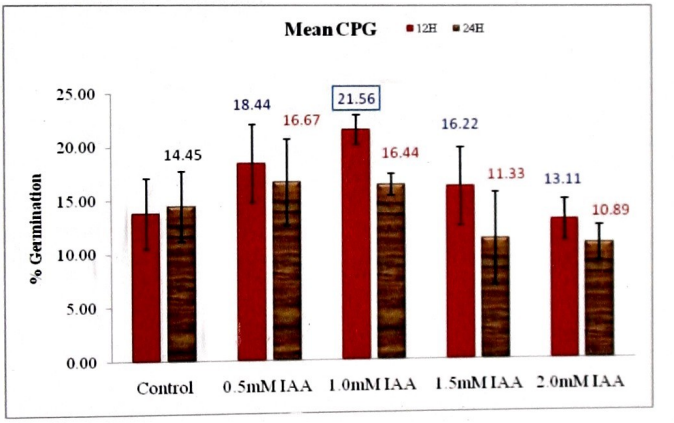
Values represent mean ± SD of three independent experiments performed on Seeds of 2007 seed lot.
* = differ significantly from control as per Dunett's test at p = 0.05

VII-8 Effect of IAA on seed germination in *Santalum album* L.

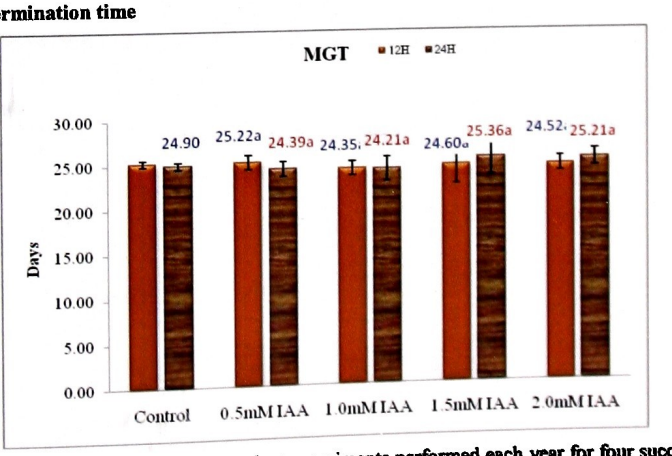
a) Cumulative percentage germination on 30th day



b) Mean Cumulative percentage germination



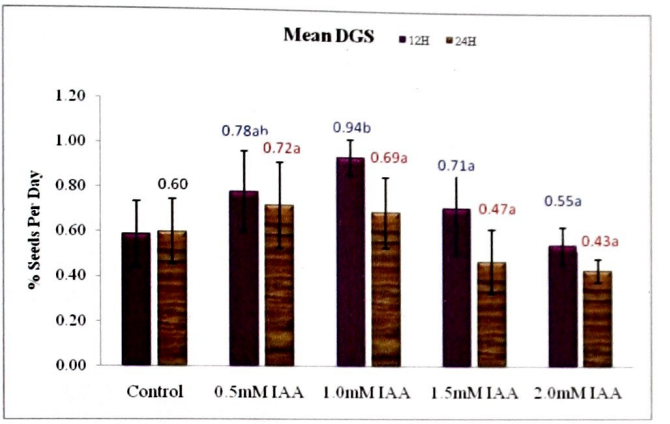
c) Mean germination time



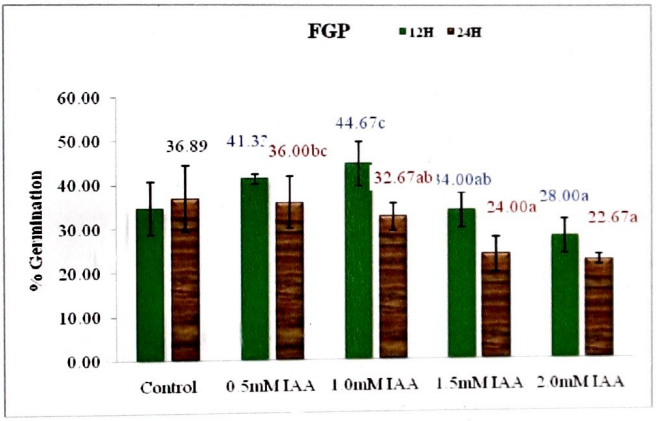
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

VII-8 Effect of IAA on seed germination in *Santalum album* L.

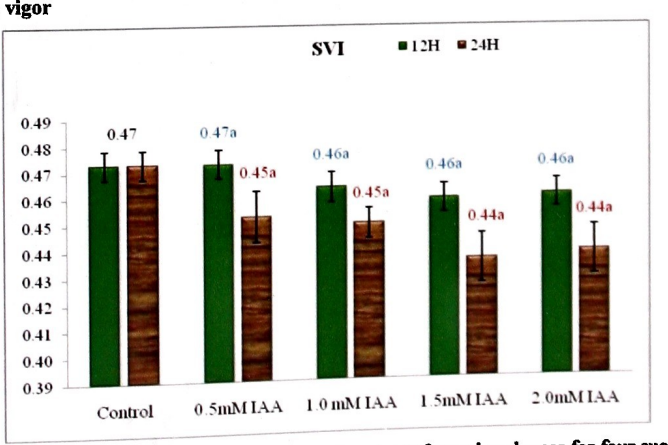
d) Mean daily germination speed



e) Final percentage germination on 60th Day



f) Seedling vigor



The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

significantly alter the speed of germination (Fig VII-8d) or the final germination percentage (Fig VII-8e). None of the treatments could reduce the spread of germination (Fig VII-8c). The higher concentration of IAA (1.5 and 2.0 mM) resulted in the restrained germination as was reflected by the lowering of CPG T30, mean DGS and FGP.

The seed germination pattern was slightly improved when the seeds were soaked in the 0.5 and 1.0 mM IAA for 24 h. But with higher concentration, there was a gradual decrease in the CPG T30, mean CPG, mean DGS and FGP indicating the delayed germination.

The seedling vigor in the seedling obtained from seeds presoaked in 0.5-2.0 mM IAA for 12 and 24 h did not deviated much from the control seedlings. All these seedlings show vigor in the range of 0.44 to 0.47 (Fig VII-8f).

In general, soaking the seeds of *Santalum album* in the solutions of IAA was not much helpful in improving the germination pattern. The lower concentrations used for less duration were slightly effective than the same concentrations given for longer duration or higher concentrations used for shorter or longer duration.

7.3.2.5 Activity of α- amylase

7.3.2.5.1 Effect of GA_3

The amylase activity in the seeds imbibed in different concentrations of $GA_3$for different durations is presented in table VII-17b.

In the seeds imbibed for 24 h in distilled water without growth regulators, the specific activity of α-amylase was 21.59 µg starch hydrolyzed min^{-1} mg^{-1} protein. The application of GA_3 resulted in stimulation of α-amylase activity over the control. There was linear increase in the activity of α-amylase increased with increased GA_3 concentration up to 4 mM GA and maximum α-amylase activity (26.23 µg starch hydrolyzed min^{-1} mg^{-1} protein) was recorded in the seeds treated

with 4 mM GA_3 for 24 h. However, the seeds soaked in GA_3 solutions for 36 h showed slightly reduced activities as compared to seeds treated with the GA_3 solutions for 24 h.

Thus, the treatment of 4 mM GA_3 for 24 h was the most effective for stimulating the α-amylase activity in the seeds of *S. album.*

7.3.2.5.2 Effect of BA and Kin

A marginal induction in the α-amylase activity was observed in the seeds treated with different concentrations of BA and Kin (Table VII-17c). The recorded activity of this enzyme in the treated seeds was not significantly higher than that observed in the seeds soaked in distilled water. The treatment of higher concentration of Kin and BA was inhibitory to α-amylase activity and germination of seeds.

7.3.2.5.3 Effect of IAA

The exogenous application of IAA was inhibitory to the amylase activity (Table VII-17c). With increase in the concentration of IAA, there was gradual decrease in the α-amylase activity from 21.74 μg starch hydrolyzed min^{-1} mg^{-1} protein in the untreated seeds to 19.64 μg starch hydrolyzed min^{-1} mg^{-1} protein in the seeds treated with 2 mM IAA for 24 h.

7.3.2.6 Activity of β- amylase

7.3.2.6.1 Effect of GA_3

In general, the activity of β-amylase was higher than the activity of α-amylase (Table VII-17b). The maximum activity of 47.78 μg starch hydrolyzed min^{-1} mg^{-1} protein was observed in the seeds imbibed with 4 mM GA_3 for 24 h. The seeds treated with higher concentration than this

showed suppressed activity. The seeds treated with 1-5 mM GA3 for 12 h also showed the similar trend.

7.3.2.6.2 Effect of BA and Kin

As compared to the untreated control seeds, the β-amylase activity was higher in the seeds treated with different concentrations of BA and Kin for different durations (Table VII-17c). In case of BA, the maximum activity of 45.75 μg starch hydrolyzed min^{-1} mg^{-1} protein was observed in the seeds treated with 2.0 mM BA for 24 h, whereas, for Kin it was 46.11 μg starch hydrolyzed min^{-1} mg^{-1} protein observed in the seeds treated with 1.5 mM Kin for 24 h. The induction in the β-amylase activity, however, was not significantly more than that observed in the seeds imbibed in distilled water.

7.3.2.6.3 Effect of IAA

The β-amylase activity decreased in the seeds treated with different concentrations of IAA (Table VII-17d). With increase in the concentration of IAA and duration of treatment, there was progressive decline in the enzyme activity. Thus, the highest activity (21.74 μg starch hydrolyzed min^{-1} mg^{-1} protein) was observed in the seeds treated with distilled water for 24 h.

7.4 Discussion

7.4.1 Effect of GA_3

The auxins, cytokinins, gibberellins, abscisic acid and ethylene are the major plant growth regulators that are involved in different phases of life of plants including seed germination. Among the plant hormones, gibberellins were the second group of plant hormones to be characterized and about 136 naturally occurring gibberellins are identified

so far (MacMillan, 2002, Taiz and Zeiger, 2006) and more than half of them have been identified in seeds (Bewley and Black, 1994). These molecules play a central role in the early germination processes by activating enzyme production and mobilizing storage reserves (Schmid, 2000). GA_3, the most active form of gibberellins (MacMillan, 2002) has the potential to regulate germination in several ways like elaboration of the endo-membrane system, and the synthesis of new mRNA types that can be used to regulate the protein synthesis required in germination (Jones and Stoddart, 1980). The role of gibberellins in the formation and secretion of many enzymes is the most thoroughly documented example of endogenously produced hormone which controls seed metabolism (Mayer and Shain, 1974). Exogenous treatments of gibberellins (usually gibberellic acid GA_3 and GA_{4+7}) have been shown to break dormancy in many seed species (Koyuncu, 2005).

In the present investigation, seeds of *Pterocarpus marsupium* presoaked in 1 and 2 mM GA_3 for 24 h (Fig VII-1a) showed a maximum increment of 78% in the CPG T15 and an increment of 105% in mean CPG over the best control response. The shortest MGT of 10.29 days was observed in the seeds treated with 4 mM GA_3 as against 11.51 days in the untreated seeds (Fig VII-1c). The highest speed (mean DGS) of 4.29% seeds per day was observed in the seeds treated with 4 mM GA_3 as compared to 1.94% seeds per day in untreated seeds. The FGP was significantly improved in the seeds treated with 3 and 4mM GA_3.

The germination in *Santalum album* was considerably improved in the seeds treated with 1-4 mM GA_3 for 24 h. The 5 mM GA_3 was slightly inhibitory as compared to 1-4 mM GA_3 treatments. A significant decline in the spread of germination was observed in the seeds treated with 2-5 mM GA_3 and the treatment of 5 mM GA_3 was best that showed about 33% reduction in MGT. Likewise, the speed of germination was also significantly improved in the seeds treated with 2-5 mM GA_3.

Maximum FGP (56.67%) was observed in the seeds treated with 4 mM GA_3.

On the basis of above results, the treatments of GA_3 facilitated seed germination in both *Pterocarpus marsupium* and *Santalum album.* These results are in accordance with the results reported in several other species. External application of gibberellins to seeds has broken seed dormancy and aided seedling establishment (Leubner Metzger, 1983; Pitel and Wang, 1988; Karssen *et al.*, 1989; Dunand, 1992; Lecat *et al.*, 1992). The exclusive role of gibberellins as the primary germination-promoting hormones has been demonstrated by Karssen *et al.* (1989).

Pre-treatment with GA_3 significantly enhanced germination of non-stratified black mulberry seeds (Koyuncu, 2005). GA_3 treatments at 1000 and 2000 mg/l concentrations yielded the highest germination percentage (60–67%) and increasing the concentration of GA_3 resulted in an increase in germination percentage. Pretreatment of blueberry seeds (*Vaccinium* sp.) with 100–500 mg/l GA_{4+7} accelerated germination (Ballington, 1984). It was reported that germination can be induced by gibberellic acid in *Vaccinium myrtillus* L. (Giba *et al.*, 1993), *Vaccinium corymbosum* L. (Dweikat and Lyrene, 1988) and *Fagus sylvatica* (Nicolás *et al.*, 1996) seeds. Gibberellic acid is known to break dormancy of several types of seeds including light-promoted seeds (*Lactuca sativa* L. var. Grand Rapids), light inhibited seeds (*Phacelia tanacetifolia* Benth), seeds requiring stratification (*Corylus avellana* L.) and seeds requiring storage at room temperature in dry condition (*Avena fatua* L.) (Chen and Chang, 1972).

Seeds of the same or different species may contain different levels of gibberellins, cytokinins and inhibitors leading to various depths of dormancy and therefore, should not be expected to give the same response to application of a gibberellin or cytokinins (Kabar, 1998). Application of gibberellic acid has been shown to enhance germination of hardwoods, but have limited effectiveness for conifer species (Leadem, 1987). *Pterocarpus*

marsupium belongs to Fabaceae while *Santalum album* is a member of family Santalaceae. In the present investigation, the best germination pattern in *P. marsupium* was observed in the seeds presoaked in the 3 mM GA_3 for 24 h while for *S. album,* seed treatment with 4 mM GA_3 for the same duration was required to give the best germination. It is likely that a specific quantity, rather than just the presence of free gibberellins, is required to break dormancy and stimulate germination.

Inhibitors of GA biosynthesis such as paclobutrazol and tetcyclasis prevent germination indicating a necessity of *de novo* biosynthesis of GAs during imbibition (Karssen *et al.*, 1989). Induction of expression of genes encoding enzymes hydrolyzing the endosperm which confers part of the mechanical resistance to radicle protrusion is a well known function of endogenous GA. This has been demonstrated in tomato (Groot and Karssen, 1987; Groot *et al.*, 1988), tobacco (Leubner- Metzger *et al.*, 1996), and barley (Schuurink *et al.*, 1992).

Gibberellins are synthesized in seeds (Bewley and Black, 1982) and their role in germination is thought to trigger hydrolysis of storage nutrients in seeds with a direct effect on the growth of the embryo (Karssen *et al.*, 1989). In the present investigation, the seeds of *P. marsupium* and *S. album* imbibed in the solution of GA_3 have shown increased activity of α-amylase as well as β-amylase. GA promotes α-amylase production for endosperm starch hydrolysis during seed germination and facilitates cell elongation in different organs and tissues throughout plant growth and development (Graebe, 1987; Karssen *et al.*, 1989). Although synthesis of amylases in cereal aleurone layers is controlled by gibberellins, Chen and Chang (1972) ruled out the possibility that GA stimulates germination via amylase production, because the sequence of events in enzyme development in germinating oat seeds showed that dormant wild oat seeds soaked in GA_3 germinated first; post-germination growth followed, and only then was an increase in amylase activity observed.

Some reports suggested that the weakening of the endosperm is necessary factor in induction of seed germination. Endosperm poses a mechanical constraint in the tip region of the radicle which contributed in the slow germination rate and weakening of the endosperm by externally applied GA occurred prior to germination (Watkins & Cantlife, 1983; Watkins *et al.*, 1985). Andreoli & Khan (1993) showed the pericarp in papaya seeds split along a suture after matriconditioning and that the loosening and dissolving of the cell wall were induced by exogenous applied GA and matriconditioning resulting in substantial increase in emergence. Another role of GA is to stimulate growth potential of the embryo, as suggested for *Arabidopsis* by Karssen and Lacka (1986).

Abscisic acid (ABA) produced in the embryo restricts its growth potential (Karssen *et al.*, 1983). ABA has been suggested to induce a dormant state during the later phases of seed maturation after which its function is limited because its concentration falls below an inhibiting level. GA is required to overcome this ABA-induced dormant state (Debeaujon and Koornneef, 2000). Hormones promoting germination, particularly gibberellin and cytokinin, have been thought to interact with inhibitors so as to allow germination (Bewley & Black, 1982) whereas Khan (1970) offered a view of the existence of a promoter-inhibitor balance in which the quantitative relation of regulating substances would determine the control of dormancy and seed germination.

The results recorded on the treatments of GA_3 revealed promotion of seed germination in both *P. marsupium* and *Santalum album.* The enhanced germination behavior might be due either the induction of hydrolytic enzymes, particularly amylases, which mobilized reserved food material during early phases of germination or due to counter balancing the effect of inhibitors present in the diaspore. The inhibitors present in the seed coat or embryonic axis either interrupt gene expression or render the enzymes inactive (Karssen *et al.*, 1987).

7.4.2 Effect of cytokinins

Cytokinins get their name from their ability to promote cell division but they also have numerous other effects. The cytokinins are one of the major plant growth regulators influencing various physiological and developmental processes including apical dominance, nutrient mobilization, formation and activity of shoot apical meristem, leaf senescence and breaking of bud dormancy and seed germination (Taiz and Zeiger, 2006). After the discovery of zeatin, the first cytokinin to be discovered, more than 30 cytokinins have been isolated so far. Exogenous treatments of cytokinins (usually kinetin, benzyladenine) have been shown to break dormancy in some seed species (Koyuncu, 2005).

6-Benzylaminopurine (BA) and kinetin (Kin) belongs to cytokinins group of hormones. The quantitative and qualitative responses of plants to different cytokinins may differ considerably (Kabar, 1998). The interaction between cytokinin and auxin is well ascertained in plant propagation: a high auxin/cytokinin ratio favors root development, whereas a high cytokinin/auxin ratio favors shoot development (Hartmann *et al.*, 1997).

Although seed germination is sometimes encouraged by the exogenous application of cytokinins, both germination and seedling development may be abnormal and seeds treated with cytokinins sometimes germinate with the shoot before the radicle (Bewley and Black 1994). However, in the present investigation, the seedlings of *P. marsupium* as well as *S. album* obtained from seeds treated with various concentrations of BA and Kin showed normal phenotypes similar to that of seedlings developed from untreated seeds.

The role of cytokinins in seed germination is not fully understood. Applied cytokinins (kinetin) have been reported to overcome dormancy in lettuce seed (Smith *et al.*, 1968). In *Ziziphus mauritiana* 100 ppm BA stimulated both total germination and vigor of seedlings (Murthy and Reddy, 1989).

Thomas *et al.* (1975) and Biddington and Thomas (1976) demonstrated that BA is more active than any other cytokinin in germination and in breaking dormancy of celery and lettuce seeds

In the present investigation, both the cytokinins BA and Kin had a very little effect in the activity of α and β-amylase in the seeds imbibed with BA and Kin solutions of different concentrations and for different durations. However, cytokinins have been thought to interact with inhibitors so as to allow germination (Bewley & Black, 1982). Mikulic and Vinter (2002) studied the effect of 1mg l^{-1} kinetin and 6-(3-methoxybenzylamino) purine-riboside on senescent seeds of *Dianthus superbus.* Both the cytokinin tested were a bit weaker stimulators increasing germination capacity over 10% only and the length of treatment with these growth substances didn't correlate positively. The results of the present investigation are on the same line as the lower concentrations of both BA and Kin only marginally improved percentage germination in *P. marsupium* (19% and 16% respectively) as well as *Santalum album* (11% and 10% respectively). The higher levels of BA and Kin were inhibitory for seed germination in both the species. Weinberger (1969) has shown that cytokinin application alone failed to alleviate germination behavior in *Prunus persica.*

7.4.3 Influence of IAA

Indoleacetic acid (IAA) is the major auxin in seeds found in free as well as bound forms. Lipe *et al.* (1969) has extracted and identified it from pecan, Hopper and Vozzo (1882) from *Quercus nigra* L., Michalski (1969) from *Q. robur* L. and Bewley and Black (1994) from *Prunus cerasus* L.

In the present investigation, exogenous application of IAA to the seeds of *Santalum album* was not much effective in alleviating the germination pattern as well as in boosting the percentage germination. The possibility that indole-3-acetic acid itself has an effect on germination

has long been unresolved. Koller (1962) indicated that the divergent results obtained with exogenous application of indole-3-acetic acid on seed may be related to the stage of dormancy. He postulated that this compound may stimulate germination only in very dormant seeds.

Jacobsen *et al.*, (1995) have described the involvement of phytohormones in the whole process of seed germination in *Hordeum vulgare.*

Yates and Curtis (1949) found that NAA stimulated the growth of roots and tillers in *Epidendrum nocturnum* seedlings but had no effect of seed germination. Salisbury and Ross (2000) opinioned that auxins do not promote seed germination in photo-latent and non-photo-latent seeds.

Seed germination in *Hygrophila auriculata* was inhibited in the dark, as well as in the light by exogenous indole-3-acetic acid (IAA) application at concentrations greater than 1×10^{-6} M (Amritphale *et al.*, 1992). Auxin induces the synthesis of ethylene which inhibits root growth. It has been demonstrated that when the biosynthesis of ethylene was blocked, low concentrations (10^{-10} to 10^{-9}M) of auxin promote the growth of intact roots, whereas higher concentration (10^{-6}M) inhibit growth (Taiz and Zeiger, 2006). The root cap contains small amounts of IAA and abscisic acid and IAA is more inhibitory to root growth than ABA when applied directly to the elongation zone, suggesting that IAA is a root cap inhibitor (Taiz and Zeiger, 2006). These might be the reasons behind the non promotion of seed germination in *Santalum* after exogenous application of IAA in the present investigation.

Chapter VIII

Influence of Seed Storage on Germination

8.1 Introduction

On the basis of storage behavior, Roberts (1973) categorized seeds as 'orthodox' and 'recalcitrant'. By the end of the process of seed development, the orthodox seeds undergo maturation drying (desiccation) on the parent plants as a terminal event in the seed development (Black, 1991; McCarty, 1995; Kermode, 1995). As the seed maturation progresses, the embryo reaches its lowest water content (as low as 10% of the original water remains), becomes desiccated, and enters a period of developmental and metabolic arrest (Leprince *et al.*, 1993; Bartels *et al.*, 1996).

On the other hand, recalcitrant seeds cannot withstand desiccation even when they reach maturity, and they do not survive drying which is normally associated with seed development and maturation (Chandler and Robertson, 1994). Viability in the recalcitrant seeds is lost if moisture levels are lower than those considered critical. The seed viability is completely lost when seed moisture is at or below the levels considered lethal (Probert & Longley, 1989; Pritchard, 1991; Hong & Ellis, 1992). The process of dehydration of plant cells can also be imposed environmentally (drought, cold, heat shocks) (Nedeva and Nikolova, 1997) or dehydration may occur during seed storage. Seed deterioration can be quantitatively defined as an increased probability of death of an individual seed as deterioration proceeds. Seed death is indicated by the failure to germinate and seed longevity is the period until seed death occurs (Tang *et al.*, 1999).

The loss of viability and vigor compromises the usefulness of seeds and can also affect their nutritional properties (Cortelazzo *et al.*, 2005). From productivity point of view, the total germination is as important as rapid and uniform germination. Seeds of many species deteriorate with storage time and age of seeds. In the present chapter we report the influence of storage duration on seed health, viability and germination in *P. marsupium* and *S. album*.

8.2 Materials and methods

8.2.1 *Pterocarpus marsupium* Roxb.

8.2.1.1 Seed storage

The fruits of *P. marsupium* were collected in the month of March 2004, March 2005, March 2006 and March 2007. The pods were cleaned and stored in polythene bags. Each bag contained 500 pods. The bags containing pods were placed in corrugated box and stored under ambient conditions.

Before the experiments, the wing of the fruit was cut with sharp scissors and such dewinged pods were considered as seed in the present investigation. Germination of the stored seeds was compared with germination in the fresh seeds collected in the year 2007. The seeds collected in the year 2004, 2005, 2006 and 2007 were termed as SL-04 (Seed Lot of 2004 season), SL-05, SL-06 and SL-07 respectively.

8.2.1.2 Seed viability

The viability of the stored and fresh seeds was determined by performing the TZ test that was conducted as described in 3.7.2.

8.2.1.3 Moisture content (MC)

The true seed was separated from the seed case. Three replicates of fifty seeds from each seed lot were used to determine the moisture content (MC) of seeds. The seed were weighed before and after drying at 60 °C in hot air oven till no further decline in the weight was observed (Sestak, 1971). The MC of seeds was expressed on fresh weight basis.

8.2.1.4 Seed leachates

Membrane permeability of stored and fresh seeds was studied by recording the electrical conductivity of seed leachates. To analyze the electrical conductivity in the untreated seeds, the true seed enclosed in the

central seed case was separated manually. After rinsing in distilled water, three replications of 10 g seeds from each seed lot (SL-04, SL-05, SL-06 and fresh seed lot SL-07) were soaked separately in 100 ml distilled water at 25 ^{0}C. The electrical conductivity in leachates from the seeds pre-soaked in 3 mM GA_3 for 24 h was analyzed after separating the true seed from the seed case and soaking 10 g seeds in 100 ml distilled water. Electrical conductivity (EC) of steep water was measured using conductivity meter (Elico India Ltd., Model CM 180) after 24 h of soaking and expressed as mS/cm (Basra *et al.*, 2002).

8.2.1.5 Amylase activity

The α- amylase (EC 3.2.1.1) and β- amylase (EC 3.2.1.2) activities were studied in the germinating seeds. These enzymes were extracted and assayed as described earlier in 7.2.3.

The results were expressed as mean ± standard deviation.

8.2.1.6 Analysis of PGRs

The GA_3 and indole-3-acetic acid (IAA) were analyzed by HPLC performed on a crude extract of SL-07 and SL-04 seeds.

8.2.1.6.1 Extraction of GA_3 and IAA

Twenty five milliliter ice cold methanol was added to 5 g seed sample powdered in liquid nitrogen. The sample thus processed was stored at 4 ^{0}C for 24 h in dark followed by homogenization by using tissue homogenizer. The resultant homogenate was filtered through Whatman No. 1 filter paper. The filtrate was condensed to 1 ml at 35 ^{0}C. Ten microlitre samples were subjected to HPLC analysis.

8.2.1.6.2 Assay of GA_3 and IAA

The chromatographic analysis was performed on a Shimadzu Model LC (UFLC Shimadzu). The equipment consist of LC-20 AD pump

with manual injector and SPD-20A uv-vis detector. The equipment has column oven CTO-20A and a degasser system DGU-20 A3. The column used was a Luna C_{18} (2) 100A of dimensions 250 mm × 4.6 mm and pore size of 5 μM (Phenomonex). The acetonitrile (30%) was used as a mobile phase. The flow rate was 0.8 ml min^{-1}. The wavelength used for detection was 208 nm for GA_3 and 280 nm for IAA (Kelen *et al.*, 2004). During the chromatographic separation, the column temperature was maintained at 29±1^{0}C. An injection volume of 10 μL was used for analysis. The standard GA_3 and IAA used were of HPLC grade (SRL India Ltd). The standards were prepared by dissolving the chemicals in the mobile phase. Ten microgram standard GA_3 and IAA was injected for analysis. Peak identification was based on retention time and quantification was based on peak area.

8.2.2 *Santalum album* L.

8.2.2.1 Seed storage

The fruits of *Santalum album* were collected in the month of April 2004 (SL-04), April 2005 (SL-05), April 2006 (SL-06) and April 2007 (SL-07) from naturally grown sandal trees on the campus of University of Pune, India. The drupes were cleaned and stored in polythene bags under ambient conditions. Each bag contained 100 drupes. The seeds (true seeds enclosed in hard woody endocarp) were isolated as described in the Chapter III. Germination of such stored/aged seeds was compared with the seeds collected in the month of April 2007 (SL-07).

8.2.2.2 Seed viability

The viability of the stored and fresh seeds was determined by performing the TZ test conducted as described in 3.7.2.

8.2.2.3 Moisture content (MC)

The seeds along with stony endocarp were weighed and the weight was noted as initial weight. The seeds were subjected to drying at

60 °C in hot air oven till constant weight was observed (Sestak, 1962) and noted as final weight. The MC of seeds was expressed on fresh weight basis.

8.2.2.4 Seed leachates

To analyze the seed leachates in stored and fresh seeds of *S. album,* the hard endocarp was cracked open and the enclosed true seed was manually separated. For analyzing the effect of GA_3 on seed health, the seeds were soaked in 3 mM GA_3 solution for 24 h after which the endocarp was manually removed and true seed was separated from the endocarp. After rinsing in distilled water, three replications of 10 g seeds (untreated and GA_3 soaked) from each seed lot (SL-04, SL-05, SL-06 and fresh seed lot SL-07) were soaked separately in 100 ml distilled water at 25 ^{0}C. Electrical conductivity (EC) of steep water was measured and expressed as mS/cm (Basra *et al.*, 2002).

The details of design of experiments and collection of data on seed germination behavior in fresh and stored seeds of both these species were followed as described in Chapter III.

8.2.2.5 Amylase activity

α- amylase (EC 3.2.1.1) and β- amylase (EC 3.2.1.2) activities were studied in the germinating seeds. These enzymes were extracted and the activity was estimated as described in 7.2.3. The results were expressed as mean ± standard deviation.

8.2.2.6 Analysis of PGRs

The GA_3 and IAA in the fresh and stored seeds were extracted and quantified by following HPLC technique as described in 8.2.1.6.

8.3 Results

8.3.1 *Pterocarpus marsupium* Roxb.

8.3.1.1 Seed viability

The highest seed viability (96%) was noticed in the fresh seeds of SL-07. With increase in the storage duration there was gradual decline in the percentage of viable seeds. After one year of storage under ambient conditions, 74% tested positive with TZ test and therefore viable. In the seeds stored for two years the viability was reduced to 52% and the seeds stored for three years showed the least viability of 32%.

8.3.1.2 Moisture content (MC)

The moisture content in the pods of *P. marsupium* gradually decreased with increase in the storage duration (Table VIII-1a). The fresh seeds of SL-07 seed lot had maximum MC of 14.21% which was significantly reduced to 6.12% and 4.45% by two and three years of storage respectively.

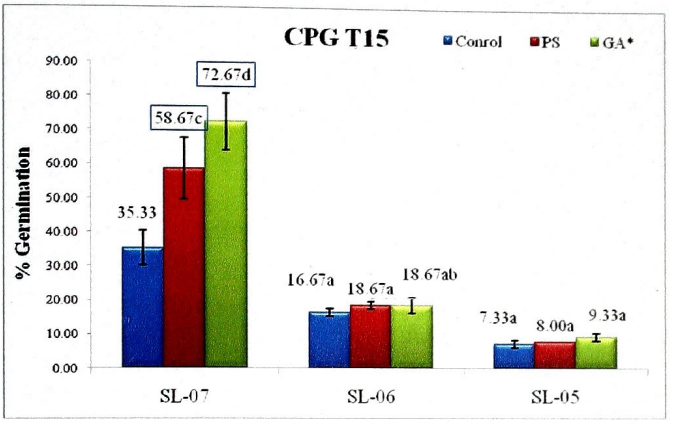
8.3.1.3 Seed leachates

The seeds of *P. marsupium* belonging to the stored seed lots SL-04, SL-05 and SL-06 showed increased electrolyte conductivity in seed leachates compared to electrolyte conductivity in leachates from SL-07 seeds. The electrical conductivity (measured after 24h of soaking) of SL-04 was maximum (5.50 mS/cm). The EC in leachates from SL-05 seeds was 1.39 times more than the SL-06 seeds, which in turn was 1.46 times more compared to the fresh seeds of SL-07 (Fig VIII-3a). The leachates from the freshly collected seeds of SL-07 showed least EC of 2.12 mS/cm.

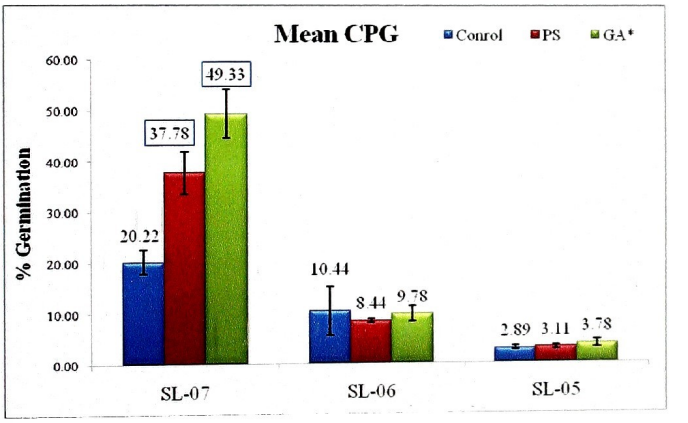
The soaking of seeds in GA_3 solution for 24 h substantially reduced the EC only in the fresh seed lot of SL-07; wherein about 43% reduction in the EC values was noted. Such reduction in the EC values of seed leachates from SL-06 and SL-05 seeds was only 4.5% and 1.4% respectively.

VIII-1 Effect of seed storage on seed germination in *Pterocarpus marsupium* Roxb.

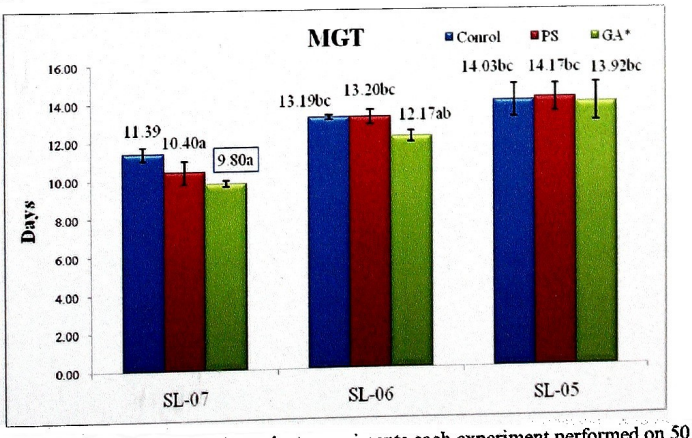
a) Cumulative percentage germination on 15th day



b) Mean Cumulative percentage germination



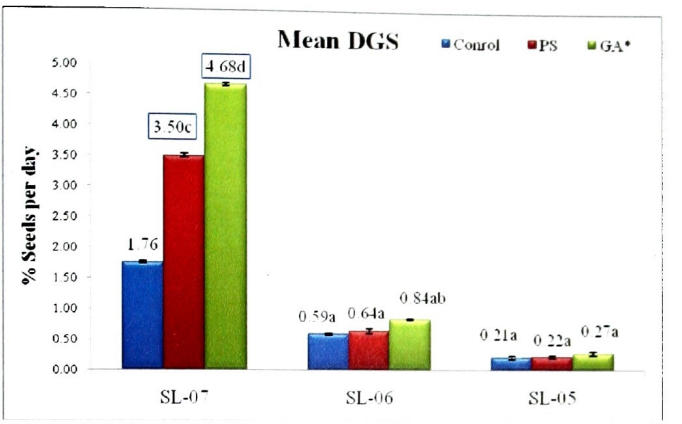
c) Mean germination time



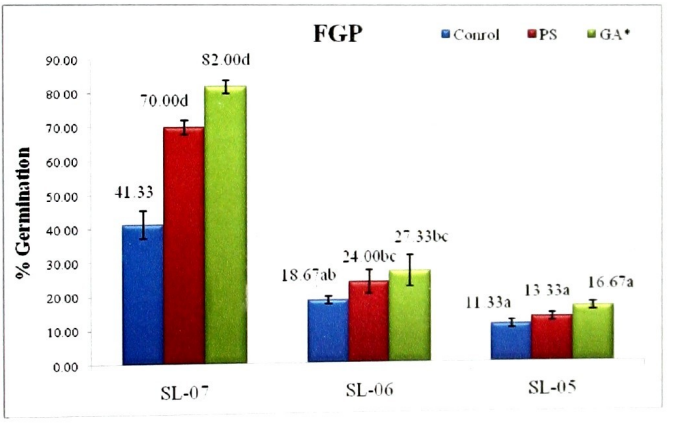
The values represents (mean ± SD) of three independent experiments each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

VIII-1 Effect of seed storage on seed germination in *Pterocarpus marsupium* Roxb.

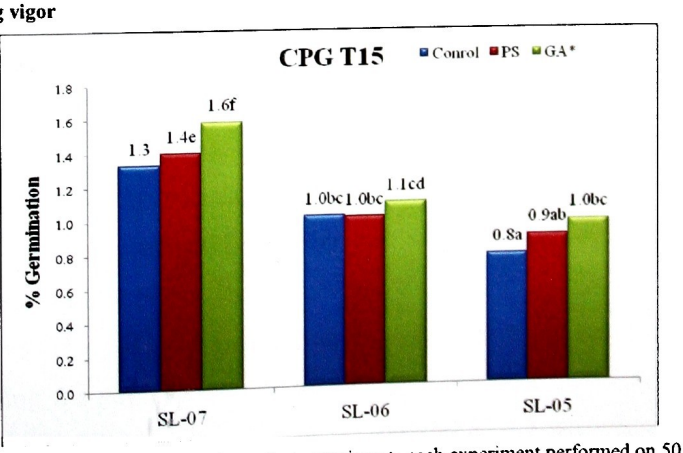
d) Mean daily germination speed



e) Final percentage germination on 30th Day



f) Seedling vigor



The values represents (mean ± SD) of three independent experiments each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

8.3.1.4 Seed germination

The results on the germination behavior of stored seeds and fresh seeds are depicted in Table VIII-1b and Fig VIII-1a-f.

The seeds of SL-04 did not germinate. The germination behavior of untreated seeds (control) of SL-05 and SL-06 was compared with untreated SL-07. A significantly inferior pattern was observed in the stored seeds of the SL-05 and SL-06. The CPG T15 was significantly reduced by about 53% and 80% in the seeds of SL-06 and SL-05 respectively (Fig VIII-1a). A similar pattern was observed with the mean CPG values. The highest mean CPG of 20.22% was observed in SL-07 seeds as compared to 10.44% and 2.89% mean CPG in the seeds from SL-06 and SL-05 respectively (Fig VIII-1b). The seeds of SL-05 showed significantly longest MGT of 14.03 days (Fig VIII-1c) as compared to 11.39 days in the fresh seeds of SL-07. The seeds of SL-06 showed intermediate value. The fastest germination was shown by the seeds of SL-07 as the mean DGS was maximum (1.76% seeds per day) as compared to the stored seeds SL-05 and SL-06 (Fig VIII-1d). The FGP also declined with age of the seeds and only 11.33% seeds of SL-05 germinated as compared to 41.33% FGP in the SL-07 seeds (Fig VIII-1e).

It was established in Chapter IV that the physical scarification significantly improves seed germination pattern in *Pterocarpus marsupium.* Therefore its effect on the stored seeds was analyzed. In the fresh seeds of SL-07, physical scarification dramatically improved the germination pattern by alleviating CPG T15 by about 66%, mean DGS by 99% and FGP by 98% over the untreated seeds of SL-07 (Fig VIII-1a, d and e respectively).

The physical scarification, however, was not much effective in improving the germination pattern in stored seeds of SL-05 as well as SL-06. The CPG T15 in the seeds of SL-06 and SL-05 was improved by only 11% and 9% respectively whereas the same treatment in the fresh

seeds of SL-07 resulted in 66% increase in CPG T15. Likewise, the mean DGS in the seeds of SL-06 and SL-05 was increased only by 8% and 5% respectively. The FGP also revealed the same trend. The seeds of SL-07 after physical scarification showed an increment of about 98% over control, whereas the seeds of SL-06 and SL-05 showed only 24% and 23% increment respectively over their control.

The presoaking in 3 mM GA_3 solution for 24 h was established as an optimum GA_3 treatment in *P. marsupium* for seed germination (Chapter VII) and therefore was tested for its ability to enhance seed germination in the stored seeds.

The fresh seeds of SL-07 after presoaking in GA_3 solution showed about 166% increase in the mean DGS whereas the same treatment could boost the mean DGS by only 42% and 28% respectively in the seeds of SL-06 and SL-05. The FGP also revealed the same trend. The SL-07 seeds after GA_3 treatment showed 82% germination whereas only 27.33% seeds from SL-06 and 16.67% seeds from SL-05 season could germinate within the period of 30 days.

The seedling vigor declined with increase in the age of the seeds. The maximum vigor was shown by the seedlings obtained from the SL-07 seeds (Fig VIII-1f).

8.3.1.5 Amylase activity

The results on the amylase activity in the fresh and seeds stored under ambient conditions are recorded in table VIII-1a.

It was observed that the amylase activity in the germinating stored seeds significantly decreased with the age of the seeds. The activity of β-amylase was more than the activity of α-amylase. The maximum activity of α-amylase (21.34 μg starch hydrolyzed min^{-1} mg^{-1} protein) and β-amylase (43.11μg starch hydrolyzed min^{-1} mg^{-1} protein) was observed in the fresh seeds (SL-07) of *P. marsupium* and there was gradual decline in

VIII-1a Effect of seed storage on germination in *Pterocarpus marsupium* Roxb.

Seed lot	Seed viability (% TZ estimate)	% Moisture content	Amylase activity	
			α-amylase (µg starch hydrolyzed min^{-1} mg^{-1})	β-amylase (µg starch hydrolyzed min^{-1} mg^{-1})
SL-07	96.0 ± 0.0	14.21 ± 1.2	21.34 ± 2.3	43.11 ± 4.3
SL-06	74.0 ± 3.1*	10.32 ± 2.8	14.12 ± 1.1*	22.89 ± 2.9*
SL-05	52.0 ± 0.6*	6.12 ± 3.8*	4.32 ± 0.9*	8.45 ± 2.1*
SL-04	32.0 ± 0.6*	4.45 ± 1.1*	--	--

The values represent (mean± SD) of three independent experiments, each experiment performed on 50 seeds.
*= Significantly different from SL-07 seeds as per Dunett's test at p=0.05

VIII-1b Effect of seed storage on germination in *Pterocarpus marsupium* Roxb.

DAS	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
5 Days	SL-07 Control	2.00 ± 0.0	4.00 ± 0.0	2.00 ± 0.0	4.00 ± 0.0
	SL-07+ PS	5.67 ± 3.1	11.33 ± 6.1	5.67 ± 3.1	11.33 ± 6.1
	SL-07 + GA_3	8.33 ± 0.6	16.67 ± 1.2	8.33 ±0.6	16.67 ± 1.2
	SL-06 Control	0.33 ± 0.6	0.67 ± 1.2	0.33 ± 0.6	0.67 ± 1.2
	SL-06+ PS	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
	SL-06+ GA_3	1.00 ± 0.0	2.00 ± 0.0	1.00 ± 0.0	2.00 ± 0.0
	SL-05 Control	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
	SL-05+ PS	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
	SL-05+ GA_3	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
10 Days	SL-07 Control	8.67 ± 1.2	17.33 ± 2.3	10.67 ± 1.2	21.33 ± 2.3
	SL-07+ PS	16.00 ± 1.7	32.00 ± 3.5	21.67 ± 4.7	43.33 ± 9.5
	SL-07 + GA_3	21.00 ± 2.0	42.00 ± 4.0	29.33 ± 2.5	58.67 ± 5.0
	SL-06 Control	2.33 ± 1.2	4.67 ± 2.3	2.67 ± 0.6	5.33 ± 1.2
	SL-06+ PS	3.33 ± 0.6	6.67 ± 1.2	3.33 ± 0.6	6.67 ± 1.2
	SL-06+ GA_3	3.33 ± 1.2	6.67 ± 2.3	4.33 ± 1.2	8.67 ± 2.3
	SL-05 Control	0.67 ± 0.6	1.33 ±1.2	0.67 ± 0.6	1.33 ± 1.2
	SL-05+ PS	0.67 ± 0.6	1.33 ± 1.2	0.67 ± 0.6	1.33 ± 1.2
	SL-05+ GA_3	1.00 ± 1.0	2.00 ± 2.0	1.00 ± 1.0	2.00 ± 2.0
15 Days	SL-07 Control	7.00 ± 1.7	14.00 ± 3.5	17.67 ± 2.5	35.33 ± 5.0
	SL-07+ PS	7.67 ± 1.2	15.33 ± 2.3	29.33 ± 4.5	58.67 ± 9.0
	SL-07 + GA_3	7.00 ± 1.7	14.00 ± 3.5	36.33 ± 4.2	72.67 ± 8.3
	SL-06 Control	5.67 ± 1.2	11.33 ± 2.3	8.33 ± 0.6	16.67 ± 1.2
	SL-06+ PS	6.0 ± 1.0	12.0 ± 2.0	9.33 ± 0.6	18.67 ± 1.2
	SL-06+ GA_3	5.00 ± 0.0	10.00 ± 0.0	9.33 ± 1.2	18.67 ± 2.3
	SL-05 Control	3.00 ± 1.0	6.00 ± 2.0	3.67 ± 0.6	7.33 ± 1.2
	SL-05+ PS	3.33 ± 0.6	6.67 ± 1.2	4.00 ± 0.0	8.00± 0.0
	SL-05+ GA_3	3.67 ± 1.2	7.33 ± 2.3	4.67 ± 0.6	9.33 ± 1.2

The values represent (mean± SD) of three independent experiments, each experiment performed on 50 seeds.

the activity of these enzymes with increase in the duration of seed storage. The seeds stored for two years showed the least activity.

8.3.1.6 Effect of age on endogenous levels of GA_3 and IAA

The results of HPLC analysis of GA_3 and IAA in the standard GA_3 and IAA are presented in Fig VIII-$3b_1$. The results on HPLC analysis on GA_3 and IAA and stored and fresh seeds of *P. marsupium* are presented FigVIII-$3b_2$ and $3b_3$. It was observed that the quantity of GA_3 as well as IAA in the seeds of *P. marsupium* decreased with increase in the duration of storage under ambient conditions. The quantity of GA_3 and IAA noticed in the SL-07 seeds was 18.4 and 164 $\mu g\ g^{-1}$ tissue respectively, whereas in the SL-04 seeds, GA_3 and IAA were not be detected.

In conclusion, the quality and germinability in the stored seeds of *Pterocarpus marsupium* reduced gradually with increase in the storage time. Though the physical scarification and GA_3 treatment improved germination pattern in the stored seeds, the germination was still poor as compared to the fresh seeds. Most of the stored seeds remained ungerminated even after the application of physical scarification and GA_3 treatment. This indicated that in the seeds of *P. marsupium,* the germinability and vigor decreases with storage time and there is progressive deterioration of seeds as indicated by reduced moisture content, reduced amylase activity, reduced amount of GA_3 and IAA in the stored seeds and increased electrical conductivity in the seed leachates. Therefore, for nursery germination in *Pterocarpus marsupium* Roxb. the freshly collected seeds are more suitable than the stored seeds.

8.3.2 *Santalum album* L.

8.3.2.1 Seed viability

There was gradual decline in the seed viability with increase in the duration of storage. The seed viability as indicated by TZ test in the stored seeds of *S. album* was 41% for SL-04, 62% for SL-05, 79% for SL-06 and 95% for SL-07.

8.3.2.2 Moisture content (MC)

The initial moisture content in the seeds of sandalwood was recorded as 7.12% (SL-07 seeds). With increase in the duration of storage under ambient conditions, a progressive decline the MC was observed and the seeds of SL-05 and SL-04 had significantly reduced MC as compared to fresh seeds of SL-07 seed lot.

8.3.2.3 Seed leachates

The SL-04, SL-05 and SL-06 seed leachates showed higher EC values as compared to the freshly collected seeds of SL-07 (Fig VIII-3c). The EC value in SL-07 seed leachates was least (2.10 mS/cm). With increase in the storage duration, the leachate conductivity also increased. The SL-04 seeds released maximum leachates as indicated by EC value of 6.22 mS/cm. The leachates of seeds from SL-05 lot showed electrical conductivity of 5.34 mS/cm whereas the leachates from SL-06 seeds showed intermediate value of 4.14 mS/cm.

Pre-soaking of seeds in GA_3 was helpful in reducing the EC values of seed leachates. The reduction observed was about 43% in SL-07 seeds, 22% in SL-06, 5% in SL-05 and 2% in SL-04 seeds of *S. album*.

8.3.2.4 Seed germination

The results on the seed germination behavior of stored and fresh seeds of *Santalum* are presented in Table VIII-2b and Fig VIII-2a-f.

VIII-2a Effect of seed storage on germination in *Santalum album* L.

Seed lot	Seed viability (% TZ estimate)	% Moisture content	Amylase activity	
			α-amylase ($\mu g\ min^{-1}\ mg^{-1}$)	β-amylase ($\mu g\ min^{-1}\ mg^{-1}$)
SL-07	95.0 ± 3.7	7.12 ± 2.0	11.33 ± 2.3	29.56 ± 4.3
SL-06	79.0 ± 4.2	6.23 ± 2.1	9.18 ± 2.1	17.23 ± 3.2*
SL-05	62.0 ± 3.6*	4.39 ± 2.2*	4.23 ± 1.9*	9.32 ± 1.1*
SL-04	41.0 ± 2.6*	3.12 ± 1.8*	--	--

The values represent (mean± SD) of three independent experiments, each experiment performed on 50 seeds.

*= Significantly different from SL-07 seeds as per Dunett's test at p=0.05

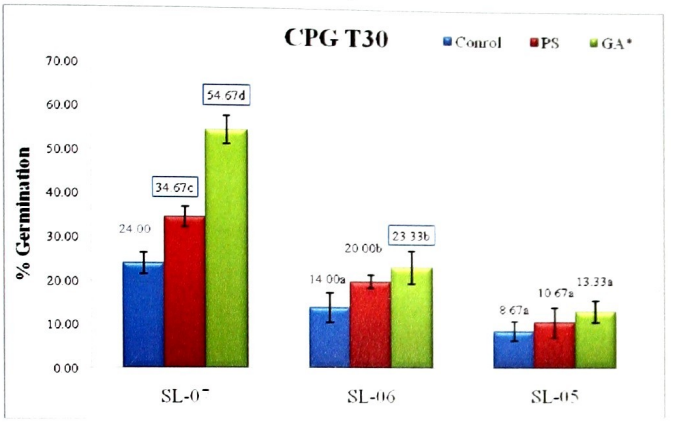
VIII-2b Effect of seed storage on germination in *S. album*

DAS	Treatment (for 24h)	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
	SL-07 Control	1.67±0.67	3.33± 1.2	1.67±0.6	3.33±1.2
	SL-07+ MS	2.67±0.6	5.33±1.2	2.67±0.6	5.33±0.2
	SL-07 + GA	6.33±1.5	12.67±3.1	6.33±1.5	12.67±3.1
	SL-06 Control	0.33±0.1	0.67±0.2	0.33±0.1	0.67±0.2
10	SL-06+ MS	1.33±1.2	2.67±2.3	1.33±1.2	2.67±2.3
Days	SL-06+ GA	2.33±0.6	4.67±1.2	2.33±0.6	4.67±1.2
	SL-05 Control	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
	SL-05+ MS	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
	SL-05+ GA	0.00±0.00	0.00±0.0	0.00±0.0	0.00±0.0
	SL-07 Control	4.33±0.6	8.67±1.2	6.00±1.0	12.00±2.0
	SL-07+ MS	10.67±2.1	21.33±4.2	13.33±1.5	26.67±3.1
	SL-07 + GA	13.00±1.7	26.00±3.5	19.33±1.2	38.67±2.3
	SL-06 Control	3.67±1.5	7.33±3.1	4.00±2.0	8.00±4.0
	SL-06+ MS	3.00±1.0	6.00±2.0	4.33±2.1	8.67±4.2
20 Days	SL-06+ GA	7.33±0.2	14.67±2.3	9.67±0.6	19.33±1.2
	SL-05 Control	1.00±0.0	2.0±0.0	1.00±0.0	2.0±0.0
	SL-05+ MS	1.33±0.6	2.67±1.2	1.33±0.6	2.67±1.2
	SL-05+ GA	6.00±0.0	12.00±1.0	6.0±0.0	12.00±0.0
	SL-07 Control	6.00±1.0	12.00±2.0	12.00±1.7	24.00±3.5
	SL-07+ MS	4.00±1.0	8.00±2.0	17.33±0.6	34.67±1.2
	SL-07 + GA	8.00±1.7	16.00±3.5	27.33±2.9	54.67±5.8
	SL-06 Control	3.00±1.7	6.00±3.5	7.00±1.0	14.00±2.0
30 Days	SL-06+ MS	5.67±1.2	11.33±2.3	10.00±1.0	20.00±2.0
	SL-06+ GA	2.00±1.0	4.00±2.0	11.67±1.2	23.33±2.3
	SL-05 Control	3.33±0.6	6.67±1.2	4.33±1.5	8.67±3.1
	SL-05+ MS	4.00±1.0	8.00±2.0	5.33±1.2	10.67±2.3
	SL-05+ GA	0.67±0.6	1.00±1.0	6.67±0.6	13.33±1.2

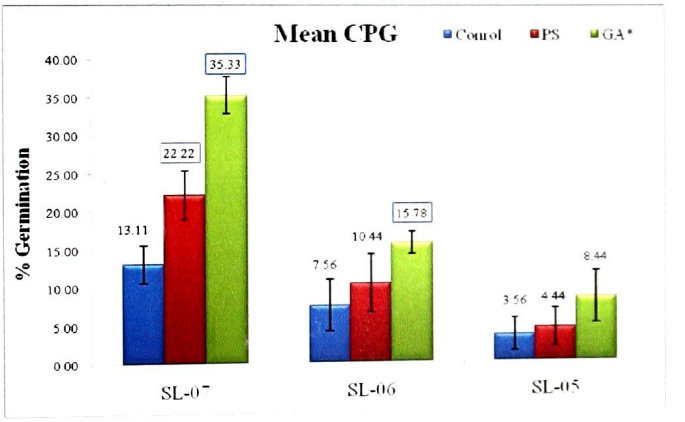
The values represent (mean± SD) of three independent experiments, each experiment performed on 50 seeds.

VIII-2 Effect of seed storage on seed germination in *Santalum album* L.

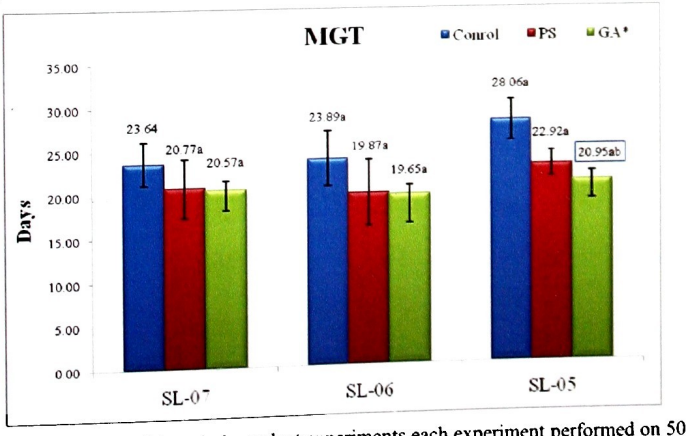
a) Cumulative percentage germination on 30th day



b) Mean Cumulative percentage germination



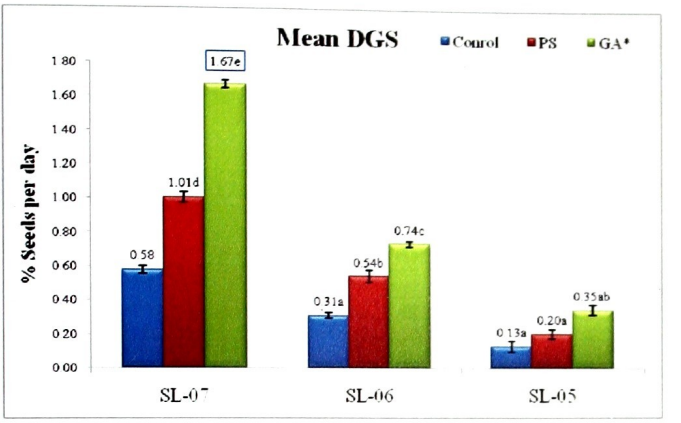
c) Mean germination time



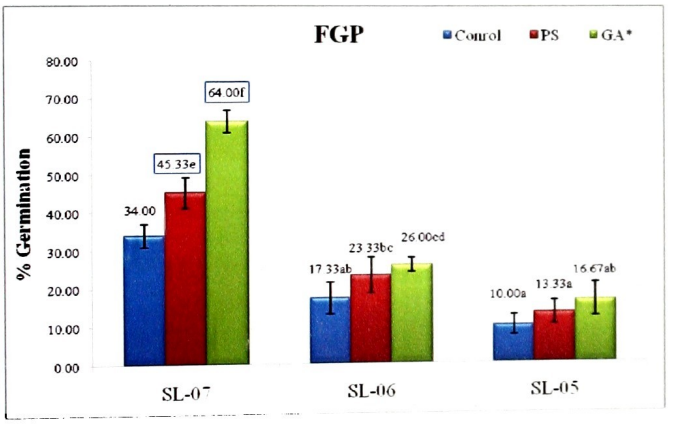
The values represents (mean ± SD) of three independent experiments each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

VIII-2 Effect of seed storage on seed germination in *Santalum album* L.

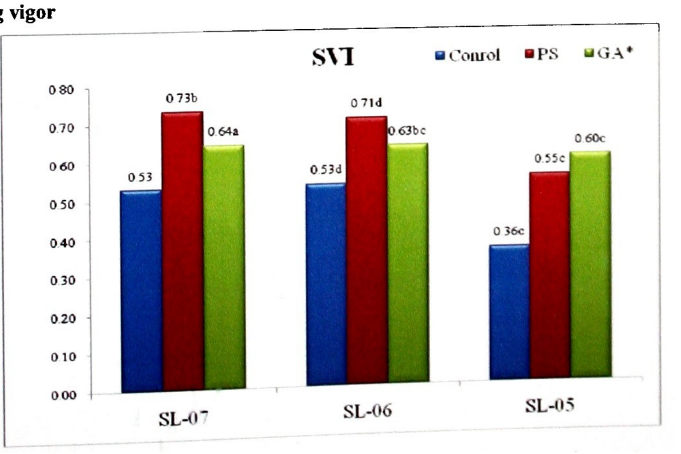
d) Mean daily germination speed



e) Final percentage germination on 60th Day

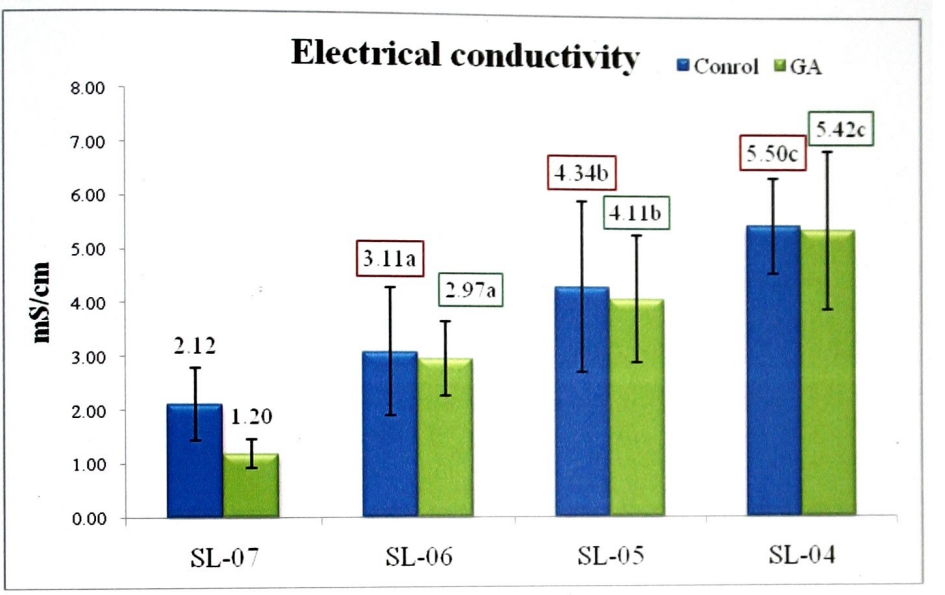


f) Seedling vigor



The values represents (mean ± SD) of three independent experiments each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

VIII-3a Electrical conductivity of seed leachates in stored and fresh seeds of *Pterocarpus marsupium* Roxb.



The values represents (mean ± SD) of three independent experiments each experiment performed on 10 g seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

Fig. VIII - $3b_1$: HPL Chromatogram of standard GA_3 and IAA showing retention time.

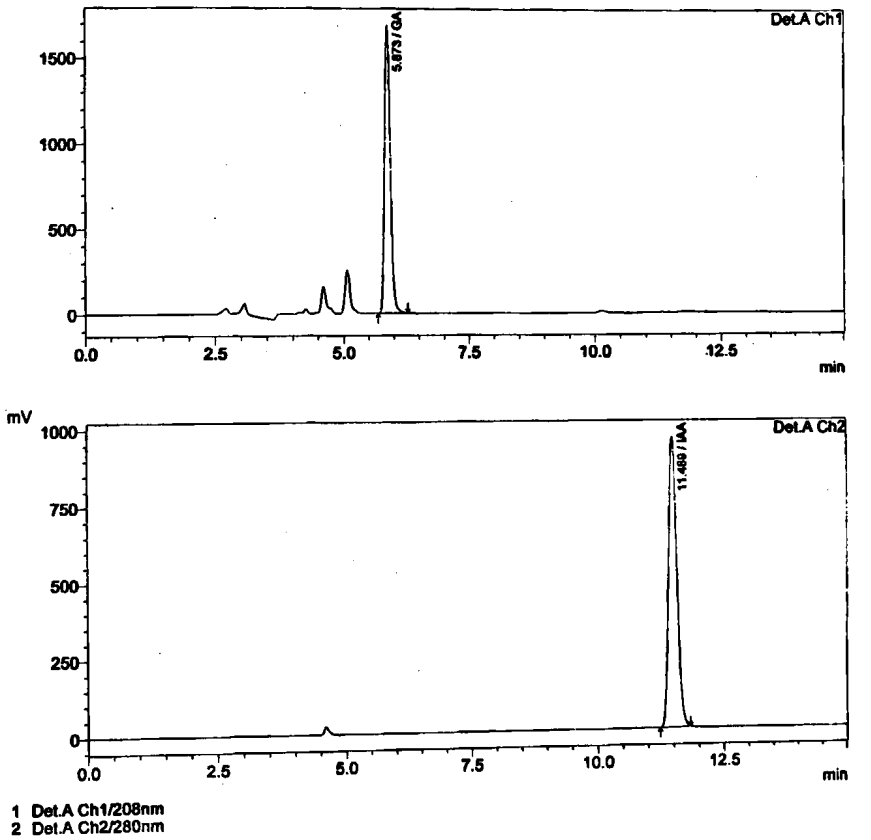


Fig. VIII - $3b_2$: HPL Chromatogram showing presence of and retention time of GA_3 and IAA in the SL-07 seeds of *Pterocarpus marsupium* Roxb.

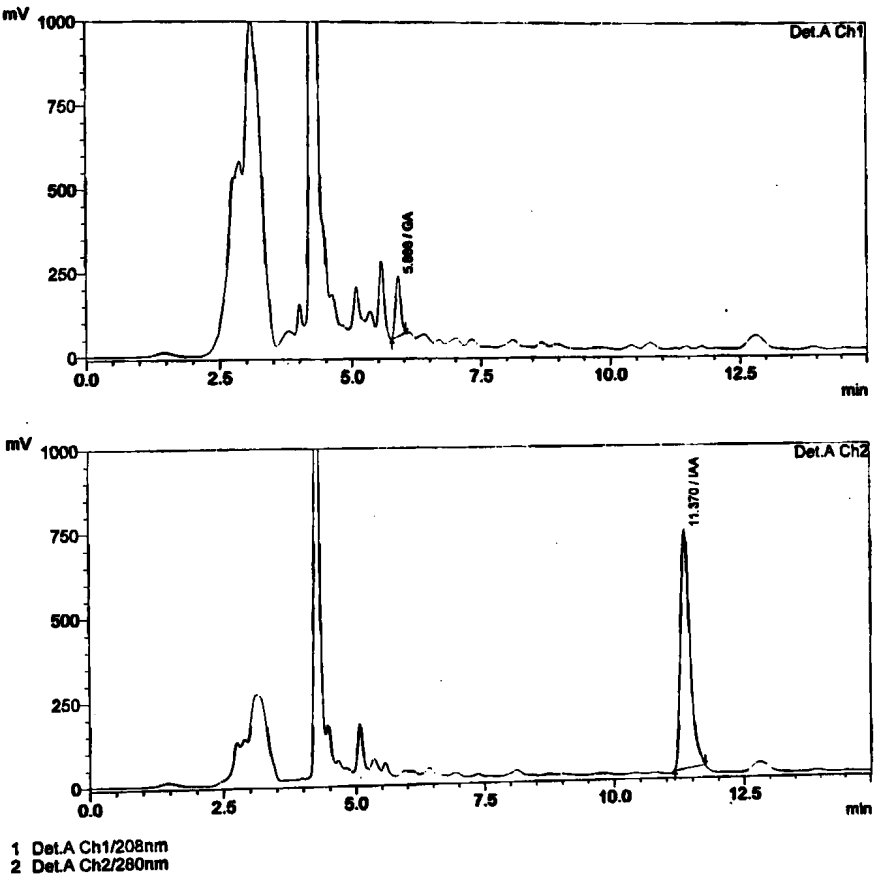
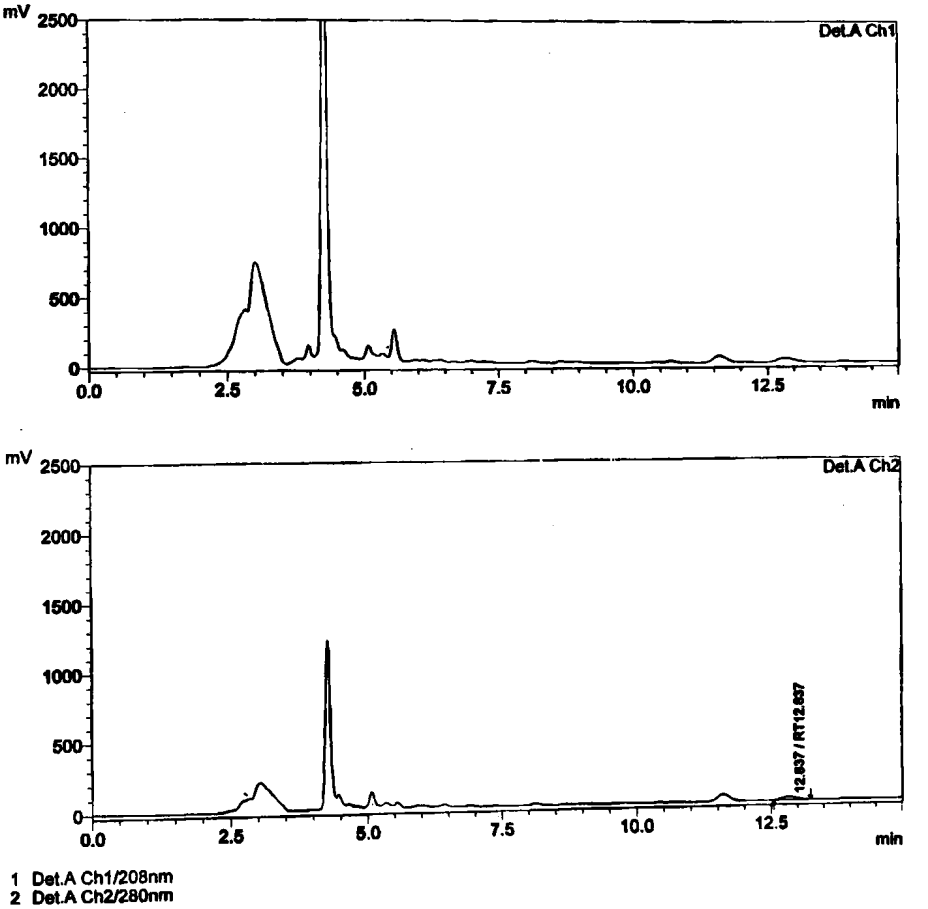
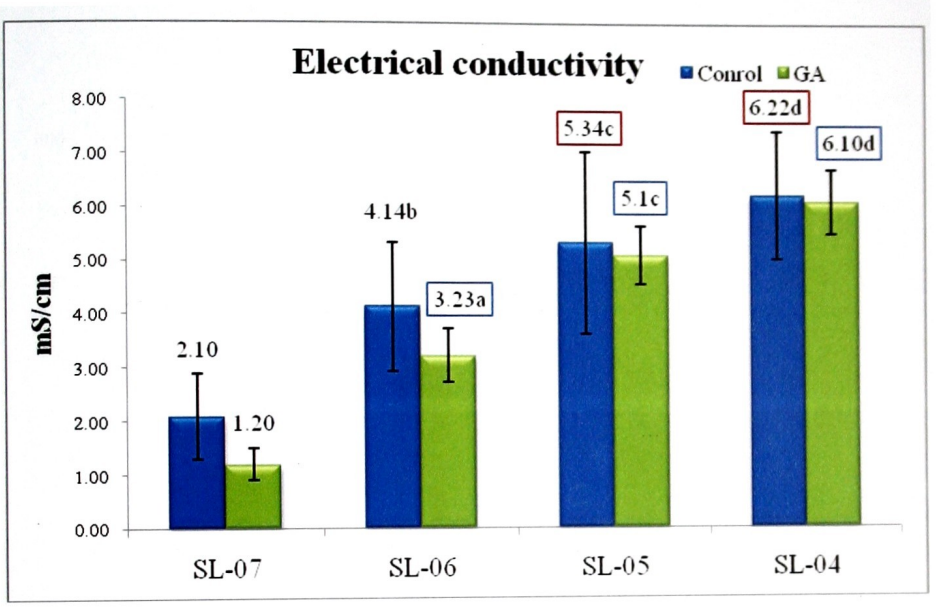


Fig. VIII - $3b_3$: HPL Chromatogram showing absence of GA_3 and IAA in the SL-04 seeds of *Pterocarpus marsupium* Roxb.



VIII-3c Electrical conductivity of seed leachates in stored and fresh seeds of *Santalum album* L.



The values represents (mean ± SD) of three independent experiments each experiment performed on 10 g seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

Fig. VIII - $3d_1$: HPL Chromatogram showing presence of and retention time of GA_3 and IAA in the SL-07 seeds of *Santalum album* L.

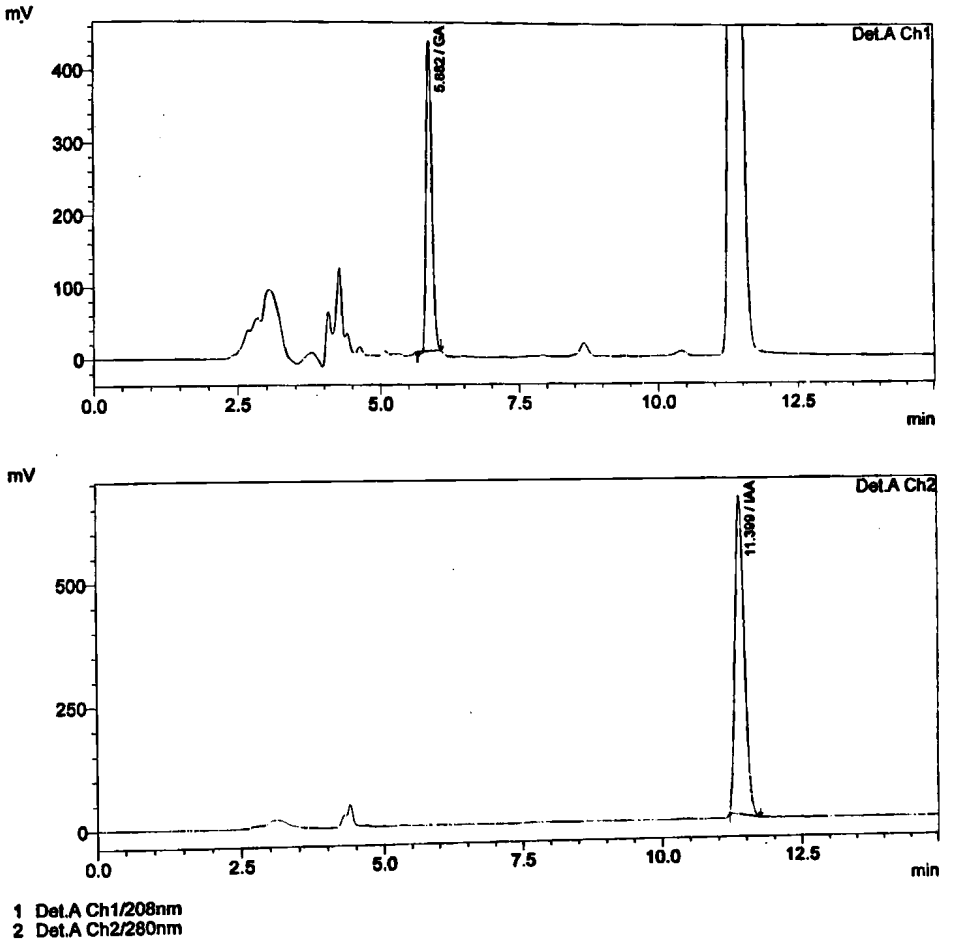
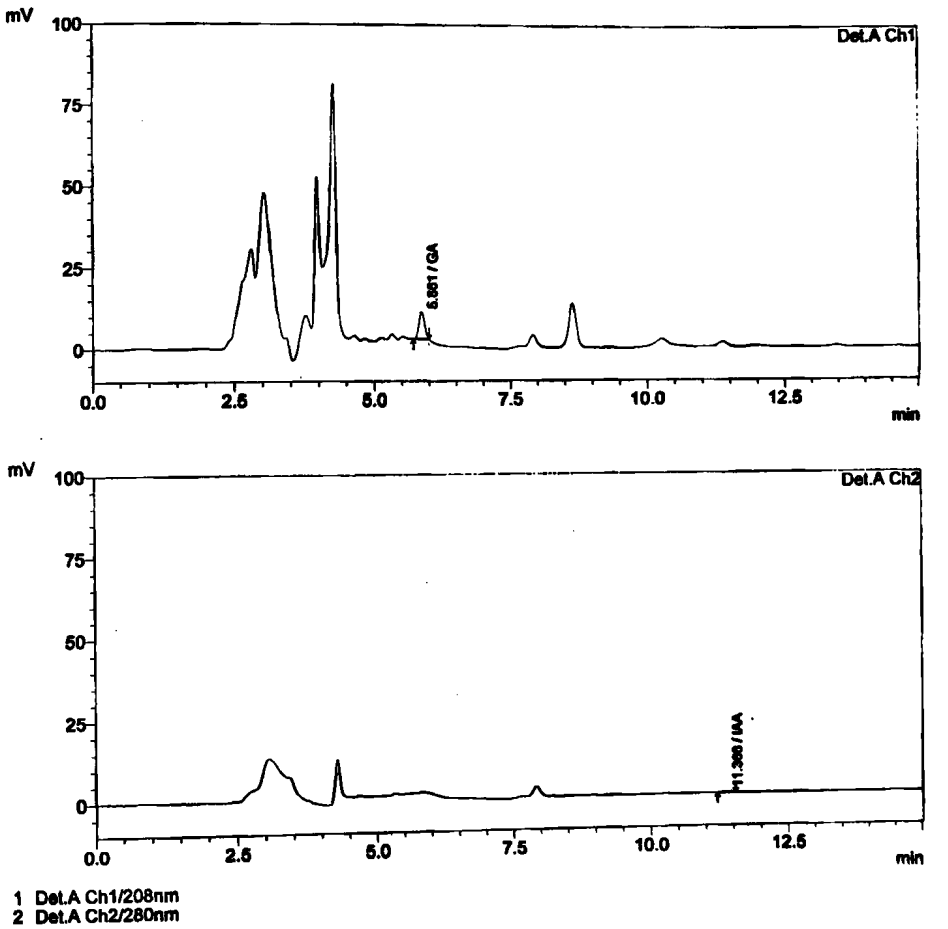


Fig. VIII - $3d_2$: HPL Chromatogram showing presence of and retention time of GA_3 and IAA in the SL-04 seeds of *Santalum album* L.



None of the seeds of SL-04 seed lot germinated in the 60 day germination run. Among the untreated seeds of SL-05, SL-06 and SL-07, the seeds of SL-06 and SL-05 showed progressively substandard germination pattern as compared to the seeds of SL-07. All the parameters computed showed inferior values in the seeds of SL-06 and SL-05.

The CPG T30 in the SL-07 was 24% (Fig VIII-2a) which declined significantly by 42% in SL-06 and by 64% in SL-05. The mean CPG also showed similar pattern (Fig VIII-2b). The MGT increased with increase in the storage period wherein the SL-07 seeds exhibited least MGT (23.64 days) than SL-06 (23.89 days) and SL-05 seeds (28.06 days), (Fig VIII-2c). The speed of germination (mean DGS) significantly dropped down from 0.58% seeds per day (SL-07) to 0.31% (SL-06) and 0.13% seeds per day (Fig VIII-2d). The seeds of SL-07 showed maximum FGP (34%) among the three seed lots which was reduced significantly by 50% in SL-06 and by 70% in SL-05.

The effectiveness of physical scarification and treatment with 3 mM GA_3 for 24 h decreased with increase in the storage duration. The seeds of SL-07 responded better to these treatments than the seeds of SL-06 and SL-05 (Fig VIII-2a-f). The increments over control observed in CPG T30, mean CPG, mean DGS and FGP were of higher magnitude in SL-06 than those of SL-05. Between the SL-06 and SL-05 seeds, the SL-05 seeds showed least sensitivity to these methods. The MGT, however, was effectively reduced in fresh as well as stored seeds by both the treatments (Fig VIII-2c).

The seedling vigor declined with the age of the seeds (Fig VIII-2f). The fresh seeds (SL-07) produced the most vigorous seedlings as compared to SL-06 and SL-05 seeds. Physical scarification as well as application of GA_3 enhanced the vigor in the seedlings irrespective of the age of the seeds. However, the magnitude of effectiveness of these treatments deceased with increase in the age of the seeds.

8.3.2.5 Amylase activity

The activity of α-amylase and β-amylase in the germinating stored and fresh seeds of *S. album* is recorded in table VIII-2a. It was observed that with increase in the age of the seeds, there was gradual decline in the specific activity of these enzymes during germination of sandal seeds. The maximum activity of α-amylase was 11.33 µg starch hydrolyzed min^{-1} mg^{-1} protein and that of β-amylase was 29.56 µg starch hydrolyzed min^{-1} mg^{-1} protein in the fresh seeds of SL-07 seed lot. In the seeds stored for two years, these values were 4.23 and 9.32 µg starch hydrolyzed min^{-1} mg^{-1} protein, respectively thereby indicating reduced seed vigor.

8.3.2.6 Effect of age on endogenous levels of GA_3 and IAA

The results on the HPLC analysis of GA_3 and IAA contents in the SL-07 and SL-04 seeds are presented in the Fig VIII-$3d_1$ and $3d_2$. The concentration of GA_3 as well as IAA in terms of µg per gram tissue was found to decrease with increase in the duration of seed storage. The amount of GA_3 observed in the fresh seeds of SL-07 seed lot was 45 µg per gram tissue, whereas the concentration of IAA was 137 µg per gram tissue in the seeds belonging to the same seed lot. With increase in the duration of storage under ambient conditions, the quantities of these PGRs declined with increase in the duration of storage. The seeds belonging to SL-04 seed lot showed significantly reduced concentrations of GA_3 (0.06 µg per gram tissue) and IAA (0.135 µg per gram tissue).

In conclusion, the quality and germinability in stored seeds of *Santalum album* was reduced with increase in the duration of storage under ambient conditions. The physiological quality of seeds progressively deteriorated with increase in the duration of storage as evident from the

gradual decline in the seed moisture content, specific activity of α and β amylase, the concentration of GA_3 and IAA in the stored seeds and increase in the conductivity of seed leachates from the stored seeds. The physical scarification as well GA_3 treatment was not effective to induce germination in the seeds stored for up to three years. To raise the seedlings of this species in a nursery, therefore, the fresh seeds should used and the seed lot of a season should be used within next 1 to 1.5 years.

8.4 Discussion

The natural habitats of *P. marsupium* and *S. album* are found in the tropical regions of the world. The multiple values of tropical tree species for humankind and the undeniable importance for the protection of the environment make them potentially suitable for use in reforestation programs and agroforestry systems. Unfortunately, difficult seed storage behavior has thus far limited the utilization of tropical tree species in such programs.

Seeds stored under different conditions like seeds in soil banks, warehouses or liquid nitrogen are exposed to natural physiological deterioration or ageing, which ultimately leads to loss of viability (Smith and Berjak, 1995; Schmidt, 2000; Atici, 2007). With advancement in seed age, membranes become leaky, enzymes loose catalytic activity, chromosomes accumulate mutations and ultimately germination ratios reduce (Rao and Kalpana, 1994; Sivritepe and Dourado, 1998). However, the rate at which seeds age depends upon their physiological status, their genetic constitution and the storage conditions (Nowakowska and Rakowski, 2002; Al-Maskari *et al.*, 2003).

Sometimes the seeds retain full viability but with abnormal development of the seedling reflecting reduced seed vigor (Bewley & Black 1994). In the present investigation, however, the germinability in the seeds of *Pterocarpus marsupium* and *Santalum album* decreased with

increase in the storage time, but the seeds which germinated produced seedlings with normal morphology.

The results of the present investigation on seed germination in the stored seeds of *Pterocarpus marsupium* and *Santalum album* have exhibited longer MGT and less mean DGS as compared to fresh seeds. This reduced germinability may be attributed to reduction in vigor/viability. A loss of seed viability during storage has been demonstrated in many species (Ellis and Roberts, 1981; Ross, 1982; Rao *et al.,* 1987). The natural aged seeds of *Linum usitatissimum* displayed a marked decline in germinability (Sammour, 1989). Atici *et al.* (2007) have reported significantly lower percentage germination in the 37-year-old seeds of *Medicago sativa, Trifloliium reprens* and *T. pratense.* Cottonseeds subjected to accelerated ageing showed depression of germination ability (Iqbal *et al.,* 2002). In the seeds of *Rhododendron maddenii* and *R. niveum,* seed germination percentage and percentage survival of germinated seedlings decreased with increase of storage time (Tiwari and Chuhan, 2007). The stored or aged seeds show loss of vigor (Trawatha *et al.*, 1995). The loss in vigor is reflected in delayed germination and emergence, slower growth, a loss of germination capacity and viability (Byrd & Delouche, 1971; Woodstock, 1973; Douglas, 1975; Rice and Dyer, 2001).

According to Smith and Berjak (1995), progressive membrane deterioration, accompanied by damage to repair mechanisms results in declined vigor and loss of seed viability with age (Hendry, 1993; Smith & Berjak 1995). Measuring conductivity of the leachate from plant tissue is a long-standing method for estimating membrane permeability and seed leachate electrical conductivity (EC) is considered as an effective indicator of seed germination (Waters and Blanchette, 1983). The EC is based on the fact that seeds, when soaked in water, exude ions, sugars and other metabolites, from the starting of the soaking period, due to changes in the integrity of the cell membranes, as a function of water amount and of the level of seed deterioration (Fessel *et al.,* 2006). Seed deterioration

involves many biochemical and biophysical changes, including membrane integrity (Stewart and Bewley, 1980; Priestley and Leopold, 1983; Ferguson et al., 1990).

The stored seeds from SL-04, SL-05 and SL-06 of both the species showed increased electrolyte conductivity of seed leachates. These results are suggestive of the loss of tissue integrity and membrane damage of endosperm and/or embryo cells of the stored seeds. In deteriorated seeds, the repair mechanism is absent or inefficient, or the membranes are completely damaged (Bewley and Black, 1982), thus permitting leaching of larger electrolyte amounts. This might be the reason why the stored seeds of *P. marsupium* and *S. album* have exhibited reduced percentage germination. The percentage seed germination in the fresh seeds was increased only in the fresh seeds and the stored seeds of *P. marsupium* and *S. album* did not show significantly increased germination from control after the treatment of the seeds with GA_3

After harvest, seeds start deteriorating, moving unavoidably towards death. According to Khurana and Singh, (2001) seed longevity is generally lower for wet tropical species than for dry tropical. However, the *P. marsupium* and *S. album* growing in the tropical region showed the decline in germinability after seed storage.

In the present investigation it was observed that seed moisture content (MC) in stored seeds of *P. marsupium* and *S. album* decreased with increase in the storage duration. The reduced germination in stored seeds may be because of reduced moisture content since seed longevity during storage is strongly dependent on water content and temperature (Kamra, 1990; Hong et al., 1996; IPGRI/DFSC, 1998, 1999; Ouédraogo et al., 1999; Flynn et al., 2004). Although under dry storage conditions longevity is enhanced, eventually seeds deteriorate and lose the capability to germinate. Such deterioration might be another reason behind reduced germinability in the stored seeds of these species. The gradual decline in moisture content during the storage period might have changed

their germination requirements, which reflects the nature of non-hermetic storage. The seeds of many tropical tree species are thought to display such intermediate storage behavior (Hong *et al.*, 1996, Flynn *et al.*, 2004). According to the seed information database of the Kew Gardens, the seeds of *S. album* and some *Pterocarpus* species are orthodox (http://data.kew.org/sid/sidsearch.html)

Seed ageing results in loss of integrity of a number of structural and functional components of seed tissues, including chromosomal and DNA damage which can reduce transcription fidelity and membrane deterioration accelerated by free radical production (Hendry, 1993). Aged seeds may also show reduced viability or germinability or retention of full viability but with abnormal development of the seedling thereby expressing reduced seed vigor (Bewley & Black, 1994). Seed germination is regulated by interactions between different PGRs and environmental factors and occurs only when the conditions are favorable. Gibberellic acid is one of such intrinsic factors and the embryo is its source during germination. Brits *et al.* (1995) are of the opinion that the levels of PGRs need to be correlated with known morphological, structural and ultrastructural levels in a seed. In the present investigation, we observed a decline in the endogenous levels of GA_3 and IAA in the stored seeds of *P. marsupium* as well as *S. album.* Atici *et al.* (2007) have also reported decline in the levels of endogenous GA_3, IAA and zeatin during the germination of stored seeds of *Medicago sativa, Trifolium repens and Trifolium pretense.* Such decline in the endogenous levels of these PGRs might be one of the other reasons in reduced germinability in the seeds stored for different durations. There was decline in the percentage of viable seeds of both species as inferred by TZ test. This result also suggests a decline in physiological quality of seeds, even though higher viability was indicated by TZ test.

In the present investigation, the activity of amylases was found to decrease with increase in the duration of seed storage under

ambient conditions. Rame Gowda (1992) reported a decrease in the activity α-amylase coupled with progressive ageing and further authenticated that amylase is more directly involved in the maintenance of better germination of differentially aged seeds. In the present investigation, the decline in the specific activity of both the α-amylase as well as β-amylase indicate decline in the protein synthesis. The decreased enzyme synthesis may be because of impairment of postribosomal supernatant and ribosomal fractions (Bryan *et al.*, 1973). Impairment of transcription mechanism which in turn is a consequence of damage to nuclear DNA can also reduce the capacity for protein synthesis (Bewly and Black, 1982).

According to Fountain et al. (2002), the conductivity test allows the seed lots to be further distinguished, and raises questions about the relationship of germinability and viability to cell and tissue function in seeds. The tetrazolium estimate of seed viability in the stored seeds though was less compared to fresh seeds; it was well above the germination percentage shown by respective seed lots suggesting that some of the seeds contained living tissues but of insufficient quantity to support germination (Fountain et al., 2002). Viability loss is often attributed to the loss of barrier function of the plasma membrane (Roberts, 1972; Maguire, 1977; Bewley and Black, 1994; Golovina *et al.*, 1997). A similar relationship between elevated leakage and viability loss has been found for many other seed species (Sung and Chiu, 1995; Tammela *et al.*, 2000; Corbineau *et al.*, 2002).

The loss of germinability in the stored seeds of *P. marsupium* and *S. album* may be due to the stress associated with rehydration in water. Seed rehydration characteristics or germination requirements may change during drying and/or dry storage, particularly in tropical and sub-tropical species (Roberts *et al.*, 1984; Wood *et al.*, 2000). According to Ramanandane and Ponnuswamy (2004) because of the prevailing sub-tropical climate in the major part of India, seeds of most species with large seeds show rapid deterioration. The seeds of *Pterocarpus marsupium* and

Santalum album also of considerably large size and they are also located in the tropical regions of India, which might be the reasons behind decline in germinability of stored seeds of *P. marsupium* and *S. album.*

Germination and storability of tree seeds is an equally important aspect for ex-situ conservation of forest resources. Seeds of many tropical tree species cannot withstand dehydration below critical level of desiccation (Kamra, 1990; Hong et al., 1996; IPGRI/DFSC, 1998, 1999; Ouédraogo et al., 1999; Flynn et al., 2004), which jeopardizes their long-term storability. The better a seed survives dehydration; the longer it can be stored at a broad range of conditions without losing viability (Roberts, 1973; Dickie *et al.,* 1990; Ellis *et al.,* 1992; Mwasha *et al.,* 1996). The progressive decline in the percentage seed viability, germination and moisture content accompanied by increase in the EC of seed leachates are suggestive of deterioration of seed quality in the stored seeds of *P. marsupium* as well as *S. album.* It can be therefore concluded that the storability of seeds is either poor or require specific conditions of relative humidity and temperature to retain germinability during storage. Both these species produce thousands of seeds in each season but the natural resurgence is poor because of associated fruit structures and the seed banks persisting in soil cannot recruit seedlings due to reduction in seed quality over time. The natural stands of these species, however, are being continuously exploited for various reasons mentioned earlier. These might be the reasons behind the fact that *P. marsupium* and *S. album* are becoming endangered species.

Chapter IX

Effect of Combination Treatments on Seed Germination

9.1 Introduction

The seed germination is a highly complex process through which quiescent seed is transformed into physiologically active phase (Dow and Schwintzer, 1999, Kermode, 2005; Taiz and Zeiger, 2006). There are many exogenous as well as endogenous factors that affect this process. Among such intrinsic factors, plant growth substances are one of the most important. In the species with physical dormancy, the suitable methods to break the dormancy include mechanical and acid scarification, whereas the pretreatment of PGRs has overcome physiological dormancy in many species (Taiz and Zeiger, 2006; Atici, 2007). In many instances, a combination treatment has markedly improved germination. Mechanical scarification relieves physical constraint on expansion of embryonic axis and improves imbibition which helps to trigger metabolic machinery required for germination. The combination of physical scarification, plant growth regulators like GA_3, auxins and cytokinins and germination stimulants like thiourea and potassium nitrate show a synergistic effect that leads to more improved germination (Pedroza-Manrique *et al.*, 2005).

In this chapter, the effect of combination treatments on seed germination in *P. marsupium* and *S. album* are described.

9.2 Materials and Methods

The source of seeds, selection of the seeds and viability testing was followed as described in Chapter III. The wing associated with the pods of *Pterocarpus marsupium* was cut with sharp scissors (the resultant structure was considered as seed) and the seeds were used for the experiments.

The epicarp and mesocarp from the drupes of *Santalum album* were removed to separate the true seed enclosed by the endocarp. This structure (endocarp+ seed) was termed as seed.

From the Chapters IV, V, VI and VII the optimum treatments were identified for enhanced germination in *Pterocarpus marsupium* and *Santalum album.* The influence of some of these treatments in combination was studied. The combining of these treatments is outlined below.

9.2.1 *Pterocarpus marsupium* Roxb.

9.2.1.1 Effect of combination of physical scarification and GA_3

The seeds were subjected to physical scarification (PS) by nicking the edge of the central seed case by about 1-2 mm so as to expose the enclosed seed. The PS seeds were then subjected to the treatment with 4 mM GA_3 for 24 h (PS+GA) as this particular GA_3 treatment was found to be optimum for enhanced germination in *P. marsupium* (Chapter VII).

The seeds thus treated were sown in the polythene bags and cavity seeding trays containing moist garden soil. The germination pattern was observed as described in Chapter III.

9.2.1.2 Effect of combination of physical scarification and thiourea

The seeds physically scarified (PS) as mentioned earlier in 9.2.1.1 were immersed in 25 mM thiourea for 24 h (PS+THIO) (as the treatment of 25 mM thiourea for 24 h was the optimum treatment among the different treatments of thiourea, details in Chapter VI). The seeds thus treated were sown in the polythene bags and cavity seeding trays containing moist garden soil. The germination pattern was observed as described in Chapter III.

9.2.1.3 Effect of combination of physical scarification and KNO_3

The seeds of *P. marsupium* treated with 80 mM KNO_3 gave better germination pattern compared to non-treated seeds. Therefore, the seeds physically scarified (PS) as mentioned earlier in 9.2.1.1 were immersed in 80 mM KNO_3 for 24 h (PS+KNO_3). The seeds thus treated were sown in the polythene bags and cavity seeding trays containing moist

garden soil. The germination pattern was observed as described in Chapter III.

9.2.1.4 Effect of combination of acid scarification and GA_3

The seeds were scarified (AS) with concentrated H_2SO_4 (36N, 99%) for 30 min. The seed as to acid ratio was 1:2. The AS seeds were washed thoroughly in running tap water to remove the traces of acid. The AS seeds were then treated with 4 mM GA_3 for 24 h (AS+GA) as this particular GA_3 treatment was found to be optimum for enhanced germination in *P. marsupium* (Chapter VII). The seeds thus treated were sown in the polythene bags and cavity seeding trays containing moist garden soil. The germination pattern was observed as described in Chapter III.

9.2.1.5 Effect of combination of acid scarification and thiourea

The seeds scarified with acid as described in 9.2.1.4 were soaked in 25 mM thiourea for 24 h (AS+THIO) (as the treatment of 25 mM thiourea for 24 h was the optimum treatment among the different treatments of thiourea, details in Chapter VI). The seeds thus treated were sown in the polythene bags and cavity seeding trays containing moist garden soil. The germination pattern was observed as described in Chapter III.

9.2.1.6 Effect of combination of acid scarification and KNO_3

The seeds pretreated with 80 mM KNO_3 for 24 h produced the best germination pattern among the different concentrations of KNO_3 as compared to untreated seeds (Chapter VI). Therefore, the acid scarified seeds were treated with 80 mM KNO_3 for 24 h (AS+KNO_3). The treated seeds were sown in the polythene bags and cavity seedling trays containing moist garden soil.

9.2.2 *Santalum album* L.

9.2.2.1 Effect of combination of physical scarification and GA_3

The seeds were subjected to physical scarification (PS) by gently hammering the seeds with pestle in a mortar so as to induce a crack in the endocarp. The PS seeds were then treated with GA_3 by immersing the seeds in 4 mM GA_3 solution for 24 h (PS+GA). The selection of concentration of GA_3 and duration of treatment was determined on the basis of results on GA_3 treatment and germination as described in Chapter VII.

The seeds thus treated were sown in the polythene bags and cavity seeding trays containing moist garden soil. The germination pattern was observed as described in Chapter III.

9.2.2.2 Effect of combination of physical scarification and thiourea

The seeds that were physically scarified as described earlier (9.2.2.1) were treated with 75 mM thiourea for 24 h (PS+THIO). This selection was based on the effect of different concentrations of thiourea applied for soaking the seeds for different durations as described in Chapter VI. The seeds thus treated were sown in the polythene bags and cavity seeding trays containing moist garden soil. The germination pattern was observed as described in Chapter III.

9.2.2.3 Effect of combination of physical scarification and KNO_3

The seeds which were physically scarified (PS) were subjected to soaking in 75 mM KNO_3 for 12h (PS+KNO_3). The seeds presoaked in 75 mM KNO_3 for 12 h demonstrated the best germination pattern among the different combinations of concentrations of KNO_3 and duration of treatments (Chapter VI) which was the basis of this selection.

The seeds subjected to the combination treatments were sown in the polythene bags and cavity seeding trays containing moist

garden soil. After sowing the containers were maintained in the shade-net house.

9.2.2.4 Effect of combination of acid scarification and GA_3

The seeds of *Santalum album* were acid scarified (AS) with concentrated H_2SO_4 (36N, 99%) for 30 min. The seed as to acid ratio was 1:2. After acid scarification, seeds were washed thoroughly in running tap water to remove the traces of acid.

The acid scarified seeds (AS) were then soaked for 24 h in the solution of 4 mM GA_3 (AS+GA). The seeds subjected to the combination treatment were sown in the polythene bags and cavity seeding trays containing moist garden soil. After sowing the containers were maintained in the shade-net house.

9.2.2.5 Effect of combination of acid scarification and thiourea

The seeds after acid scarification as described in 9.2.2.4 were soaked in the 75 mM thiourea for 24 h (AS+THIO). The seeds subjected to the combination treatment were sown in the polythene bags and cavity seeding trays containing moist garden soil. After sowing the containers were maintained in the shade-net house.

9.2.2.6 Effect of combination of acid scarification and KNO_3

The acid scarified seeds as described in 9.2.2.4 were soaked in 75 mM KNO_3 for 12 h (AS+KNO_3). The seeds subjected to the combination treatment were sown in the polythene bags and cavity seeding trays containing moist garden soil. After sowing the containers were maintained in the shade-net house.

9.3 Results

9.3.1 *Pterocarpus marsupium* Roxb.

9.3.1.1 Effect of combination of physical scarification and GA_3

The combination of PS+ GA produced the best germination pattern (Table IX-1 and Fig IX-1a-f) than that of seeds only physically scarified or seeds treated with GA_3 alone (Fig VII-1a-e). In the seeds with

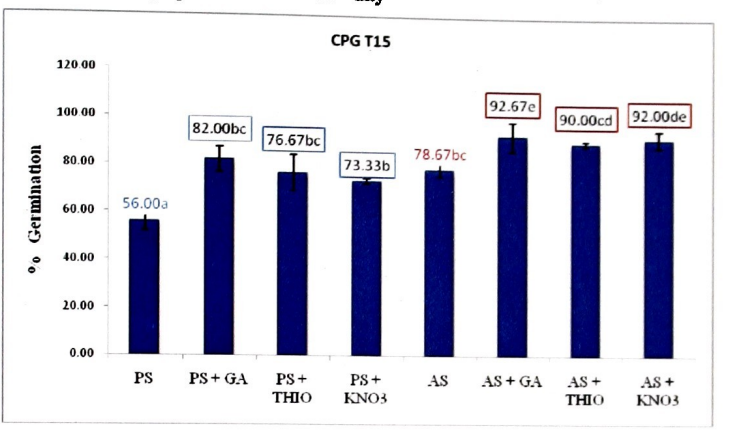
IX-1 Effect of combination treatment on germination in *Pterocarpus marsupium Roxb.*

DAS	Treatment (for 24h)	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
	PS	11.33±2.3	22.67±4.6	11.33±2.3	22.67±4.6
	PS+GA	21.33±1.1	42.67±2.3	21.33±1.1	42.67±2.3
	PS+THIO	18.33±1.1	36.67±2.3	18.33±1.1	36.67±2.3
5	PS+KNO_3	16.67±0.5	33.33±1.1	16.67±0.5	33.33±0.5
Days	AS	15.33±1.4	30.67±2.8	15.33±1.4	30.67±2.8
	AS+GA	23.00±0.0	46.00±0.0	23.00±0.0	46.00±0.0
	AS+THIO	20.67±0.0	41.33±0.0	20.67±0.0	41.33±0.0
	AS+KNO_3	22.67±0.7	45.33±1.4	22.67±0.7	45.33±1.4
	PS	13.33±0.5	26.67±1.1	24.67±2.0	49.33±4.0
	PS+GA	16.33±1.5	32.67±3.0	37.67±2.0	75.33±4.1
	PS+THIO	16.67±2.5	33.33±5.0	35.00±3.6	70.00±7.2
10	PS+KNO_3	16.00±0.0	32.00±0.0	32.67±0.5	65.33±1.2
Days	AS	22.00±4.9	44.00±9.9	37.33±3.5	74.67±7.0
	AS+GA	21.33±4.3	42.67±8.2	44.33±1.5	88.67±3.6
	AS+THIO	21.33±4.2	42.67±8.3	42.00±1.6	84.00±3.5
	AS+KNO_3	21.67±0.0	43.33±0.0	44.33±0.7	88.67±1.4
	PS	3.33±2.5	6.67±5.0	28.00±2.0	56.00±4.0
	PS+GA	3.33±1.1	6.67±2.3	41.00±2.6	75.33±4.1
	PS+THIO	3.33±1.5	6.67±3.0	38.33±3.7	76.67±7.5
15	PS+KNO_3	4.00±1.0	8.00±2.0	36.67±0.5	73.33±1.0
Days	AS	2.00±1.1	2.83±2.3	39.33±2.1	78.67±4.2
	AS+GA	2.00±1.1	4.00±2.1	46.33±2.6	92.67±5.4
	AS+THIO	3.00±0.7	6.00±1.4	45.00±3.2	90.00±6.0
	AS+KNO_3	1.67±0.5	3.33±1.2	46.00±2.4	92.00±5.0

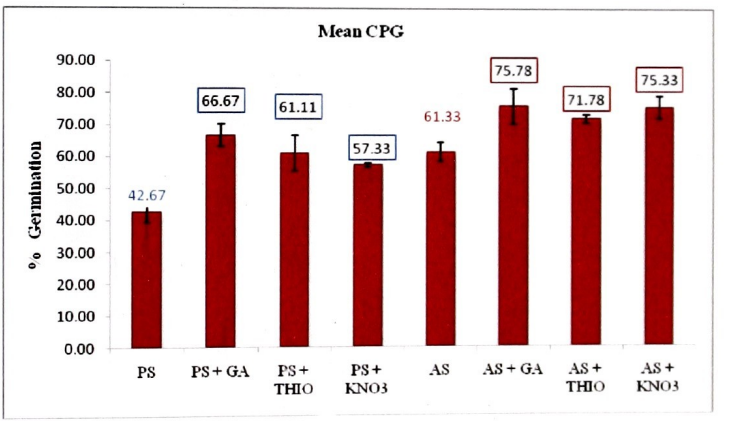
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

IX-1 Effect of combination treatments on seed germination in *Pterocarpus marsupium* Roxb.

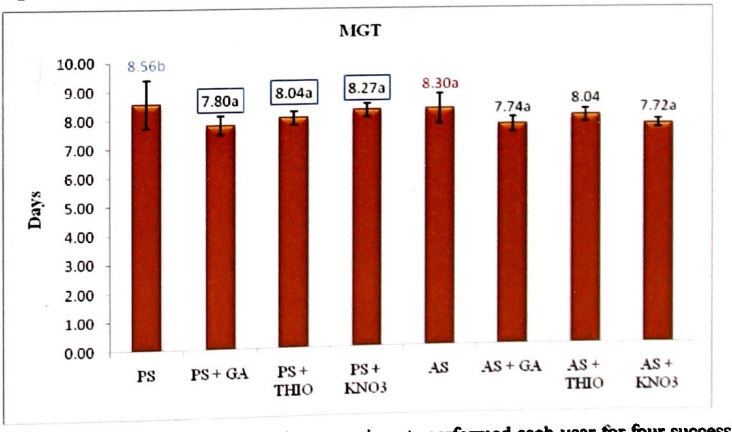
a) Cumulative percentage germination on 15th day



b) Mean Cumulative percentage germination



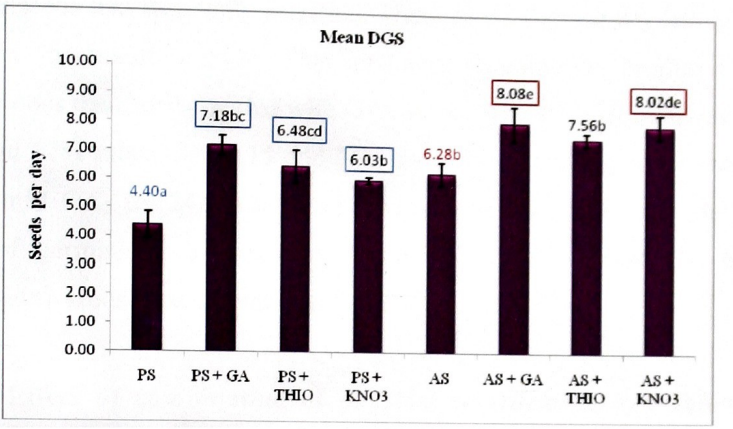
c) Mean germination time



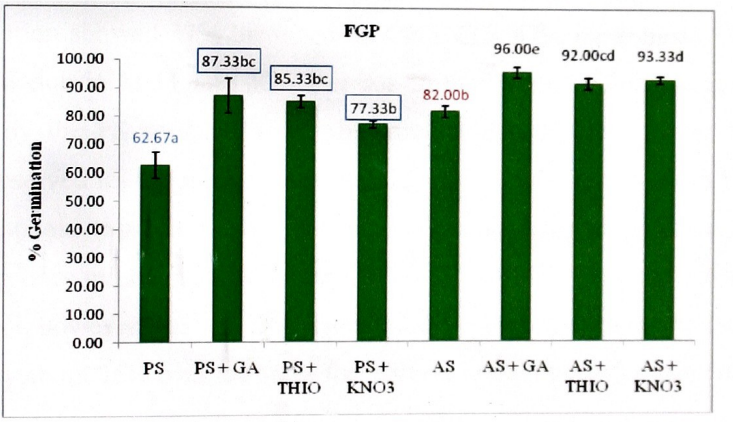
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

IX-1 Effect of combination treatments on seed germination in *Pterocarpus marsupium* Roxb.

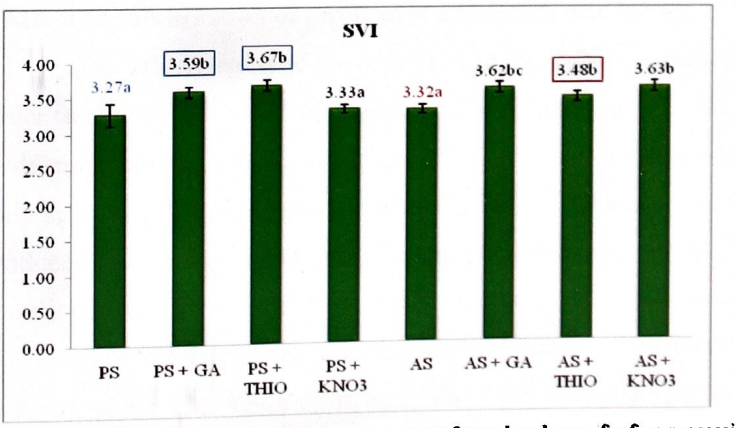
d) Mean daily germination speed



e) Final percentage germination on 30th Day



f) Seedling vigor



The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

combination treatment, the CPG T15 was significantly boosted to 82% over the seeds that were only scarified. These seeds showed 8% less MGT and 38% increment in FGP. This treatment combination produced the results better than seeds treated with GA_3 alone. The PS+GA combination produced 17% more CPG T15 and 24% more FGP than the seeds treated with 4 mM GA_3 for 24h. The MGT was reduced by about 24% and the speed of germination in terms of mean DGS was increased by 67% compared to seeds treated with GA_3 alone.

9.3.1.2 Effect of combination of physical scarification and thiourea

From the results recorded in Table IX-1 and Fig IX-1a-f, this combination showed 37% increment in CPG T15, 47% more mean DGS, 6% reduction in MGT and 30% increase in FGP compared to the values shown by the PS seeds. This combination produced results better than those observed for the seeds treated with 25 mM thiourea alone (Fig VI-1a-e). Compared to such seeds, the germination pattern was improved by showing only minor increase in the CPG T15 and FGP. The combination treatment, however, substantially increased the speed of germination (mean DGS) by about 35% over the seeds that received only thiourea treatment.

9.3.1.3 Effect of combination of physical scarification and KNO_3

The effectiveness of the combination PS+ KNO_3 was more or less similar to that of PS+ THIO combination (Table IX-1 and Fig IX-1a-f). The seeds thus treated had 32% more CPG T15 than the seeds only physically scarified. The treatment was less efficient in reducing the MGT and produced only 3% reduction. However, the mean DGS was significantly boosted by 37% in these seeds and the FGP was elevated to 77.33%. The combination treatment was better than the treatment of 80 mM KNO_3 alone (Fig VI-2a-e) as well. The CPG T15 and FGP in the seeds with combination treatment were boosted by 18% and 27% respectively compared to seeds that received only KNO_3 treatment. Further,

the MGT was reduced by 23% and the mean DGS was enhanced by 66% in the seeds with combination treatment.

9.3.1.4 Effect of combination of acid scarification and GA_3

The acid scarified seeds when treated with 3 mM GA_3 for 24 h showed 92.67% CPG T15 (Table IX-1 and Fig IX-1a-f and Plate IX-1). The MGT in these seeds was reduced by about 7% and the speed of germination (mean DGS) by about 27%. The overall germination (FGP) was also boosted to 96% thus showing an increment of 17% over FGP in the acid scarified seeds. This pattern was better than the seeds treated with GA_3 alone.

9.3.1.5 Effect of combination of acid scarification and thiourea

The acid scarified seeds treated with 25 mM thiourea showed 14% increase over the value shown by acid scarified seeds on CPG T15. In the seeds treated with combination treatment, the speed of germination in terms of mean DGS was improved by 20%. This combination was less effective in reducing the MGT and only 7% reduction was observed. The FGP was increased by 12% over seeds the seeds that were only acid scarified. The pattern of seed germination was superior to the pattern shown by seeds treated with thiourea alone (Table IX-1 and Fig IX-1a-f).

9.3.1.6 Effect of combination of acid scarification and KNO_3

As compared to only acid scarified seeds, the seeds exposed to the combination treatment showed 17%, 155% and 10% increase in CPG T15, mean DGS and FGP respectively (Table IX-1 and Fig IX-1a-f) and 47% reduction in MGT. The combination treatment was also better over the treatment of KNO_3 given alone, wherein, the CPG T15, mean DGS and FGP showed increments of 48%, 121%, and 54% respectively. The MGT was reduced by 73% in the combination treatment than in the seeds treated with KNO_3 alone.

The seedling vigor was also boosted by all the combinations of the treatments. The physical scarification alone produced the seedlings with vigor index of 3.27 which was significantly improved when the physically scarified seeds were treated with GA (SVI = 3.59) or thiourea (SVI = 3.67) and acid scarified seeds (SVI = 3.33) were treated with GA_3 (3.62) or KNO_3 (SVI = 3.63) (Fig. 9.2f).

In conclusion, among the different combination treatments tested, physical scarification followed by treatment with 4 mM GA_3 for 24 h and acid scarification in concentrated H_2SO_4 for 30 min followed by treatment with 4 mM GA_3 for 24 h were the optimum combination treatments for alleviating germination pattern in *Pterocarpus marsupium*.

9.3.2 *Santalum album* L.

The results of the effect of combination treatments are depicted in the Table IX-2 and Fig IX-2a-f.

9.3.2.1 Effect of combination of physical scarification and GA_3

When the physically scarified seeds were treated with 4 mM GA_3 for 24 h, the pattern of germination showed noteworthy improvement (Table IX-2 and Fig IX-2a-f). The CPG T30 was significantly boosted by 104%. There was 19% reduction in the MGT coupled with 190% faster speed of germination in terms of mean DGS. The overall germination was also increased as rise in the FGP to 80.67% compared to 48.67% in the seeds that were only scarified. The germination behavior was also superior to that shown by the seeds treated with GA_3alone (Fig VII-5a-e).

9.3.2.2 Effect of combination of physical scarification and thiourea

The physically scarified seeds when treated with 75 mM thiourea, the CPG T30, mean DGS and FGP were increased by 52%, 109% and 73% respectively over the physically scarified seeds. However, as compared to the seeds that received only 75 mM thiourea treatment for 24 h (Fig VI-3a-e), the combination treatment showed increments of 70%,

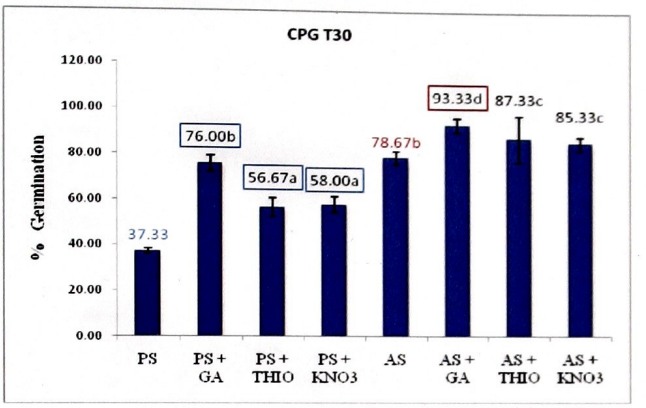
IX-2 Effect of combination treatment on germination in *Santalum album* L.

DAS	Treatment (for 24h)	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAS	Cumulative percentage germination
	PS	2.67±0.6	5.33±1.1	2.67±0.6	5.33±1.1
	PS+GA	13.67±2.0	27.33±4.1	13.67±2.0	27.33±4.1
	PS+THIO	9.33±2.3	15.67±4.6	9.33±2.3	15.67±2.3
10	PS+KNO_3	8.67±0.5	17.33±1.1	8.67±0.5	8.67±0.5
Days	AS	15.33±2.5	30.67±5.0	15.33±2.52	30.67±5.0
	AS+GA	23.00±4.5	46.00±9.0	23.00±7.0	46.00±14.1
	AS+THIO	19.33±2.4	38.67±4.4	19.33±2.4	38.67±4.4
	AS+KNO_3	20.00±0.7	40.00±1.4	20.00±0.7	20.00±0.7
	PS	7.67±0.6	15.33±1.1	10.33±0.58	20.67±1.1
	PS+GA	15.67±2.0	31.33±4.1	29.33±2.0	58.67±4.1
	PS+THIO	12.00±2.65	24.00±5.29	21.33±0.58	42.67±1.1
20	PS+KNO_3	9.67±1.5	19.33±2.0	18.33±1.5	36.67±3.0
Days	AS	22.00±3.6	44.00±7.2	37.33±2.8	74.67±5.7
	AS+GA	21.00±3.2	42.00±6.3	44.00±2.8	88.00±3.2
	AS+THIO	21.00±1 4	42.00±2.8	40.33±0.0	80.67±0.0
	AS+KNO_3	19.33±2.8	38.67±5.6	39.33±3.5	78.67±7.0
	PS	8.33±0.58	16.67±1.1	18.67±0.58	37.33±1.1
	PS+GA	8.67±0.58	17.33±1.1	38.00±1.7	76.00±3.4
	PS+THIO	7.00±2.0	14.00±4.0	28.33±2.0	56.67±4.1
30	PS+KNO_3	10.67±0.5	21.33±1.1	29.00±1.7	58.00±3.4
Days	AS	2.00±1.0	4.00±1.1	39.33±1.53	78.67±3.0
	AS+GA	2.67±0.7	5.33±1.1	46.67±2.1	93.33±4.3
	AS+THIO	3.33±1.1	6.67±2.2	43.67±2.4	87.33±4.3
	AS+KNO_3	3.33±1.4	6.67±2.8	42.67±2.1	85.33±4.3

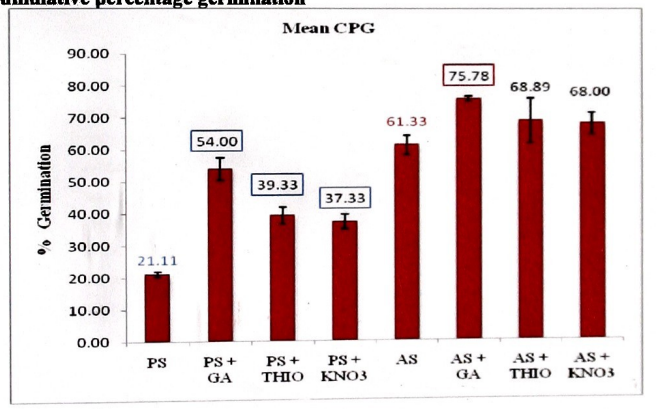
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

IX-2 Effect of combination treatments on seed germination in *Santalum album* L.

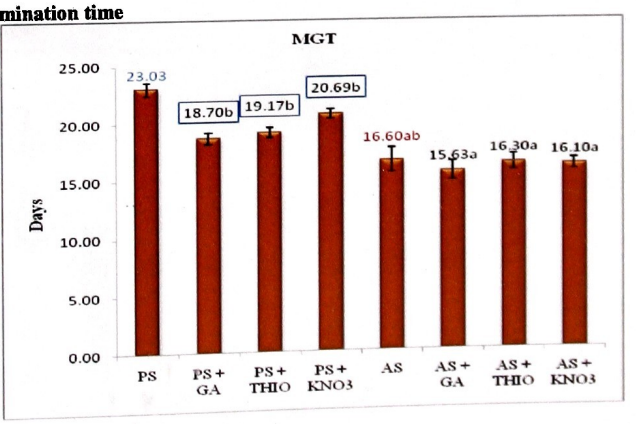
a) Cumulative percentage germination on 30th day.



b) Mean Cumulative percentage germination



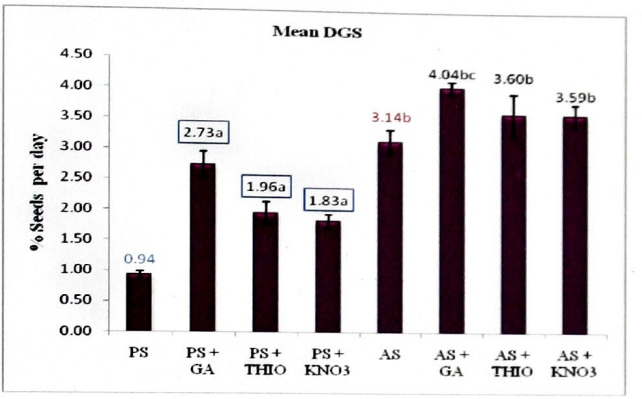
c) Mean germination time



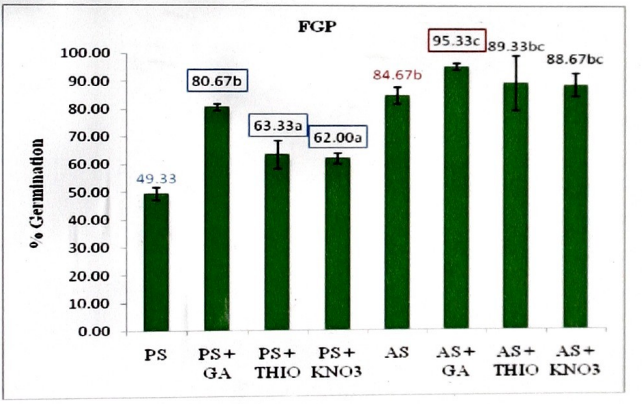
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

IX-2 Effect of combination treatments on seed germination in *Santalum album* L.

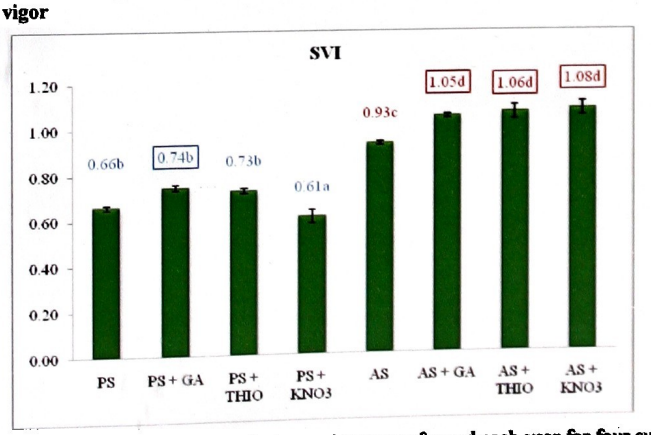
d) Mean daily germination speed



e) Final percentage germination on 60th Day



f) Seedling vigor



The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

Plate IX-1: Seedlings of *Pterocarpus marsupium* Roxb. and *Santalum album* L. from acid scarified seeds followed by treatment of GA_3

A. *Pterocarpus marsupium* Roxb.

B. *Santalum album* L

Plate IX-1

A

B

87% and 58% in CPG T30, mean DGS and FGP respectively. The reduction in the MGT shown by combination treatment was of about 17% than MGT in only physically scarified seeds and 4% than the seeds treated with 75 mM thiourea alone (Table IX-2 and Fig IX-2a-f).

9.3.2.3 Effect of combination of physical scarification and KNO_3

This combination enhanced the CPG T30 by 55% over seeds that were only scarified (Table IX-2 and Fig IX-2a-f). The mean DGS and FGP were substantially improved by 95% and 69% respectively over the values shown by scarified seeds. The MGT was shortened by 10% by the combination treatment compared to the scarified seeds. However, the combination treatment reduced the MGT by 14%, enhanced CPG T30 by 78%, mean DGS by 141% and FGP by 69% compared to the seeds that were treated with KNO_3 alone (Fig VI-4a-e)

9.3.2.4 Effect of combination of acid scarification and GA_3

The results presented in Table IX-2 and Fig IX-2a-f and Plate IX-1revealed that the seeds treated with concentrated H_2SO_4 for 30 min showed 78.67% CPG T30. The seeds germinated at the speed of 3.14% seeds per day with 16.60 days MGT and finally reached 84.67% FGP.

The acid scarified seeds treated with GA_3 showed 19% increment in CPG T30, 29% increment in mean DGS and 13% increase in FGP over only acid scarified seeds (Table IX-2 and Fig IX-2a-f). The MGT was reduced by 6%. The effectiveness of combination treatment was also better than the effect of GA given alone (Fig VII-5a-e). As compared to seeds that were treated only with 4 mM GA_3 for 24 h, the CPG T15, FGP and mean DGS were increased by 79%, 68% and 354% respectively and the MGT was reduced by 29%.

9.3.2.5 Effect of combination of acid scarification and thiourea

In the seed germination pattern exhibited by seeds treated with this combination (Table IX-2 and Fig IX-2a-f), there was 11%, 15% and 6% increase in the CPG T30, mean DGS and FGP respectively over the values obtained in seeds only acid scarified. As compared to seeds treated only with 75 mM thiourea alone (Fig VI-3a, VI-3d, VI-3e), these increments were 162%, 243% and 123% respectively along with 18% reduction in MGT.

9.3.2.6 Effect of combination of acid scarification and KNO_3

As compared to acid scarified seeds the seeds exposed to this combination of treatments showed 8%, 14% and 5% increase in CPG T15, mean DGS and FGP respectively. There was 3% reduction in MGT. The combination treatment was also better over the treatment of KNO_3 given alone (Fig VI-4a-e), wherein, the CPG T15, mean DGS and FGP showed increments of 161%, 372%, and 142% respectively. The MGT was reduced by 33% in the combination treatment than in the seeds treated with KNO_3 alone.

To summaries, in *Santalum album* the physically scarified seeds and the acid scarified seeds on treatment with GA_3 or thiourea or KNO_3 showed germination pattern superior to the pattern shown by each of the treatment alone. The seed germination pattern was better when the acid scarification was followed by GA_3 treatment, presoaking in thiourea and KNO_3 than the combination of same treatments applied to the physically scarified seeds. Among different combinations with acid scarified seeds, the combination of acid scarification followed by GA_3 treatment was the best to enhance germination in *Santalum album.*

Seedling vigor in the seedlings produced from treatments combined with physical scarification and from the treatments combined with acid scarification was remarkably different (Fig IX-2f). Among the combinations used with physical scarification, only the GA_3 treatment after

physical scarification of seeds could significantly improve the vigor compared to seedling from seeds only physically scarified or treated with GA_3 alone. On the contrary, all the combinations involving acid scarification produced more vigorous seedlings than the seedlings obtained in the seeds that were treated only with any one of the treatment. Among these combinations, the seedlings obtained from the acid scarified seeds treated with KNO_3 showed the maximum vigor that was 16% more over seedlings from acid scarified seeds and 64% more vigorous than the seedlings obtained from physically scarified seeds.

9.4 Discussion

Seed germination in species that produce seeds with thick seed coats or hard surrounding fruit structures may be improved if the seed coat is broken or scarified before planting (Crocker, 1907; Shipley and Parent, 1991). In *Pterocarpus marsupium* as well as *Santalum album* the true seed remains covered by thick pericarp and hard and woody endocarp respectively. When these seed-surrounding structures were impaired either by physical scarification or acid scarification boosting of seed germination was observed. The germination pattern in *Pterocarpus marsupium* as well as *Santalum album* was dramatically improved when the physical scarification was coupled with either GA_3, or thiourea or KNO_3 and acid scarification was followed by GA, or thiourea or KNO_3 treatment.

The physical scarification slightly opens up the central seed case in *P. marsupium* and induces a crack in the endocarp associated with the *S. album* seeds. This damage to the structures covering the true seeds facilitate the medium to have faster access to the enclosed seed. Therefore, physically scarified seeds imbibe faster than the non-scarified seeds. This might be the reason why physical scarification followed by the treatment of GA_3 or thiourea or KNO_3 resulted in the better germination pattern than the

pattern observed in the seeds that were only physically scarified or only imbibed in the solutions of GA_3, thiourea and potassium nitrate separately.

In *Pterocarpus marsupium* and *Santalum album,* the acid scarification possibly made the pericarp more permeable and also leached out the inhibitory substances, if any. Mayer and Poljakoff-Mayber (1963) postulated that in many species with poor germination the pericarp might produce an inhibitory substance, or act as a mechanical barrier to seed germination.

Secondly, the acid scarification alone improved the germination pattern more profoundly than the physical scarification in *Pterocarpus marsupium* and *Santalum album.* Acid scarification makes the seed coat permeable or reduces the seed coat thickness so that imbibition can occur at faster rate, while GA_3 overcomes embryo dormancy (Keogh and Banister, 1992; Dehgan and Pérez, 2005). This might have lead to far better germination pattern in all combination treatments involving acid scarified than when the same treatments were combined with physically scarified seeds or seeds only acid scarified.

Sequential application of acid scarification followed by imbibition with 10^{-4} M GA_3 is a technique widely used to germinate seeds with a hard seed coat and embryo dormancy (Hartmann *et al.,* 2002). This technique has proved consistently reliable for enhanced seeds germination in *Discaria toumatou* (Keogh and Banister, 1992) and has been shown to enhance embryo development in many other cases, resulting in significant improvement in germination time (Dehgan and Johnson 1982; Dehgan and Schutzman, 1983, 1989; Keeley and Fortheringham 2000; Baskin and Baskin 2001).

In *Scirpus robosfus* the acid treatment resulted in significantly higher germination percentages, indicating that probably the pericarp is removed by degrees (Dietert and Shontz, 1978).

The evidences of better output by combining two or more treatments than the treatments given individually are widespread in literature (Keogh and Bannister, 1992; Hartmann *et al.*, 1997; Rosner *et al.*, 2002; Dehgan and Perez, 2005).

Sequential application of acid scarification followed by imbibition with 10^{-4} M GA_3 is a technique widely used to germinate seeds with a hard seed coat and embryo dormancy (Hartmann *et al.*, 1997). Keogh and Bannister (1992) reported more than 80% germination in *Discaria toumatou* within 14 days of commencing treatment when scarification by immersion in 98% sulphuric acid for 24 min was followed by imbibition with a solution of 10^{-4} M GA for 24 hours.

Dehgan and Perez (2005) reported significantly enhanced germination rate in *Harrisia fragrans* when seeds were treated with a combination of H_2SO_4 and GA_3. Seeds scarified for 15, 30, and 45 s followed by 500 ppm GA_3, began to germinate faster than either of the treatment applied individually.

Rosner *et al.*, (2002) have demonstrated that the combination of a 30-min acid soak, 21-d warm stratification treatment, and 84-d cold stratification treatment (the shortest duration evaluated) was highly effective than each of the treatment applied individually in promoting germination in mountain snowberry (*Symphoricarpos oreophilus* Gray (Caprifoliaceae).

Germination in *Emmenanthe* was greatly dependant upon combination of acidity and certain anions such as nitrate or sulphate (Keeley and Fotheringham, 2000). In smoke-stimulated species nitrate in water fails to stimulate germination but under acidic condition arising due to buffers or added pyrolysis products, nitrate is stimulatory to germination suggesting that the combination of protons and the nitrate anion was involved in the germination response (Keeley and Fotheringham, 1998, 2000).

In *Medicago truncatula* seeds, the seed coat imposed dormancy is stronger than the physiological dormancy and the most efficient treatment to break the dormancy was the combination of acid scarification and cold imbibition (Faria *et al.*, 2005).

In *Opuntia ficus-indica* seeds, Altare *et al.*, (2006) have demonstrated the synergistic effect of combination of acid scarification with H_2SO_4 for 5 min followed by incubation in H_2O_2 from 1 to 5% on the germination process.

Kaye and Kuykendall (2001b) have recommended a combination of scarification and stratification to optimize germination speed and percentage in *Lupinus sulphureus*.

In conclusion, the results of the present investigation and the earlier reports mentioned above suggest that the physical scarification gives a direct channel for imbibition that results in the rapid germination in the scarified seeds than the unscarified seeds. In addition, the acid scarification reduces the thickness of the structures that surround the seeds and leaches out the inhibiting chemicals, if any. The improved imbibition after physical scarification and acid scarification facilitates better uptake of germination stimulating chemical used in the second treatment. And due to such synergistic effect a better germination pattern resulted as compared to the individual application of any of these treatments.

Chapter X

Seed germination *In Vitro*

10.1 Introduction

Santalum album L. is an important forest tree species of the family Santalaceae. The heartwood is commercially important in many parts of the world. It is valued for the rich aroma and sandal wood oil. The sandal wood and oil obtained from it is an important part of many cultural rituals in India. The species has a restricted distributed in the southern part of India. Due to great demand in the national as well as international markets, the species is over exploited which have resulted in the gradual decrease in its natural stands. The species is under the threat of extinction due to spike disease and illegal poaching (Das *et al.,* 2001) and therefore vulnerable to erosion of genetic diversity. The seed germination was found to decrease with increase in the duration of storage under ambient condition thereby excluding seed banking approaches for long term *ex situ* conservation. On the other hand, natural regeneration of *Santalum album* is poor because of low germination (10-20%) (Nagarajaiah and Rao, 1993). Secondly, seeds normally take more than 140-150 days for obtaining 80% germination (Nagaveni and Shrimathi 1981). Therefore, there is a need to study the *in vitro* germination behaviour and methods to improve it. This technique shall help not only for raising the seedlings for plantations but also would help to form the baseline for developing alternative method for its *ex situ* conservation. *In vitro* techniques offer one such alternative strategy for medium and long-term germplasm storage and as a regeneration system.

Tissue culture technology is successfully applied in the propagation of plants with poor and uncertain response to conventional propagation and for plants on the verge of extinction (Rout *et al.,* 2004; Sundarkumari *et al.,* 2005; Tsay *et al.,* 2006). Micropropagation and *in vitro* seed germination are the modern tools to produce large number of uniform plants in a short period and to overcome germination constraints in many species, notably orchids. *In vitro* seed propagation technique is a

powerful tool for *ex situ* biodiversity conservation of rare tropical orchid species suffering from over-collecting and continuous loss of their natural habitats (Stenberg and Kane 1998; Gangaprasad *et al.*, 1999). *In vitro* techniques are used not only for conservation of biological diversity, for multiplying endangered species that have extremely small populations, but also for species with reproductive problems and for recovery and reintroduction (Bromwell, 1990).

In the present investigation, physical methods like physical scarification, soaking seeds for different durations in various acids, germination stimulants and plant growth regulators helped substantially to improve seed germination in *Santalum album.* There is still a possibility to improve the germination further by using *in vitro* seed germination technique. This chapter describes the use of *in vitro* seed culture technique for rapid production of uniform seedlings in *Santalum album.*

10.2 Materials and methods

10.2.1 Source of the seeds

The completely matured black colored drupes of *Santalum album* were collected from a sandal tree growing naturally in the campus of University of Pune. The fruits were collected from off the ground. The thin pericarp and fleshy mesocarp were removed as described in the Chapter III. The true seeds still enclosed by hard endocarp were obtained and used for surface sterilization.

10.2.2 Surface sterilization of seeds

The seeds with hard endocarp were washed in 1% liquid soap for 30 min, after which they were washed thoroughly in sterile distilled water to remove the traces of soap. The seeds were surface sterilized in 0.1% $HgCl_2$ (w/v) for 10 min. The surface sterilized seeds were washed thrice with sterile distilled water. All the operations were carried out under aseptic conditions in the laminar air flow.

10.2.3 Removal of true seed

The endocarp from surface sterilized seeds was cracked open in a sterilized mortar with pestle. The cracked endocarp was manually removed and the true seed was separated out. The seeds were bulked in a conical flask containing 100 ml of sterile distilled water. The true seeds were again surface sterilized in 0.1% $HgCl_2$ (w/v) for 5 min followed by washing in sterile distilled water for three times. Such seeds were then treated with GA.

10.2.4 Soaking the seeds in GA_3

The surface sterilized seeds devoid of endocarp were treated with GA_3. In the earlier experiments (described in Chapter VII) the treatment of 4 mM GA_3 was optimum treatment for maximum seed germination. Therefore, for in vitro seed germination, 150 seeds were incubated for 12 h in 300 ml of 4 mM GA_3 solution. After incubation, the GA_3 solution was decanted off and the seeds were washed thrice with sterile distilled water.

10.2.5 Culture of excised embryo

From the seeds not treated with GA and seeds treated with 4 mM GA_3 for 12 h, the embryo was dissected out from the endosperm. Such excised embryos were inoculated on MS basal medium and MS medium fortified with 0.5 and 1.0 mg l^{-1} BA separately.

10.2.6 Culture medium and culture conditions

The GA_3-treated seeds were then inoculated on MS (Murashige and Skoog, 1962) culture medium without BA and supplemented with 0.5 and 1.0 mg l^{-1} BA separately. The MS medium contained 3% (w/v) sucrose and was gelled with 0.65% (w/v) agar. The pH of the medium was adjusted to 5.8 with NaOH or HCl. The medium was poured in the culture tubes (Borosil, 25 × 150 mm), each containing about 15 ml medium. The medium was sterilized by autoclaving at a pressure of 1.06 kg cm^{-2} for 20 min. Two seeds were inoculated in each culture tube. The seed cultures were incubated at 25±2 ^{0}C and were illuminated with

40-50 μmol m^{-2} s^{-1} intensity light from fluorescent tubes (40 W, Phillips India Ltd.) for 8 h followed by 16 h dark phase.

The seed germination counts were taken at the interval of 10 days. The cultures were maintained for a period of one month after which the seedlings were removed from the culture tubes and transferred in plastic glasses containing moist garden soil as a medium. The plants were than hardened in the shade-net house. The hardened seedlings were transplanted in the polythene bags containing moist garden soil.

10.3 Results

The results on *in vitro* seed germination in *Santalum album* are presented in Table X-1 and Fig X-1a-d and Plate X-2. The seeds used for *in vitro* germination were the 'true seeds' i.e. seeds separated from the hard endocarp case. The data on *in vitro* seed germination were recorded for a period of 30 days after which the seedlings were transferred for hardening. Therefore cumulative percentage germination recorded on 30 DAI (CPG T30) coincides with FGP.

The seeds without endocarp but not treated with GA (control) and cultured on MS salts medium showed the germination pattern more or less similar to that of untreated seeds (seeds without endocarp) sown in soil. These seeds showed least CPG T30 (46%) and mean CPG (12.67%) (Fig X-1a and b).These seeds required the longest time to complete germination (MGT = 23.28 days) with least mean DGS (0.56 % seeds per day).

The seeds treated with 4 mM GA_3 and cultured on MS medium devoid of any growth regulators showed improved germination pattern over control. All the parameters computed showed significant increment over control. The CPG T30 and mean CPG in these seeds showed about 255% and 237% increase over control (Fig. 9.1a and b). The spread of germination was significantly reduced by about 10% (Fig X-1c)

X-1 *In vitro* seed germination in *Santalum album* L.

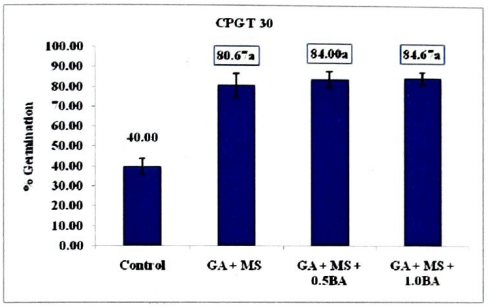
DAI	Treatment	Seeds Germinated	Percentage Germination during time interval	Total Seeds Germinated Since DAI	Cumulative percentage germination
10 Days	Control	2.33 ± 0.6	4.67 ± 1.2	2.33 ± 0.6	4.67 ± 1.2
	4 mM GA + MS	6.67± 0.6	13.33± 1.2	6.67± 0.6	13.33± 1.2
	4 mM GA + 0.5 BA	6.67 ± 1.2	13.33 ± 2.3	6.67 ± 1.2	13.33 ± 2.3
	4 mM GA + 1.0 BA	8.00 ± 1.0	16.00 ± 2.0	8.00 ± 1.0	16.00 ± 2.0
20 Days	Control	9.0 ± 0.6	18.00 ± 1.2	11.33 ± 1.0	22.67 ± 2.0
	4mM GA	23.33± 2.1	46.67± 4.2	30.00± 2.6	60.00± 5.3
	4mM GA + 0.5 BA	24.67 ± 1.5	49.33 ± 3.1	31.33 ± 2.5	62.67 ± 5.0
	4mM GA + 1.0BA	23.33 ± 1.5	46.67 ± 3.1	31.33 ± 2.5	62.67 ± 5.0
30 Days	Control	8.67 ± 0.6	17.33 ± 1.2	20.00 ± 1.2	40.00 ± 2.3
	4mM GA	10.33± 4.0	20.67± 8.1	40.33± 3.1	80.67± 6.1
	4mM GA + 0.5 BA	10.67 ± 4.5	21.33 ± 9.0	42.00 ± 2.0	84.00 ± 4.0
	4mM GA + 1.0BA	11.00 ± 3.5	22.00 ± 6.9	42.33 ± 1.5	84.67 ± 3.1

DAI = Days after inoculation

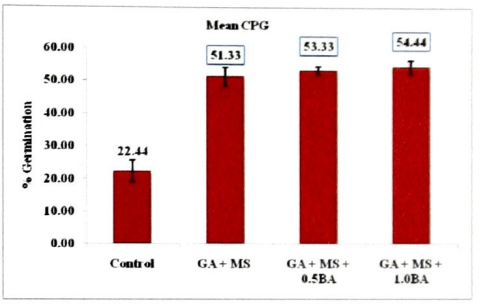
The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.

X-1 *In vitro* seed germination in *Santalum album* L.

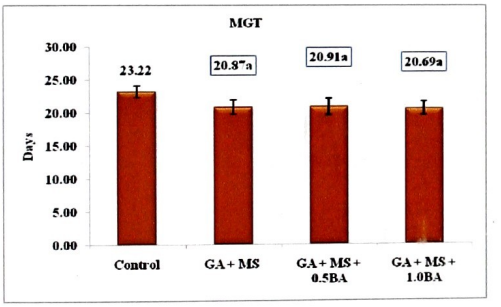
a) Cumulative percentage germination on 30th day



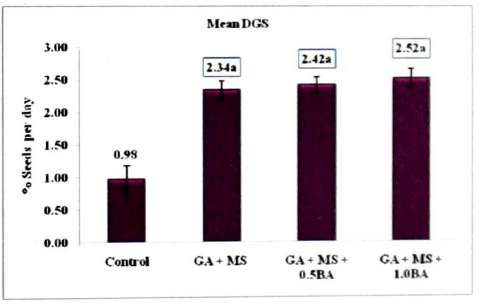
b) Mean Cumulative percentage germination



c) Mean germination time



d) Mean daily germination speed



The values represent (mean± SD) of three independent experiments performed each year for four successive years, each experiment performed on 50 seeds.
Bracketed values differ significantly from control as per Dunnett's Test at p= 0.05.
Treatment means with similar letters are not significantly different as per DMRT at p=0.05

Plate X-1: *In vitro* seed germination in *Santalum album* L.

a. *In vitro* germinated seeds of *Santalum album*

b. Twin seedlings

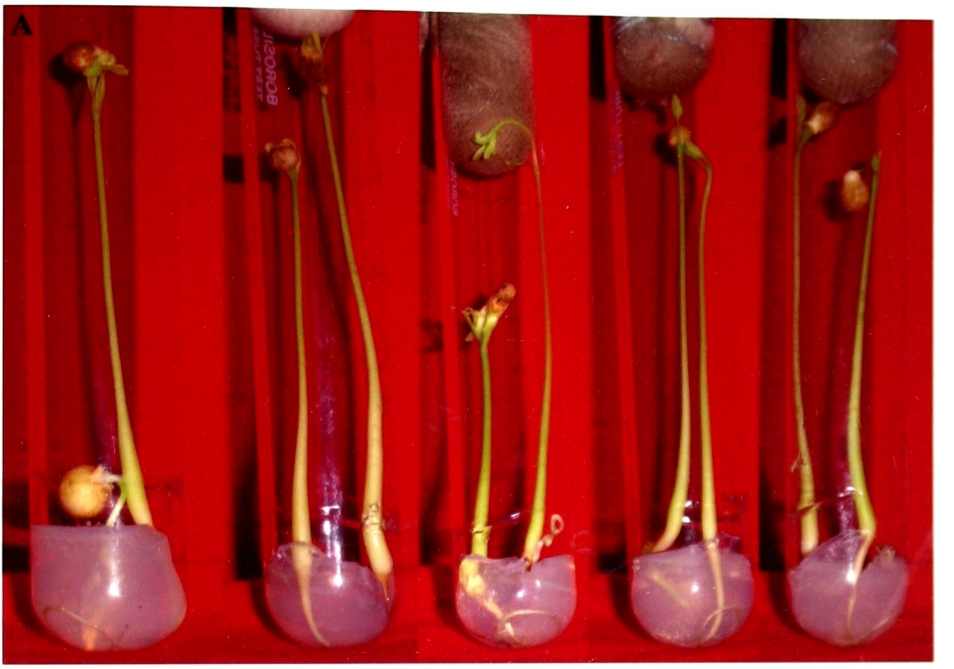
Plate X-1

A

Plate X-2: *In vitro* seed germination in *Santalum album* L.

a. *In vitro* seedling after 20 days of growth
b. Hardening off of *in vitro* seedlings

Plate X-2



and the seeds germination was faster (mean DGS = 2.34% seeds per day, Fig X-1d).

The seed germination pattern was further improved over control when the GA_3 treated seeds were cultured on MS medium fortified with 0.5 and 1.0 mg l^{-1} BA. However, the germination pattern on MS + 0.5 mg l^{-1} BA and MS + 1.0 mg l^{-1} BA did not show much variation.

The maximum CPG T30 and mean CPG observed was 84.67% and 54.44% respectively in GA treated seeds cultured on MS medium fortified with 1.0 mg l^{-1} BA (Fig X-1a and b). The mean time to complete germination was also less (MGT = 20.69 days) as compared to control (22.98 days) and the seeds germinated on MS + 0.5 mg l^{-1} BA. The fastest speed of germination in terms of mean DGS was 2.52% days for the seeds cultured on MS + 1.0 mg l^{-1} BA which was slightly better than mean DGS observed for seeds cultured on MS + 0.5 mg l^{-1} BA (2.42% seeds per day).

Occasionally, two seedlings emerged from a single seed and both the seedlings grew well and survived after transfer in the soil (Plate X-1).

In general, the *in vitro* germination was substantially increased in the seeds treated with 4 mM GA_3 for 12 h. The germination pattern was slightly improved further after fortification of MS medium with 0.5 and 1.0 mg l^{-1} BA, indicating that addition of BA to MS medium was not much helpful in improving germination pattern in GA_3 treated seeds.

The excised embryos cultured on MS basal medium as well MS medium fortified with 0.5 and 1.0 mg l^{-1} BA separately did not show any sign of growth even when maintained for a period of two months. Out of 200 embryos, only two embryos showed sign of germination i.e. slight elongation of plumule and radicle portion. However, these embryos died after 3 weeks of incubation. Therefore, the present method of excision and culture of embryo is not suitable to develop seedlings in *Santalum album.*

10.4 Discussion

GA_3 regulates germination in many different ways, especially through the elaboration of the endo-membrane system and the synthesis of new mRNAs used to regulate the protein synthesis required during germination (Jones and Stoddart, 1980). Changes in the characteristics of the membrane regulate the translocation of materials required for growth during germination (Pedroza-Manrique *et al.,* 2005). This might be the reason why exogenous applications of GA produce high percentage germination, under *in vitro* as well as field conditions. In the present investigation, the seeds treated with 4 mM GA_3 for 12 h showed significant improvement in the percentage germination as well as reduced the mean time to complete germination.

For the *in vitro* germination of *Cypripedium calceolus* and *Epipactis helleborine*, cytokinin was essential but not necessary for germination of *Dactylorhiza maculata* and *Listera ovalis* (Van Waes and Debergh, 1986). From the data recorded on seed germination in *Santalum album*, the treatment of seeds with GA_3 and subsequent culture on MS medium containing 0.5 and 1.0 mg BA l^{-1} only marginally improved germination pattern compared to that of GA_3 treated seeds cultured on MS basal medium lacking BA. This suggests that BA is neither essential nor necessary for *in vitro* seed germination in *Santalum album.* On the other hand, seed germination of *Cypripedium candidum* increased with the addition of cytokinins to the culture medium. Miyoshi and Mii (1995) also reported enhanced seed germination of *Calanthe discolor* after pretreatments with various levels of benzyladenine (BA), a common cytokinin used in plant tissue culture. These results indicate that the dependency on cytokinin during *in vitro* germination is species specific.

In vitro seed germination in *Comparettia falcata* showed a positive correlation with concentration of GA_3 present in the medium and the highest percentage of germination (91.4%) was obtained at 15μM GA_3 as compared to 53.7% germination in control (Pedroza-Manrique *et al.,*

2005). Addition of 15μM Kin to the medium containing 15μM GA_3 improved the germination further to 100%. The results obtained in the present investigation are in accordance with these results. In the present investigation, *in vitro* germination was marginally improved in GA treated seeds incubated on MS medium containing BA. Also in *Calanthe discolor* and *Cypripedium candidum,* pretreatment with cytokinin or its presence in the culture medium has improved germination (Van Waes and Debergh, 1986; Miyoshi and Mii, 1995). This improved germination may be attributed to cytokinin since cytokinins are thought to be assisting lipid mobilization in seeds with high levels of lipid reserves (Dimalla and van Staden, 1977). The excised embryo of *Santalum album* from the GA treated as well as non-treated seeds failed to grow on culture on MS basal medium without BA and containing 0.5 or 1.0 mg BA l^{-1}. This failure might be due unavailability of some factor present in the endosperm.

The seed germination behavior in root parasites such as *Sopubia delphinifolia, Orobanche aegyptiaca, Cistanche tubulosa* and *Striga asiatica,* suggest that these seeds require a stimulus present in the root exudates of the host plant for germination and seedling establishment (Kuijt, 1969; Shivanna and Mohan Ram, 2005). Seed germination in a hemi-parasitic angiosperm *Striga hermonthica* has prerequisite of a period of imbibition (2-14 d) at temperatures about 30 ^{0}C and seeds germinate only in response to compounds present in exudates from host roots and imbibed seeds simply kept moist do not germinate (Netzly *et al,* 1988; Stewart and Press, 1990). The germination of *Striga hermonthica* seeds triggered by root exudates from sorghum roots was detectable after 6 h and had reached its maximum by 20 to 24 h (Logan and Stewart, 1991).

In contrast to this, according to Rangaswamy (1967) and Shivanna and Rangaswamy (1976) it has been possible to induce seed germination *in vitro* in hemi-parasitic species in the absence of host stimulus. We agree with this assertion since though *Santalum album* is also considered as a hemi-root parasite (Scott, 1871; Barber, 1903; Kim *et al.,*

2006, Rama Rao, 1911) and in the present investigation; the results on *in vitro* germination of its seeds (and so also the nursery germinations) were achieved in the absence of any host stimulus. The developed seedlings were maintained in the polythene bags containing garden soil and in absence of any host. These plants were morphologically identical with the naturally growing seedlings.

The findings of these experiments will facilitate the development of strategies for ***ex situ*** conservation of *Santalum album* which is of significant interest to compliment ***in situ*** conservation and secure sustainable utilization of this species.

Chapter XI

Nursing The Seedlings

11.1 Introduction

Seed propagation is the principal mode of plant production in temperate as well as tropical silvicultural practices. In managing nursery operations based on seed propagation, the main objective after seedling development is to provide optimum conditions for survival and growth of seedlings into strong healthy trees.

Better understanding and implementation of nursery cultural practices to improve seedling quality enables better matching of seedlings to forest sites, reducing the chance of regeneration delay and improving future growth of forest stands (Duryea, 1984).

The cost of tree seedlings is a major component of any tree planting project. Nursery phases are an important part of the operation in the cultivation of many tropical tree crops (Ayodele, 1997). Keeping the seedlings growing in the nursery until they are big enough, tougher and more vigorous, save seeds, space and water and reduces the risk of damage to or loss of the plant (Ayodele, 1997).

This chapter describes the nursery practices administrated after the successful seed germination in *Pterocarpus marsupium* Roxb. and *Santalum album* L. for the production of quality tree seedlings with a potentially high survival rate after planting out.

11.2 Materials and methods

The seeds germinated in the cavity seeding trays and seed beds were transplanted in the polythene bags of size 4" × 6" containing moist garden soil. The transplantation was carried out in the morning so as to avoid the thermal shock to the seedlings. The polythene bags containing seedlings were maintained in the shade-net house. The shade-net house was equipped with the net that can cut 50% of the incident light.

11.2.1 Watering

The most important factor in germination and seedling production is water. The soil in the bags and cavity seeding trays was never allowed to dry. While watering, excessive watering was avoided as it is nearly always damage the seedlings by replacing the air in the soil resulting in compaction, which in turn restricts the respiration process of the plant. Therefore the seedlings were watered at the interval of 3 days.

To avoid physical injury to the seedlings the watering was done with rubber hose fitted with mist nozzles. Watering was done preferably in the mornings and avoiding the mid-day period to avoid excessive evaporation and thermal injury.

11.2.2 Control of weeds

Any herbaceous plant growing in the containers along with the seedlings were considered as weed. These weeds compete with the seedlings for nutrients, water and light. Therefore, all such weeds were immediately uprooted as they are more vigorous and grow at a faster rate. The prominent weeds observed were species of *Oxalis* and *Cyanodon dactylon.*

11.2.3 Pruning

In the seedlings of *Pterocarpus marsupium* that were more than one year old, the roots had a propensity to come out of the polythene bags. The roots were pruned only in such instances. The polythene bags were lifted gently and the roots were pruned to the level of polythene bag. Root pruning was practiced only in the rainy season. The leaves and branches that showed the signs of diseases or damaged by the insects were removed with sharp secateurs.

11.2.4 Control of insect pests

For controlling the insects that feed on the leaves, 1% Rogor was sprayed in the shade-net house at the interval of one month.

11.2.5 Hardening off

The main objective of hardening off the seedlings is to condition the plants for survival in the relentless environmental conditions in the field. It is usually done by progressively pulling out the seedlings from the more controlled and favorable conditions in which seedlings were nurtured.

After about one year of growth in the shade-net house (Plate XI-2 and Plate XI-4), the seedlings were shifted to the open space conditions in the garden. The quantity of water and frequency of watering the seedlings was reduced and intermittently the soil was allowed to become dry. The watering was done just before the plants start showing the signs of wilting.

11.2.6 Assessment of seedling quality

The seedlings of *Pterocarpus marsupium* and *Santalum album* produced in the various experiments were evaluated for their quality on the basis of water content (WC) and root to shoot ratio. The water content was analyzed on the basis of entire seedling.

An integrated approach of Dickson *et al.* (1960) to quantify morphological quality was followed to formulate a quality index (QI) which included morphological features of height, weight of aerial parts of seedling, diameter of the seedling at soil level and weight of root system. The QI was calculated as

QI = seedling dry weight (g) / [height (cm) + shoot weight (g) /diameter (mm) + root weight (g)]

The QI was calculated separately for 25 randomly selected seedlings obtained from different experiments. The seedlings of *Pterocarpus marsupium* at one, two and three years of age and seedlings of

Santalum album at one and two years of age were subjected to QI analysis. The QI was expressed as mean ± standard deviation.

11.3 Results

11.3.1 *Pterocarpus marsupium* Roxb.

The seedling survival was 100% in the seedlings generated in different experiments and transplanted in the polythene bags. The data on seedling height and number of leaves is presented in Table XI-1, Plate XI-3.

Irrespective of the initial treatment given to the seeds for seed germination, all the seedlings showed similar morphology. The seedlings obtained from seeds treated with GA_3 showed slightly longer stem in the initial stages of growth. After about one year of growth, there was not much variation in the seedlings with respect to height and number of leaves.

All the seedlings of *Pterocarpus marsupium* gradually shed their leaves in the summer season (March, April and May) and by the end of the month of May, the seedling is almost completely devoid of leaves. During this period, the necessity of water was very low. The seedlings quickly regained the vitality with the onset of monsoon season in the month of June.

The results on evaluation of seedling quality are presented in Table XI-1. The 1-3 years old seedlings showed WC in the range of 88-90%. The shoot : root ratio for these seedlings remained in the range of 1 to 1.5. The roots in two and three year old seedlings showed coiling in the polythene bags. The seedling quality index was in the range of 1-2 for 1-3 years old seedlings.

11.3.2 *Santalum album* L.

The seedling survival was 100% in the seedlings generated in different experiments and transplanted in the polythene bags. The data on seedling height and number of leaves is presented in Table XI-2 and Plate XI-4.

XI -1. Evaluation of seedlings of *Pterocarpus marsupium* Roxb.

Seedling characteristic	Seedling age (years)		
	One	Two	Three
Height (cm)	28.3 ± 5.8	48.8 ± 3.4	89.9 ± 5.3
Weight of shoot (g)	28.12 ± 7.4	48.45 ± 9.3	89.78 ± 10.8
Weight of root system (g)	9.34 ± 5.2	11.56 ± 4.9	21.67 ± 6.4
Diameter of stem (mm)	4.2 ± 1.5	6.7 ± 2.4	7.4 ± 2.8
Dry weight (g)	4.16 ± 1.2	7.72 ± 1.8	11.18 ± 1.3
Water content (%)	88.81 ± 4.5	89.31 ± 4.3	88.44 ± 5.2
Shoot : root ratio	1.2 ± 0.2	1.4 ± 0.5	1.5 ± 0.5
Number of leaves	12.0 ± 3.0	21.0 ± 5.2	29.0 ± 7.6
Quality index (QI)	0.92 ± 2.2	1.26± 1.9	1.95 ± 2.7

The values represent (mean± SD) of three independent experiments.

XI -2. Evaluation of seedlings of *Santalum album* Roxb.

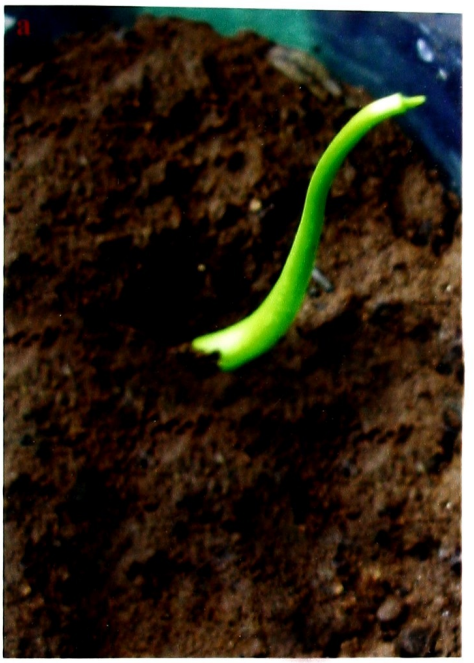
Seedling characteristic	Seedling age (years)	
	One	Two
Height (cm)	25.3 ± 5.8	45.6 ± 3.4
Weight of shoot (g)	10.12 ± 3.4	14.45 ± 3.7
Weight of root system (g)	3.34 ± 1.2	4.56 ± 1.3
Diameter of stem (mm)	3.2 ± 1.5	4.7 ± 2.4
Dry weight (g)	1.76 ± 0.2	2.72 ± 0.3
Water content (%)	86.81 ± 4.5	84.38 ± 4.3
Shoot : root ratio	1.2 ± 0.2	1.4 ± 0.5
Number of leaves	13.0 ± 4.2	24.0 ± 3.1
Quality index (QI)	0.29 ± 2.2	0.46 ± 1.9

The values represent (mean± SD) of three independent experiments.

Plate XI-1: Regenerative capacity in *Santalum album* L.

a. Decapitated seedling
b. Shoot primordia
c. Development of shoots

Plate XI-1



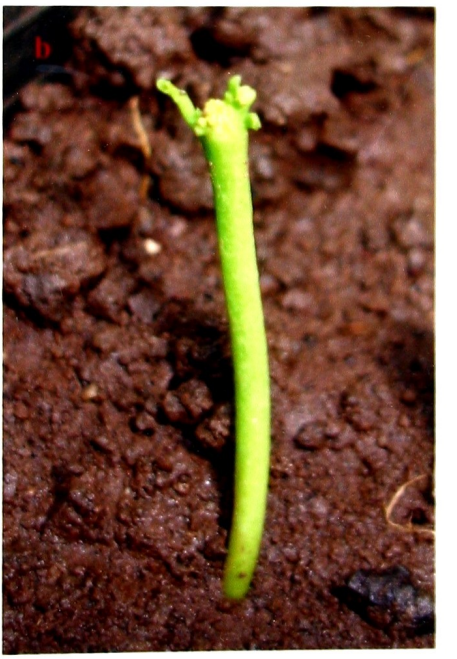


Plate XI-2: Seedlings in shade-net house

a. Seedlings of *Pterocarpus marsupium* Roxb.

b. Seedlings of *Santalum album* L.

Plate XI-2

Plate XI-3: Well developed seedlings of *Pterocarpus marsupium* Roxb.

1. One year old

2. Two year old

Plate XI-3

Plate XI-4: a) Well developed seedlings of *Santalum album* L.

b) Shade-net house

Plate XI-4

After six month of growth all the seedlings of *Santalum* derived from seeds treated differently exhibited same general vigor. The seedlings obtained from seeds treated with GA_3 showed slightly longer stem in the initial stages of growth. The data on seedling characteristics is presented in the Table XI-2.

The seedlings of *Santalum album* generated in these experiments are about 2 - 2.5 year old. These seedlings have survived without any host.

The seedlings obtained from the experiments on *in vitro* seed germination were also nurtured along with the seedlings raised in the soil. There was no difference in the morphology of seedlings grown *in vitro* and seedlings raised from the seeds sown in the soil.

In about 8% seedlings, the leaves showed curling because of some disease. The regenerative capacity of the *Santalum* seedlings was very high. The apical meristem was consumed by insects in some seedlings. In such seedlings, numerous shoot primordia were observed in the area just below the region of shoot apical meristem (Plate XI-1). One or two such primordia developed the shoot and the seedling continued the growth.

The results on evaluation of seedling quality are presented in Table XI-3.The one and two years old seedlings showed WC in the range of 84-86%. The shoot : root ratio for these seedlings remained in the range of 1.2 to 1.4 indicating slow growth of the roots. The seedling quality index in the two year old seedlings was 0.46.

11.4 Discussion

A nursery is a place where plants are propagated and grown to suitable size. The main objective in managing nursery operations based on seed propagation is to obtain good germination and provide optimum conditions for survival of seedlings and their growth into strong healthy trees (Hartman *et al.*, 1997).

The cost of tree seedlings is a major component of any plantation program and therefore quality tree seedlings having a high survival rate after planting out is of crucial importance. The quality of seedlings depend upon two factors- the seeds from which the seedling is produced and the aftercare extended to ensure better health of seedling. High quality in plant materials is achieved through nursery practices. The seedlings maintained out of the ground have high mortality due to improper care and handling (Duryea, 1984).

Once seeds have germinated and the seedlings have been transplanted, there is need to prevent damping-off and to develop healthy plants capable of withstanding further transplanting in the field without affecting the growth (Hartman *at al.,* 1997). In the present investigation, the seedling of *Pterocarpus marsupium* and *Santalum album* were transplanted in the garden soil without humus and watered adequately, and the out set of damping-off was prevented. However, in case of *Santalum album* excess watering caused stunted growth of the seedlings and yellowing of the leaves. On the other hand, excess watering did not affect the health of the *Pterocarpus marsupium* seedlings. These seedlings also tolerated infrequent watering even in the summer season. The coiling in the roots of 2 and 3 years old seedlings suggest that these seedlings should be used for reforestation and similar uses when they are about 1-1.5 years old.

In conclusion, the *Santalum album* seedlings required less frequent watering while excess water damaged the seedlings. In *Pterocarpus marsupium* the seeding could tolerate lees frequent watering as well as excess watering.

The seedling quality index (QI) in the seedlings of *Pterocarpus marsupium* and *Santalum album* indicated the appropriateness of the nursery practices followed for tending these seedlings.

The seedlings of *Santalum album* were maintained in the polythene bags containing garden soil and in the absence of any host. These plants were morphologically identical with the naturally growing

seedlings. Baskin and Baskin (2001) have put forth that *Santalum album* do not require host stimulus for germination of its seeds. The results of the present investigation indicate that that for seed germination as well as during the early stages of seedling development in *Santalum album* presence of host is not required.

Chapter XII

Summary And Conclusions

The natural forest covers are under pressure due to various human activities like urbanization, industrialization, deforestation, and agriculture. The extensive deforestation and habitat conversion is recognized as the biggest factor in the biological diversity crisis (May *et al.*, 1995, Khurana and Singh, 2001). It has been predicted that the forest cover will drastically decline in the next few decades and the tropical areas of Asia and Africa continents would suffer most. Seed biology of many temperate species has been thoroughly investigated but the research on tropical and subtropical species is lagging behind; thus our knowledge about tropical tree seed physiology is still inadequate (Phartyal *et al.*, 2002). Knowledge on germination and seedling establishment is of pivotal importance for understanding such community processes as plant recruitment and succession and for the management of plant populations (Khurana and Singh, 2001).

The increased interest in tree seeds may be attributed not only to the large-scale practices of artificial regeneration, but also to the growth of the agroforestry, social forestry, commercial nursery operation, watershed management and restoration of degraded areas (Phartyal *et al.*, 2002). Therefore there have been high demands for seedlings of different tree species for plantation work.

To cope up with the increasing demands for tree saplings from various sectors, we need basic knowledge about seed harvesting, processing, germination, seed storage, viability, dormancy, vigor and seed storage physiology, preparation of uniform seedlings, maintenance of seedlings and their transfer to the field conditions. Giving the due consideration to these facts, the present investigation was focused on the seed germination pattern and its alleviation in commercially important indigenous forest tree species *Pterocarpus marsupium* Roxb. and *Santalum album* L. with the following objectives:

1. To assess the viability in the seeds
2. To study the germination behavior
3. To break the dormancy and reduce the germination time

4. To develop efficient procedures for uniform and fast seed germination
5. To study the effect of storage period on seed germination
6. To assess efficiency of *in vitro* seed germination
7. Preparation and maintenance of seedlings in nursery
8. Evaluation of seedling quality

The seed sources were authenticated from the Botanical Survey of India, Western Circle, Pune, India.

The seeds of *P. marsupium* were collected in the month of March 2004, 2005, 2006 and March 2007 whereas the seeds of *S. album* were collected in the month of May 2004, 2005, 2006 and May 2007. The experiments were carried out on the freshly collected seeds of each season. The seeds were stored under ambient conditions. The experiments were repeated at least thrice. A completely randomized design of experiments was adopted. The data was analyzed for variance by following ANOVA. The treatment means were then compared with the control mean by using Dunnett's Test at p=0.05 (Dunnett, 1964). The effectiveness of the treatments was assessed by comparing the treatment means by following Duncan's Multiple Range Test (DMRT) (Duncan, 1955).

Following are the important findings in the present investigation.

***Pterocarpus marsupium* Roxb.**

1. The seed viability tested with the help of 2,3,5-Triphenyl Tetrazoliumchloride (TZ) indicated higher viability of 96% whereas the results on nursery germination revealed that only about 42% seeds could germinate and to achieve this level of germination 30 days period was required. The remaining of the seeds was sound and healthy but

failed to germinate. This indicated that though the seed viability is very high in these species, there are certain constrains required to be overcome for seed germination to occur. This also indicated that the species has poor seedling vigor in addition to dormancy.

2. To separate the seeds from samara with a very thick pericarp, the central seed case was incised with secateurs and the gap was manually widened to open the seed case and separate the seed. All the separated seeds soaked in water showed rapid imbibition stress that was evident from cracking of testa and cotyledons. The seeds are thus very fragile. These injured seeds failed to germinate. To separate the seeds from the fruit was laborious, time consuming and the seed were often injured during separation. Therefore using seeds separated from fruit for raising seedlings in the nursery is impractical. It indicates that the support of the surrounding hard seed case is required during initial imbibition and seed germination to prevent physical damage caused by rapid imbibition.

3. Improved germination pattern was observed in *P. marsupium* on the removal of orbicular wing of the pod (dewinged seed), exposure of dewinged seeds to hot and boiling water and physical scarification. This was evident by increase in final germination percentage by 50%, 20%, 35% and 60% respectively. Among these, the physical scarification not only effectively enhanced germination percentage (FGP = 67.33%) but it was also coupled with reduced germination spread of 8.43 days and higher germination speed (mean DGS = 4.41% seeds per day) than the control seeds (FGP = 42%, MGT = 11.33 days, mean DGS = 1.80% seeds per day).

Since the seed coat of *P. marsupium* is hard, it takes more time to germinate with lower germination percentage in nursery establishment. In scarified seeds the improved pattern of seed

germination in respect to faster germination, higher germination percentage and seedling vigor index in comparison to control thus suggest that in *P. marsupium* there is physical dormancy which could be alleviated by artificial rupturing of the central seed case of the pod (considered as seed in the present investigation).

4. Presoaking the seeds in concentrated H_2SO_4 for 30 and 45 min resulted in 85.33 and 87.33% FGP respectively, which was significantly more than the best control response (FGP = 43.33%). The treatment of concentrated HCl for 30 min also gave significantly higher germination percentage as compared to control. The acid scarification carried out for these durations might have partially dissolved the seed coat and/or created minute pores in the seed coat thereby enhancing its permeability to water and/or oxygen. This might be the reason behind the faster seed germination.

5. Compared to control, the germination pattern was very poor in the seeds scarified with concentrated HNO_3. This suggests that HNO_3 appears to be inhibitory to seed germination. The seeds presoaked in concentrated HCl and H_2SO_4 for more than 45 min either suppressed the germination or produced damaged seedlings with high mortality. During prolonged immersion in concentrated acids, the embryo might have exposed to acids that resulted in injuries to its vital parts.

6. Generally thiourea has been used to alleviate poor germinability related to salt stress. In the present investigation, though the seeds were not subjected to salt stress, the presoaking of seeds in thiourea (5-25 mM) improved germination pattern. The treatment of 25 mM thiourea for 24 h significantly improved CPG T15, mean DGS and FGP. This suggests that thiourea is also effective in promotion of seed germination even when the seeds are not subjected to salt stress.

7. The KNO_3 treatments significantly affected the germination pattern. Earlier germination was recorded in seeds treated with 80 mM KNO_3 for 24 h. This was indicated by higher values on CPG T15, and lower MGT accompanied by higher value of FGP. These seedlings had higher vigor index compared to non treated seeds. However, the treatment of thiourea was superior to KNO_3 for promotion of seed germination.

The physical treatments like cutting of wing, manual cutting of seed case to expose seed (physical scarification), immersion of seeds in hot water and boiling water were effective in promoting seed germination pattern. The diaspore in *P. marsupium* possesses a tough and densely fibrous fruit coat which has high mechanical resistance which makes physical scarification tedious, laborious and time consuming.

The acid scarification with concentrated H_2SO_4 for 30 min resulted in 85.33% final germination percentage, but the method required a very careful handling and over exposure to the acid resulted in damaged seedlings. About 2000 ml of concentrated H_2SO_4 (costing about Rs. 400/-) would be required for scarifying 1000 seeds.

The use of germination stimulant thiourea (25 mM for 24 h) resulted in 86% germination within 30 days. This method was relatively simple, safe and convenient. The cost involved in this method was about Rs. 25/- for the treatment of 1000 seeds. The use of germination stimulants is therefore recommended over physical scarification as well as acid scarification method for improving seed germination in *Pterocarpus marsupium*.

8. Exogenous GA has been used for many species to satisfy the needs of embryo for stimulating germination and to increase the embryo growth potential. In the present investigation, application of GA_3 increased germination and decreased mean germination time. The 3 mM GA_3

treatment for 24 h was the best treatment and maximum seed germination of 70% was observed in the treated seeds. The presence of GA_3 appeared to speed up germination over control but the response was inferior as compared to treatment of thiourea.

The treatment of presoaking the seeds in cytokinin (BA and Kin) solutions was slightly effective for improving seed germination pattern. The treatments of Kin (3.0 mM for 12 h) and BA (0.25 mM BA for 12 h) gave final germination percentage of 52.67% and 58.67% respectively. The cytokinins are therefore not very effective in stimulating the germination in this species.

The treatments of 2.5 – 10.0 mM IAA given to the seeds of *Pterocarpus marsupium* resulted in reduced germination and poor seed germination pattern compared to the untreated seeds. This is suggestive of prevention of germination due to exogenous IAA. In the early stages of germination the embryos require very little or no IAA because hormonal receptors of IAA are not well developed. This might be the reason for the inhibition of seed germination at the tested concentration.

The exogenous application of auxin IAA was inhibitory to seed germination in *P. marsupium*. The cytokinin could only marginally improve the germination pattern and the GA_3 treatment was slightly superior over cytokinin treatment to stimulate seed germination. The treatment of thiourea was much more effective than the use of Kin, BA as well as GA_3.

9. The results on the activity of α-amylase and β-amylase in the seeds imbibed in the solutions of GA_3, BA, Kin and IAA at different concentrations and durations indicated that among the PGRs used, the GA_3 significantly enhanced the activity of these enzymes, whereas BA and Kin could only marginally induce the activity. The exogenous

application of IAA was inhibitory to the activity of α-amylase as well as β-amylase in the seeds of *P. marsupium.* These results were also reflected in the germination pattern, wherein the GA_3 treatment significantly improved germination than the BA or Kin and the germination was retarded due to exogenous application of IAA.

10. The seed germination pattern was studied in the fresh seeds and seeds stored for one, two and three years under ambient conditions. The seed germination behavior in untreated seeds and seeds treated with physical scarification and most optimum treatment of GA_3 was studied.

The germination percentage declined in the stored seeds as compared to fresh seeds. The percentage germination in untreated seeds was 35% which declined by about 25%, 55% and by about 80% in seeds stored for one, two and three years respectively. The physical scarification and the optimum treatment of GA_3 (4 mM for 24 h) which was effective in alleviating germination in fresh seeds were unable to improve the germination in stored seeds.

The seed viability in the stored seeds assessed with TZ test indicated higher viability than the observed percentage germination. However, the percentage viability in stored seeds was less as compared to fresh seeds. The electrical conductivity test has demonstrated higher contents of seed leachates from the stored seeds as compared to the fresh seeds. The endogenous levels of GA_3 and IAA were found to be lower in the stored seeds than the fresh seeds. These are indications of physiological deterioration of the seed tissues which might be responsible for inferior germination in the stored seeds. Therefore the freshly collected pods should be used for raising seedlings of *Pterocarpus marsupium* in nurseries.

11. The acid scarified and physically scarified seeds were treated with optimum treatment of GA_3 (4 mM for 24 h) and thiourea (25 mM for 24 h). The performance of such seeds was better than seeds that received each of this treatment individually. Among the different combination treatments tested, physical scarification followed by treatment with 4 mM GA_3 for 24 h and acid scarification in concentrated H_2SO_4 for 30 min followed by 4 mM GA_3 for 24 h were the optimum combination treatments for alleviating germination pattern in *Pterocarpus marsupium.*

The germinated seeds from the laboratory experiments and seeds sown in the seeding trays were transferred to the polythene bags containing garden soil showed the lag phase of growth of about 2 weeks after which the rapid elongation of seedlings was observed. The seedlings attained the maximum mean height of 10 cm in 20 days after transfer to the polythene bags.

The seedlings of *Pterocarpus marsupium* growing in the polythene bags in rainy season showed rapid growth and attained the height of 25-30 cm in one year after sowing the seeds. Similar to mature trees of this species, all the seedlings of *Pterocarpus marsupium* gradually shed their leaves in the summer season (March, April and May) and by the end of May, the seedlings were almost completely devoid of leaves. The seedlings quickly regained the vitality with the onset of monsoon season in the month of June. The seedlings developed during winter and summer showed comparatively slow growth.

The nursery practices used for this species were sufficient to give vigor to the seedlings as indicated by the low moisture content, high quality index and root : shoot ratio. The seedling survival was 100% in the seedlings generated in different experiments. The one year growth of seedlings in nursery appears to be sufficient before transfer to

the field. The seedlings of two and three year's age attained the height of 48 cm and 89 cm.

Santalum album L.

1. In the nursery germination experiments, only about 2-3% seeds showed germination in 10-15 days. There was maximum of 25% germination by the end of 60 days. The remaining seeds did not germinate even after 90 days from sowing. However, the 2,3,5-Triphenyltetrazolium chloride (TZ) test indicated higher seed viability of 94%. This indicated that though the seed viability in fresh seeds is very high, most of the seeds are unable to germinate naturally. This might be because of involvement of some dormancy principle and therefore directed efforts are required to shift the seed from dormant to quiescent state.

2. As compared to untreated seeds, only marginal improvement in the seed germination pattern was observed with wet heat treatment (hot water for 20-40 min). Seeds subjected to boiling water treatment showed less germination as compared to untreated seeds indicating possible thermal injury to seeds.

   As compared to seeds with intact endocarp, simple cracking of endocarp as well as its complete removal almost doubled the germination percentage. However, the reduction in the mean germination time was not observed. Therefore, the stony endocarp might be acting as one of the barrier in seed germination. Thus, there exists some degree of physical dormancy in *S. album*.

3. The acid scarification of seeds resulted in slightly improved germination pattern. Among the various treatments of concentrated HCl, H_2SO_4 and HNO_3, treatment with concentrated H_2SO_4 for 45 min was better for induction of germination. The acid hydrolysis of the

endocarp might have improved its permeability to water and at the same time must have weakened the endocarp. These two factors synergistically might have assisted in improving seed germination. This can also be taken as an indication of presence of physical dormancy in the species.

Taking in to account the risks in handling the acids, injury to the seeds and very little effectiveness of the acid scarification, the removal of endocarp is convenient for induction of germination in *S. album*.

4. Thiourea has been known as a germination stimulant. In the present investigation improved germination was observed with the treatments of thiourea and the treatment of 100 mM thiourea for 24 h was more effective for stimulation of germination. The mean germination time was reduced by 6 days and germination percentage was increased by 48% as compared to control. The seeds were treated with different concentrations of KNO_3 (25-100 mM) and the treatment of 100 mM KNO_3 slightly alleviated the germination over control but the pattern was similar to that observed in seeds treated with 100 mM thiourea for 24 h. However, the germination pattern in the seeds treated with thiourea and KNO_3 was more or less similar to that observed after acid scarification of seeds.

In short, the use of wet heat (hot water), physical and acid scarification of endocarps and presowing treatment of thiourea and potassium nitrate did not improve the germination substantially and therefore these treatments have less practical applications in the nursery germination in *Santalum album*. Compared to these treatments, the seeds separated from the endocarp gave higher germination percentage. Therefore, though this method is time and labor consuming, is a

relatively better alternative to above methods to enhance nursery germination in *Santalum album.*

5. The plant growth regulators (Kin, BA, and IAA) tested for stimulatory effect on germination showed only 11% (BA), 9% (Kin) and 29% (IAA) increment in germination percentage, whereas there was 85% gain in germination percentage after treating the seeds with 4 mM GA_3 for 24 h. Since the treatment of GA_3 showed significant improvement in germination percentage, it is a better option among the plant growth regulators for alleviating germination pattern in *S. album.*

6. The α and β-amylase activity was found to increase with increasing concentration of GA_3. The amylases are hydrolyzing enzymes, the activity of which results in the release of simple sugars. The availability of simple sugars in this case may be attributed to improved germination in the seeds treated with GA_3.

7. In embryo culture experiments, the embryos excised from GA_3 treated seeds as well as from the seeds not treated with GA_3 failed to grow on MS basal medium and on MS medium containing 0.5 or 1.0 mg l^{-1} BA. The inability of growth in the excised embryo may be due to non-availability of some factor present in the tissues surrounding embryo. These results suggest that there is a need of intactness of seed tissue and embryo for commencement of germination.

8. *In vitro* seed germination technique was useful to improve seed germination pattern in *S. album.* The seeds separated from the endocarp and sown in soil showed 36% germination in 30 days, whereas the seeds without endocarp and cultured on MS basal medium showed 40% germination in 30 days.

The seeds without endocarp were treated with GA_3 and cultured on MS medium without BA showed improved germination pattern and 80% seeds germinated within 30 days. The GA_3 treated seeds cultured on MS medium containing 0.5 or 1.0 mg l^{-1} BA improved germination but the increment was only marginal over the GA_3 treated seeds cultured on MS basal medium. Therefore treatment of seeds with 4 mM GA_3 for 12 h is optimum for enhanced seed germination.

The results on *in vitro* seed germination indicate that for maximum germination and obtaining uniform seedlings, removal of endocarp before the treatment of GA_3 and germinating the seeds in more controlled conditions is beneficial for germination.

Earlier it was reported that the *S. album* tree is a hemi- root-parasite and requires a host for its growth. In the experiments of present investigation the germination *in vitro* as well as in soil was observed in the absence of host. Furthermore, the seedlings were nurtured for three years in the absence of host. These plants were morphologically identical with the naturally growing seedlings. These results indicate that host is not required for seed germination and at least during the early stages of seedling development in *Santalum album*.

9. The seed germination pattern was improved when the acid scarified seeds were subjected separately to GA_3 treatment, soaking in thiourea and KNO_3. The weakening of endocarp due to acid scarification might have resulted in faster and effective uptake of these chemicals by the seeds that ultimately resulted in higher germination.
10. The seed germination percentage decreased with increase in the duration of storage under ambient conditions. The seeds showed 24%, 14%, 8% and 3% germination after one, two, three and four years of storage respectively. The seed viability as indicated by TZ test was

higher than the observed percentage germination, both in the fresh as well as stored seeds. However, there was gradual decline in the percentage of viable seeds with increase in the storage duration. The physical scarification as well the GA_3 treatment was beneficial in optimizing the germination behavior only in the fresh seeds but was less effective in the stored seeds and the effectiveness of these methods decreased with increase in storage time.

The electrical conductivity test has demonstrated higher contents of seed leachates from the stored seeds. Similar to TZ test, this is also an indication of physiological deterioration of the seed tissues which might be responsible for inferior germination pattern in the stored seeds. The endogenous levels of GA_3 and IAA were found to be lower in the stored seeds than the fresh seeds. These results suggest that the storability is less in the seeds of *Santalum album* and for raising seedlings; the collected seeds should be used within a year.

The seedlings developed in the polythene bags and those transferred in the polythene bags from *in vitro* germinated seeds and other laboratory experiments, attainted the height of about 24 cm in one year. The nursery practices used for tending the seedlings were appropriate to impart vigor in the seedlings as indicated by higher dry mass, root shoot ratio and quality index. The seedlings are sensitive to water stress and seedlings under water stress slowly shed their leaves from bottom towards shoot apex.

In brief, the studies on germination and physiological health of fresh and stored seeds pertaining to viability, moisture content, seed leachates and enzyme activities has helped in understanding the requirements of the seeds for germination.

The seeds subjected to different treatments, individually and in combinations, like physical and acid scarification, wet heat treatment, application of chemical stimulants and use of plant growth regulators

and *in vitro* germination significantly improved nursery germination in *Pterocarpus marsupium* and the treatment of physical scarification followed by application of 3 mM GA_3 for 12 h and physical scarification followed by application of 25 mM thiourea for 24 h were optimum treatments.

Among the different treatments applied to the seeds of *Santalum album,* the manual removal of seed from the hard endocarp and application of 4 mM GA_3 for 12 h followed by germination *in vitro* on MS medium lacking growth regulators was the best to overcome the limitations in the seed germination and seedling preparation.

The poor natural resurgence and indiscriminate cutting of *Pterocarpus marsupium* Roxb. and *Santalum album* L. have greatly reduced their natural stands and these species are now considered as threatened. On the other hand, the illegal poaching of these trees for their unique wood, pharmaceutical importance and the increasing demand for internationally acclaimed unique persistent sweet and woody fragrance of sandalwood oil have put further pressure on these species. The methods described in the present investigation will be highly useful to make available the seedlings for plantation programs and conservation of these threatened species. Both these plant species are known to thrive well in the draught-prone areas. The methods developed in the present investigation can make available the seedlings in large scale for reforestation programs and for the sustainable use of the important tropical forest tree species, *Pterocarpus marsupium* Roxb. and *Santalum album* L.

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Bibliography

Abbott I and Van Heurck P (1988). Widespread regeneration failure of *Persoonia elliptica* (Proteaceae) in the northern Jarrah forest of Western Australia. *Journal of the Royal Society of Western Australia,* 71:15-22.

Abdullah MM, Jones RA, El Beltagy AS. (1989). A method to overcome dormancy in cotchbroom (*Cytisus scoparius*). *Environmental and Experimental Botany,* 29: 499-505.

Adams L, Stefanescu E, Dunaway DJ. (1961). Gibberellin and thiourea break seed dormancy in California ceanothus. Res. Note PSW-178. Berkeley, CA: USDA Forest Service, Pacific Southwest Forest and Range Experiment Station, 4 p.

Aduradola AM, Adeola BF and Adedire MO (2005). Enhancing germination in seeds of African star apple, *Chrysophyllum albidum* (G. Don). *Journal of Food, Agriculture & Environmen,* 3 (2): 292-294.

Agboola DA, Adedire MO (1998). Response of treated dormant seeds of three species to germination promoters. *Nig. J. Bot.,* 11:103 – 109.

Agboola, DA, Etejere EO (1991). Studies on seed dormancy of selected economic tropical forest species. *Nig. J. Bot.*, 4:115 – 125.

Agrawal PK, Dadlani M (1995). Techniques in Seed Science and Technology. (2e) South Asian Publishers New Delhi International Book Company Absecon Highlands, pp 109–113.

Agrawal PK, Dadlani M (1995). Techniques in Seed Science and Technology. (2e) South Asian Publishers New Delhi International Book Company Absecon Highlands, pp 109–113.

Akamine EK (1947). Germination and viability studies of forest tree seeds. Rep. Hawaii agric. Exp. Sta. 1945-6, (116-18).

Aliero BL (2004). Effects of sulphuric acid, mechanical scarification and wet heat treatments on germination of seeds of African locust bean tree, *Parkia biglobosa. African Journal of Biotechnology,* 3(3): 179-181.

Al-Maskri AY, Khan MM, Khan IA and Al-Habsi K (2003). Effect of Accelerated Ageing on Viability, Vigor (RGR), Lipid Peroxidation and Leakage in Carrot (*Daucus carota* L.) Seeds. *International Journal Of Agriculture & Biology,* 5(4): 580-584.

Altare M, Trione S, Guevara JC, and Cony M (2006). Stimulation and promotion of germination in *Opuntia ficus-indica* seeds. *J.PACD,* 1:91-100.

Amritphale D, Singh B and Gutch A (1992). Inhibition of red light-induced seed germination by indole-3-acetic acid in *Hygrophila auriculata. Journal of Plant Growth Regulation,* 11(4): 203-208.

Ananthapadmanabha HS, Nagaveni HC and Rai-SN (1988). Dormancy principles in sandal seeds (*Santalum album* Linn.). Myforest. 24(1): 22-24.

Andreoli C, Khan AA (1993). Improving seedling emergence of papaya, *Carica papaya* L., by combining matriconditioning and gibberellin treatment. *Hort Science*, 28(7):708-709.

Anis M, Husain MK and Shahzad A (2005). *In vitro* plantlet regeneration of *Pterocarpus marsupium* Roxb., an endangered leguminous tree. *Curr. Sci.,* 88: 861-863.

Anonymous (1998) *In* The Wealth of India- A dictionary of Indian raw materials and industrial products. Vol. VIII, Krishnamurthi A (Chief Editor). National Institute of Science Communication, CSIR, New Delhi. pp 300-307.

Anonymous (1999) *In* The Wealth of India- A dictionary of Indian raw materials and industrial products. Vol. IX, Chadha YR (Chief Editor). National Institute of Science Communication, CSIR, New Delhi. pp 208-224.

Anuradha M and Pullaiah T (1998). Investigations on germination of *Pterocarpus santalinus* (red sanders) with special reference to in-vitro seed culture. *Indian Forester*, 124(5): 309-314.

Anuradha M and Pullaiah T (1999). In vitro seed culture and induction of enhanced axillary branching in *Pterocarpus santalinus* and *Pterocarpus marsupium*: a method for rapid multiplication. *Phytomorphology,* 49(2):157-163.

AOSA (2002). Association of Official Seed Analysts Inc., Rules for testing seed. Las Cruses, NM.

Argel PJ and Paton CJ (1999). Overcoming legume hardseededness. *In* Forage seed production: Tropical and sub-tropical species (D.S. Loch & J.E. Ferguson, eds.). v.2, chapter 14, CAB International, Wallingford, p.247-265.

Atici Okkes, Agar G and Battal P (2007). Influence of long term storage on plant growth substance levels, germination and seedling growth in legume seeds stored for 37 years. *Indian J. Plant Physiol.*, 12(1): 1-5.

Atwater BR (1980). Germination, dormancy and morphology of the seeds of herbaceous ornamental plants. *Seed Science and Technology*, 8(4): 523-573.

Ayodele VI (1997). Substrates for production of ornamentals in Nigeria. Proceedings 15th Hortson Conference, Ago – Iwoye, Ogun State, Nigeria.

Bagchi SK and Kulkarni HD (1985). Germination of open-pollinated seeds and survival of seedlings from the selected trees of *Santalum album*. *Myforest*, 21(3): 221-224.

Baker KS, Steadman KJ, Plummer JA and Dixon KW (2005). Seed dormancy and germination responses of nine Australian fire ephemerals. *Plant and Soil*, 277:345–358.

Ballard LAT (1973). Physical barriers to germination. *Seed Science and Technology*,1(2): 285-303.

Ballington JR (1984). Greenhouse forcing to reduce the time between generation in blueberry (*Vaccinium* spp) breeding. *Hort. Science*, 19: 542.

Banerjee S, Ecavade A and Rao AR (1993). Modulatory influence of sandalwood oil on mouse hepatic glutathione-*S*-transferase activity and acid soluble sulphydryl level. *Cancer Letters*, 68:105-109.

Barber CA (1903). Report on spike disease of sandalwood tree in Coorg. *Indian Forester*, 29:21-31

Bartels DA, Furini A, Ingram J and Salamini F (1996). Responses of plants to dehydration stress: A molecular analysis. *Plant Growth Regulation*, 20(2): 111–118.

Baskin C, Baskin J. (2001). Seeds: Ecology, Biogeography And Evolution Of Dormancy And Germination. San Diego (CA): Academic Press. 666 p.

Baskin JM and Baskin CC (1989). Physiology of dormancy and germination in relation to seed bank ecology. pp. 53-66. In: Ecology of Soil Seed Banks. M. A. Leck, V. T. Parker & R. L. Simpson (eds.) Academic Press, San Diego.

Basra SMA, Zia MN, Mahmood T, Afzal I and Khaliq A (2002). Comparison of different invigoration techniques in wheat (*Triticum aestvum* L.). *Pak. J. Arid. Agric.*, 5: 325-329.

Belcher E. (1985). Handbook on seeds of browse-shrubs and forbs. Tech. Pub. R8-TP8. Atlanta: SDA Forest Service, Southern Region. 246 p.

Berg AR (1974). *Arctostaphylos* Adans., manzanita. *In*: Schopmeyer CS, tech. coord. Seeds of woody plants in the United States. Agric. Handbk. 450. Washington, DC: USDA Forest Service: 228-231.

Bewley JD (1997). Breaking down the walls - a role for endo-ß-mannanase in release from seed dormancy? *Trends in Plant Science,* 2: 464-469.

Bewley JD and Black M. (1982). Physiology and biochemistry of seeds in relation to germination. Vol. 2, Springer Verlag, New York, 375p.

Bewley, JD and Black M (1994). *In* Seeds: Physiology of Development and Germination (2e.) Plenum Press, New York & London.

Bhatia NP, Nkang A, Walsh KB, Baker AJM, Ashwath N, and Midmore DJ (2005) Successful seed germination of the Nickel hyperaccumulator *Stackhousia tryonii. Annals of Botany* 96: 159-163.

Bianchetti A (1981). Comparação de tratamentos para superar a dormência de sementes de bracatinga (*Mimosa scabrella* Benth.). Boletim de Pesquisa Florestal, Colombo, n.2, p.57-67.

Bianchetti A and Ramos A (1982a). Comparação de tratamentos para superar a dormência de sementes de acácia-negra (*Acacia mearnsii* De Wild.). Boletim de Pesquisa Florestal, Colombo, n.4, p.101-111.

Bianchetti A, Ramos A (1981). Quebra de dormência de sementes de guapuruvu (*Schizolobium parahyba* (Vellozo) Blake). Boletim de Pesquisa Florestal, Colombo, v.?, n.3, p.69-76.

Biddington NL, Thomas TH (1976). Influence of Different Cytokinins on the Germination of Lettuce (Lactuca sativa) and Celery (*Apium graveolens*) Seeds. *Physiol. Plant.,* 37: 12-16.

Black M (1991). Involvement of ABA in the physiology of developing and mature seeds. *In*: Abscisic Acid: Physiology and Biochemistry. W. J. Daviesa and H. G. Jones (Eds.), Oxford, Bios Scientific, pp. 99–124.

Bonner FT, Mclemore BF, and Barnett JP (1974) Presowing treatment of seed to speed germination. Pp. 126-135, *In*: Seeds of woody plants in the United States (C. S. Schopmeyer, ed.). USDA-Forest Serv. Agric. Handbook No. 450.

Boubriak I, Kargiolaki H, Lyne L and Osborne DJ (1997). The requirement of DNA repair in desiccation tolerance of germinating embryos. *Seed Sci. Res.,* 7: 97-105.

Bradford KJ, May DJ, Hoyle BJ, Skibinski ZS, Scott SJ, and Tyler KB (1988). Seed and soil treatments to improve emergence of muskmelon from cold or crusted soils. *Crop Sci.*, 28:1001–1005.

Brant RE, McKee GW, and Cleveland RW (1971). Effect of chemical treatment on hard seed on Pengrift Crownvetch. *Crop Science*, 11(1): 1-6.

Brinkman KA. (1974). *Amorpha amorpha*, false indigo. *In*: Schopmeyer CS, tech. coord. Seeds of woody plants in the United States. Agric. Handbook 450. Washington, DC: USDA Forest Service: 216-219.

Brits GJ, Cutting JGM Brown NAC and Vanstaden J. (1995) Environmental and hormonal regulation of seed dormancy and germination in cape fynbos *Leucospermum rbr* (Proteaceae) species. *Plant Grow. Regul.* 17: 181-193

Bromwell D (1990). *In:* The role of in vitro cultivation in the conservation of endangered species. Conservation Techniques in Botanic Gardens. Koeningstein, Germany, Koeltz Scientific books.

Bryan ER, Peter IP and Daphne JO (1973). Protein synthesis and the viability of Rye grains. *Biochem. J.* 131: 275- 286

Burgass RW and Powell AA (1984). Evidence for repair processes in the invigoration of seeds by hydration. *Ann. Bot.*, 53: 753-757.

Butola JS and Badola HK (2004). Effect of pre-sowing treatment on seed germination and seedling vigour in *Angelica glauca*, a threatened medicinal herb. *Curr. Sci.*, 87(6): 796-799.

Byrd HW and Delouche JC (1971). Deterioration of soybean seed in soybean seed in storage. *Proc. Assoc. Official Seed Anal.*, 61: 41–57

Carlson JR, Sharp WC (1975). Germination of high elevation manzanitas. *Tree Planters' Notes,* 26(3): 10-11, 25.

Çetinbaş M and Koyuncu F (2006). Improving germination of *Prunus avium* L. seeds by gibberellic acid, potassium nitrate and thiourea. *Hort. Sci. (Prague),* 33(3): 119–123.

CFIA (1997). Canadian Food Inspection Agency ,Canadian methods and procedures for testing seed. Publ. CFIA0018E. Central Seed Laboratory, Ottawa, Available on line at http://www.inspection.gc.ca/english/plaveg/seesem/lab/meh/mnpe.pdf

Chandler PM, Robertson M (1994) Gene expression regulated by abscisic acid and its relation to stress tolerance. *Annu. Rev Plant Physiol. Plant Mol. Biol.*, 45: 113–141.

Chaudhari AB and Sarkar DD (2002). *In* Biodiversity endangered: India's threatened wildlife and medicinal plants. Scientific Publishers, Jodhapur, pp. 169-172.

Chauhan, SVS and Chandra Smiriti (2003). Cultivation of sandal (*Santalum album* L.) hydroponically without host. *Annals of forestry* 11(185-87).

Chen SSC and Chang JLL (1972). Does gibberellic acid stimulate seed germination via amylase synthesis? *Plant Physiol.*, 49:441-442.

Choudhary DK, Kaul BL, and Khan S. (1996). Breaking seed dormancy of *Podophyllum hexandrum* Royle ex. Camb. (syn. *P. emodi* Wall. ex. Honigberger). *J. Non Timber For. Prod.*, 3:10–12.

Come D (1970). *In*: Les Obstacle a la Germination. Paris: Massonet cie.

Conner LN and Conner AJ (1988). Seed biology of Chordospartium stevensonii. *New Zealand Journal of Botany,* 26: 473-475.

Conner LN, Conner AJ (1988). Seed biology of *Chordospartium stevensonii. New Zealand Journal of Botany*, 26: 473-475.

Corbineau F, Gay-Mathieu C, Vinel D and Come D (2002) Decrease in sunflower (*Helianthus annuus*) seed viability caused by high temperature as related to energy metabolism, membrane damage and lipid composition. *Physiologia Plantarum* 116: 489-496.

Corner EJH (1976). 'The seeds of dicotyledons '. 2 Vols. Cambridge University Press, Cambridge, UK.

Cortelazzo AL, Coutinho J and Granjeiro AP (2005). Storage and ageing of french beans (*Phaseolus vulgaris* L.): Effect on seed viability and vigor. *Braz. J. Morphol. Sci.,* 22(2): 121-128

Cox RA and Klett JE. (1984). Seed germination requirements of native Colorado plants for use in the landscape. *Plant Propagator*, 30(2): 6B10.

Crocker W (1907). Germination of seed of water plants. *Bot. Gaz.* 44:375-380.

Crocker W and Barton LV (1957). *In*: Physiology of seeds. Waltham, Massachusetts, Chronica Botanica Company. 267 p.

Cruz ED, Martins FO, Carvalho JEU (2001). Biometria de frutos e sementes e germinação de jatobá-curuba (*Hymenaea intermedia* Ducke, Leguminosae – Caesalpinioideae). Revista Brasileira de Botânica, São Paulo, 24(1):161-165.

Danthu P, Roussel J, Gaye A, and Mazzoudi E H EL (1995). Baobab (*Adansonia digitata* L.) seed pretreatments for germination improvement. *Seed Sci. and Technol.*, 23, 469–475.

Das S, Ray S, Dey S and Dasgupta S (2001) Optimization of sucrose, inorganic nitrogen and abscisic levels for *Santalum album* L. somatic embryo production in suspension culture. *Process Biochemistry,* 37: 51-56.

Das T and Chatterjee A (1993). *In vitro* studies of *Pterocarpus marsupium* – An endangered tree. *Indian J Plant Physiol.,* 36(4): 269-272

Das T, Chatterjee A, Das T (1993). In vitro studies of *Pterocarpus marsupium* - an endangered tree. *Indian Journal of Plant Physiology,* 36: 4.

Davis TD, George SW, Upadhaya A, and Parsons JM. (1991). Improvement of seedling emergence of *Lupinus texensis* following seed scarification treatments. *J. Environ. Hort.*, 9:17-21.

Daws MI, Terry, Janet, Miles, Stephanie, Davies, Hannah & Wood, Christopher B (2004). Applicability of the topographical tetrazolium test for seed viability testing of wild plant species. 27th ISTA Congress Seed Symposium Budapest, Hungary May 17th – 19th.

Dayanand T; Lohidas T (1988). Effect of different treatments on pod germination of red sanders (*Pterocarpus santalinus* Linn.). *Indian J Forestry.*,11:1, 87-88

De La Rosa-Ibarra M, Garcia H (1994). Estimulación de la germinación de cinco especies de Cactáceas consideradas en peligro de extinction. *Phyton Buenos Aires,* 56:147–150.

Debeaujon I, and Koornneef M (2000). Gibberellin requirement for *Arabidopsis* seed germination is determined both by testa characteristics and embryonic abscisic acid. *Plant Physiology,* 122: 415–42.

Dehgan B and Schutzman B (1989). Embryo development and germination of *Cycas* seeds. *Journal for American Society for Horticultural Science,* 114:125–129.

Dehgan B, and Pérez H (2005). Preliminary study shows germination of Caribbean applecactus (*Harrisia fragrans*) improved with acid

scarification and gibberellic acid. *Florida Agricultural Experiment Station Journal Series No. R-10423*:91-96.

Dehgan B, Johnson CR (1982). Improved seed germination of *Zamia floridana* (sensu lato) with sulfuric and GA_3. *Scientia Horticulture,* 19:357–361.

Dehgan B, Johnson CR (1982). Improved seed germination of *Zamia floridana* (*sensu lato*) with sulfuric and GA_3. *Scientia Horticulture 19:357–361.*

Dehgan B, Norcini JG, Kabat SM, and Pérez HE (2003) Effect of Seed Scarification and Gibberellic Acid Treatment on Seedling Emergence of Sky-Blue Lupine (*Lupinus diffusus*) Journal of Environmental Horticulture 21(2):64-67, June 2003.

Dehgan B, Schutzman B (1983). Effect of H_2SO_4 and GA_3 on seed germination of *Zamia furfuraceae. Hort Science*, 18:371–372.

Dehgan B, Schutzman B (1983). Effect of H2SO4 and GA_3 on seed germination of *Zamia furfuraceae. Hort Science 18:371–372.*

Dell B (1980). Structure and function of the strophiolar plug in seeds of Albizia lophantha. *Am. Jour. Bot.*, 67(4): 556-561.

Demel, T and Teketay D (1996). The effect of different pre-sowing seed treatments, temperature, and light on the germination of five *Senna* species from Ethiopia. *New Forests,* 11(2): 155-171.

Dewan A, Nanda K, Gupta SC (1992). *In vitro* propagation of *Acacia nilotica* subsp. Indica Brenen via coteledonary nodes. *Plant Cell Rep.*,12: 18-21.

Dickie JB, Ellis RH, Kraak HL, Ryders K and Tompsett PB (1990) Temperature and seed storage longevity. *Annals of Botany* 65: 197-204.

Dickson A, Leaf AL and Hosner JF (1960). Quality appraisal of white spruce and white pine seedling stock in nurseries. *For.Chron.* 36:10-13.

Dietert MF and Shontz JP (1978). Germination of Saltmarsh Ecology of a Maryland Population Bulrush (*Scirpus robustus*). *Estuaries,* 1(3):164-170.

Dimalla GG and J van Staden (1977). The effect of temperature on the germination and endogenous cytokinin and gibberellin levels of pecan nuts. *Zeitschrift für Pflanzenphysiologie* 82: 274-280.

Dirr MA, Heuser CW Jr. (1987). *In* The reference manual of woody plant propagation: from seed to tissue culture. Athens, GA: Varsity Press.239 p.

Douglas JE (1975). Seed storage and packaging. *In*: Feistritzer, W.P. (Ed.) *Cereal Seed Technology,* pp: 87–107. Food Agriculture Organization-United Nations, Rome.

Dow MA and Schwintzer CR (1999). Seed germination, seedling emergence and seed bank ecology of sweet fern (*Comptonia peregrine* L. Coult.). *Can. J. Bot.,* 77: 1378-1386.

Duffus C and Rosis (1973) Starch hydrolyzing enzymes in developing barley grains. *Planta* 109: 153-160.

Dunand RT (1992). Enhancement of seedling vigor in rice (*Oryza sativa* L.) by seed treatment with gibberellic acid. *In* Progress in plant growth regulation. (eds. C.M. Karssen, L.C. van Loon and D. Vreugdenhil). pp 835-841, Kluwer Academic Publishers, London.

Duncan DB (1955) Multiple range and multiple F tests. *Biometrics,* 11: 1-42.

Dunnett CW (1964) New tables for multiple comparisons with control. *Biometrics*, 20: 482-491.

Duryea ML (1984). Nursery Cultural Practices: Impacts on Seedling Quality. *In* Forest Nursery Manual: Production of Bareroot Seedlings. Duryea ML., and Thomas DL (eds.). Martinus Nijhoff/Dr W. Junk Publishers. The Hague/Boston/Lancaster, for Forest Research Laboratory, Oregon State University. Corvallis. pp. 143

Dweikat IM, and Lyrene PM (1988). Response of highbush blueberry seed germination to gibberellin A_3 and 6N-benzyladenine. *Canadian Journal of Botany,* 67: 3391–3393.

Effendi M, Garsetiasih R, Ramdhani N (1996) Effect of incising and soaking on seed germination of sandalwood (*Santalum album* L.). *Buletin-Penelitian-Kehutanan -Kupang.*, 1(2): 70-78.

Ellis RH and Roberts EH (1981). The quantification of ageing and survival in orthodox seeds. *Seed Sci. & Technol.,* 9:373-409.

Eplee RE & Norris RS (1987). Field research techniques. In: Parasitic Weeds in Agriculture, Vol. 1 Striga (ed. LJ Musselman), pp. 271–80. CRC Press, Boca Raton, FL, USA.

Esashi Y, Ohara Y, Okzaki M & Hishinuma K (1979). Control of cocklebur seed germination by nitrogenous compound like nitrite, hydroxylamine, thiourea, azide and cyanide. *Plant and Cell Physiol.*, 20: 349-361.

Evenari M, Klein S, Anchori H and Feinburn N (1957). The beginning of cell division and cell elongation in germinating lettuce seeds. *Bull. Res. Council Israel,* 6D: 33-37.

FAO (2005) Global Forest Resources Assessment 2005- Progress towards sustainable forest management, FAO Forestry Paper 147, available at http://www.fao.org/docrep/008/a0400e/a0400e00.htm. Food and Agriculture Organization of the United Nations Rome, 2005.

Faria JMR, Buitink J, Andre' AM van Lammeren and Hilhorst HWM (2005). Changes in DNA and microtubules during loss and re-establishment of desiccation tolerance in germinating *Medicago truncatula* seeds. *Journal of Experimental Botany*, 56(418): 2119–2130.

Ferguson JM, Tekrony DM, and Egly DB (1990) Changes during early soybean seed and axes deterioration. II. Lipids. *Crop Science* 30: 179-182.

Fessel SA, Vieira RD and Pessoa da Cruz MC (2006). Electrical conductivity testing of corn seeds as influenced by temperature and period of storage. *Pesq. agropec. bras., Brasília*, 41(10): 1551-1559.

Fischer CEC (1938). Where did the sandalwood tree (*Santalum album* L.) evolve? *J Bombay Nat. Hist. Soc.*, 40: 458-466.

Fisher, JB (2002). Effect of sulfuric acid treatment on *Harrisia fragrans* seed germination. *In*: Maschinski, J., M.Q.N. Fellows, and J. Possley (ed.). Final Report to the Endangered Plant Advisory Council, Florida Department of Agriculture and Consumer Services.

Flemion F (1941). Further studies on the rapid determination of the germinative capacity of seeds. *Contributions from Boyce Thompson Institute*,11: 455-464

Flynn S, Turner RM and Dickie JB (2004) Seed Information database (release 6.0, Oct. 2004) http://www.kew.org/data/sid.

Foley ME. (2001) Seed dormancy: an update of terminology, physiological genetics, and quantitative trait loci regulating germinability. *Weed Science, 49*: 305-317.

Fountain DW, McGill CR, Southward RC, Sproul RV, Mackay, AC and Outred HA (2002). Assessment of germination and vigour status of 24-year-old seeds of a *Sophora* hybrid. *New Zealand Journal of Botany,* 40: 383–387

Frett JL, Dirr MA (1979). Scarification and stratification requirements for seeds of *Cercis Canadensis* L., (redbud), *Cladrastis lutea* (Michx. F.) C. Koch (yellowwood), and *Gymnocladus dioicus* (L.) C. Koch (Kentucky coffee tree). *Plant Propagator,* 25(2): 4-6.

Fu JR, Lu XR, Chen RZ, Zhang BZ, Ki ZS and Cai CY (1988). Osmoconditioning of peanut (*Arachis hypogaea* L.) seeds with PEG to improve vigor and some biochemical activities. *Seed Sci. Technol.,* 16: 197-212.

Funes G, and Venier P. (2006) Dormancy and germination in three *Acacia* (Fabaceae) species from central Argentina. *Seed Science Research* 16: 77-82.

Gangaprasad AN, Decruse WS, Seeni S, Menon S. (1999). Micropropagation and restoration of the endangered Malabar daffodil orchid *Ipsea malabarica. Lindleyana* 14: 38–46.

Gardner WA (1921) Effect of light on germination of light-sensitive seeds. *Bot. Gaz.,* 71: 249-288

Giba Z, Grubišic′ D, and Konjevic′ R. (1993). The effect of white light, growth regulators and temperature on the germination of blueberry (*Vaccinium myrtillus* L.) seeds. *Seed Science and Technology,* 21: 521–529.

Godínez-Alvarez H, Valiente-Banuet A (1998). Germination and early seedling growth of Tehuacan Valley cacti species: the role of soils and seed ingestion by dispersers on seedling growth. *Journal of Arid Environments,* 39:21–31.

Golovina EA, Tikhonov AN and Hoekstra FA (1997) An electron paramagnetic resonance spin-probe study of membrane permeability changes with seed aging. *Plant Physiology* 114: 383–389.

Gordon AG and Rowe DCF (1982). Seed manual for ornamental trees and shrubs. *For. Comm. Bull.* 59, London.

Graebe JE (1987). Gibberellin biosynthesis and control. *Annu. Rev. Plant Physiol.* 38:419–465.

Groot SPC, Karssen CM (1987). Gibberellins regulate seed germination in tomato by endosperm weakening: a study with gibberellin-deficient mutants. *Planta,* 171: 525–531.

Groot SPC, Kieliszewska-Rokicka B, Vermeer E, Karssen CM (1988). Gibberellin-induced hydrolysis of endosperm cell walls in gibberellin-deficient tomato seeds prior to radicle protrusion. Planta, 174: 500–504

Gul B, Weber DJ. (1999). Effect of salinity, light and thermo period on the seed germination of *Allenrolfea occidentalis. Can J Bot.,* 77: 1-7.

Gunn BV (1990). Germination pretreatments for selected acacia species from the Pilbara region of Western Australia. ACIAR Proceedings Series (Australia) 28: 46-50.

Hamilton DF, Carpenter PL (1975). Regulation of seed dormancy in *Cercis canadensis* L. *Journal of the American Society for Horticultural Science,* 100(6): 653-656.

Hannerz M (2006). 'EDITORIAL', *Scandinavian Journal of Forest Research,* 21:1, 1 – 2

Harborne, JB (1994) Phytochemistry of the Leguminosae. *In Phytochemical Dictionary of the Leguminosae,* Bisby, FA *et al.*(eds.) London: Chapman & Hall.

Hartmann HT, Kester DE, Davies FT, Geneve RE (1997a). *In* Plant propagation-Principles and Practices (6 Ed.) Prentice – Hall, Inc., Upper Saddle River, New Jersey, USA., pp 125-144.

Hartmann HT, Kester DE, Davies FT, Geneve RE (1997b). *In* Plant propagation-Principles and Practices (6 Ed.) Prentice – Hall, Inc., Upper Saddle River, New Jersey, USA., pp 218

Hasegawa R, Maruyama A, Sasaki H, Tada T and Esashi Y (1995). Possible involvement of ethylene-activated CAS in the regulation of cocklebur seed germination. Exp. Bot. 46: 286, 551-556.

Hegarty, TW (1977). Seed activation and seed germination under moisture stress. *New Phytol.*, 78:349-359.

Heit CE (1967). Propagation from seed: 8. Fall planting of fruit and hardwood seeds. *American Nurseryman,* 126(4): 12-13, 85-90.

Hendricks SB, and Taylorson RB (1975). Breaking of Seed Dormancy by Catalase Inhibition. *Proc. Nat. Acad. So. USA*, 72(1):306-309

Hendricks, S. B. and Taylorson, R. B. (1974). *Plant Physiol.*, 54: 304-309.

Hendry GAF (1993) Oxygen, free radical processes and seed longevity. *Seed Science Research,* 3: 141–153.

Hendry GAF (1993). Oxygen, free radical processes and seed longevity. *Seed Science Research, 3*:141-153.

Hermansen LA, Dureya ML, West SH, White TL, Malavasi MM (200) Pretreatments to overcome seed coat dormancy in *Dimorphandra mollis.* Seed Science and Technology, Zürich, 28(3): 581-585

Hilhorst HWM (1995). A critical update on seed dormancy. I. Primary dormancy. *Seed Science Research,* 5: 61-73.

Holmquist J de Dios and J Popenoe (1967). The effect of scarification on the germination of seed of *Acrocomia crispa* and *Arenga engleri. Principes,* 11:23-25.

Hong TD and Ellis RH (1992). Optimum air-dry seed storage environments for arabica coffee. *Seed Science and Technology,* 20(3): 547-560.

Hong TD and Ellis RH (1996) A protocol to determine seed storage behaviour. IPGRI Technical Bulletin 1. 62 p.

Hong TD, Linington S and Ellis RH (1996) Seed storage behaviour: a compendium. Handbooks for germbanks No 4. Rome, Italy, International Plant Genetic Resources Institute (IPGRI).

Hopper GM, Vozzo JA. (1982). Auxin, gibberellic acid and abscisic acid in water oak embryos. In: Thielges BA, ed. Physiology and genetics of intensive culture. Proceedings, 7th North American Forest Biology Workshop. 1982 July 26–28; Lexington, KY. Lexington: University of Kentucky: 327–331.

Hosokawa J, Wick D, and Luna T(2001). Propagation protocol for production of container *Lupinus sericeus* Pursh. plants (172 ml containers); Glacier Natl. Park, West Glacier, MT. *In*: Native Plants Network. http://www.nativeplantnetwork.org

Howes MJR, Simmonds MSJ and Kite GC (2004) Evaluation of the quality of sandalwood essential oils by gas chromatography-mass spectrometry. *Journal of Chromatography* 1028:307-312.

Husain MK, Anis M and Shahzad A (2007). *In vitro* propagation of Indian Kino (*Pterocarpus marsupium* Roxb.) using thidiazuron. *In Vitro Cell Dev. Biol.-Plant,* 43:59-64.

Ibanez A, Passera CB (1997). Factors affecting the germination process of Albaida (*Anthyllis cytisoides* L.) a forage legume of the Mediterranean coast. *Journal of Arid Environments* 35: 225–231.

IPGRI/DFSC (International Plant Genetic Resources Institute / Danida Forest Seeds Centre) (1998) The project on handling and storage of recalcitrant and intermediate tropical forest tree seeds. IPGRI Newsletter 4.

IPGRI/DFSC (International Plant Genetic Resources Institute / Danida Forest Seeds Centre) (1999) The project on handling and storage of recalcitrant and intermediate tropical forest tree seeds. IPGRI Newsletter 5.

Iqbal N, Basra SMA And Khalil-Ur-Rehman (2002). Evaluation of vigour and oil quality in cottonseed during accelerated ageing. *Int. J. Agri. Biol.*, 4(3): 318-322

ISTA [International Seed Testing Association] (1985). International rules for seed testing. *Seed Sci. Technol.*, 13:299-355.

ISTA [International Seed Testing Association] (1993). Rules for testing seeds: Rules 1993. *Seed Science and Technology,* 21 (Suppl.): 1-259.

Jacobsen JW, Gubler F and Chandler PM (1995). *In*: Plant Hormones, Davies P. J. [ed.], Kluwer Acad. Publ., Dordrecht, pp 246.

Jain AK (1996).Improvement of natural regeneration in *Pterocarpus santalinus*. *Indian Forester*, 122(9):783-786.

Jones HD (1999) Seeds get a wake-up call. Dormancy, a survival mechanism for seeds, or a useful tool for agriculture. *The Biologist* 46: 65-69.

Jones R and Stoddart J (1980). Gibberellins and seed germination. *In:* The physiology and biochemistry of seed dormancy and germination. Khan A (Ed). Amsterdam: North Holland Publishing Company. pp 97-104.

Juao Correia de Araujo Neto and Ivor Bergemann (2000). Germinative pretreatments to dormancy break *Guazuma ulmifolia* Lam. seeds. *Scientia Forestalis,* 58: 15-24.

Junttilla O, and Nilsen AJ (1980). Stimulation of *Phalaris* seed germination by respiratory inhibitors and oxidizing agents. *Z. Pflanzenphysiol.*, 97:429-435.

Kabar K (1998). Comparative effects of kinetin, benzyladenine, and gibberellic acid on abscisic acid inhibited seed germination and seedling growth of red pine and arbor vitae. *Tr. J. of Botany,* 22: 1-6.

Kabar KS and Baltepe S (1989). Effect of kinetin and gibberellic acid in overcoming high temperature and salinity (NaCl) stresses on the germination of barley and lettuce seeds. *Phyton*, 30: 30-65.

Kalimuthu K and Lakshmanan KK (1994). Preliminary investigation on micropropagation of *Pterocarpus marsupium* Roxb. Indian J. For., 17: 192-195.

Kalimuthu K, Lakshmanan KK (1995). Effect of different treatments on pod germination of *Pterocarpus* species. *Indian Journal of Forestry*, 18(2):104-106.

Kamra SK (1990) Improving the forest seed situation in some African countries. *Tropical Tree Seed Research* 28:126–131.

Karssen CM and Lacka E (1986). A revision of the hormone balance theory of seed dormancy: studies on gibberellin and/or abscisic acid-deficient mutants of Arabidopsis thaliana. *In* Plant Growth Substances, M Bopp (ed.). Springer-Verlag, Berlin, pp 315–323.

Karssen CM, Brinkhorst-van der Swan DLC, Breekland AE, Koornneef M (1983). Induction of dormancy during seed development by endogenous abscisic acid: studies on abscisic acid deficient genotypes of *Arabidopsis thaliana* (L.) *Heynh. Planta* 157: 158–165

Karssen MC, Groot SPC and Koornneef M (1987). *In* Developmental Mutants in Higher Plants, Thomas H and Grierson D. [eds.], Cambridge University Press, London.

Karssen, CM, Zagórski S, Kepczynski J and Groot SPC (1989). Key role for endogenous gibberellins in the control of seed germination. *Annals of Botany*, 63: 71–80.

Kaye TN and Kuykendall K (2001a). Germination and propagation techniques for restoring rare pacific northwest prairie plants. *In* Conservation of Washington's Native Plants and Ecosystems. (Edited by S.H. Reichard, P.W. Dunwiddie, J.G. Gamon, A.R. Kruckeberg, and D.L. Salstrom.) Washington Native Plant Society, Seattle, Washington. Pp 216.

Kaye TN and Kuykendall K. (2001b). Effects of scarification and cold stratification on seed germination of *Lupinus sulphureus* ssp. *Kincaidii. Seed Sci. & Technol.*, 29: 663-668.

Keeley JE and Fotheringham CJ (1998). Mechanism of smoke induced germination in post fire chaparral annual. *Journal of Ecology,* 86: 27-36.

Keeley JE, Fortheringham CJ (2000). Role of fire in regeneration from seed. *In*: Seeds: the ecology of regeneration in plant communities. (Fenner M, Editor), 2e, Wallingford, United Kingdom: CAB International. p 311–330.

Keeley JE, Fortheringham CJ (2000). Role of fire in regeneration from seed. In: Seeds: the ecology of regeneration in plant communities. (Fenner M, Editor) 2e Wallingford, United Kingdom: CAB International. p 311–330.

Kelen M, Cubuk Demiraly E, Sen Songul and Ozkan G. (2004) Separation of abscisic acid, indole-3-acetic acid, gibberellic acid in 99 R (*Vitis berlandieri* × *Vitis rupestris*) and rose oil (*Rosa damascene* Mill) by reversed phase liquid chromatography. *Turk J Chem,* 28: 603-610.

Keogh JA and Bannister P (1992). A method for inducing rapid germination in seed of *Discaria toumatou* Raoul. *New Zealand Journal of Botany,* 30: 113-116.

Kermode AR (1995). Regulatory mechanisms in the transition from seed development to germination: Interaction between the embryo and the seed environment. *In*: Seed Development and Germination. J. Kigel and G. Galili (Eds.), Marcel Dekker Inc., New York, pp. 273–332.

Kermode AR (2005). Role of abscisic acid in seed dormancy. *J Plant Growth Regul.*, 24:319-344

Khan AA (1968). Inhibition of gibberellic acid-induced germination by abscisic acid and reversal by cytokinins. *Plant Physiol.*, 43:1463-1465

Khan AA (1970). ABA and kinetin induced changes in cell homogenates, chromatin - bound RNA polymerase and RNA composition. *In*: CARR, D.J. (Ed.) Plant growth substances. New York: Springer-Verlag, p.207-215.

Khan MA & Ungar IA (2001). Alleviation of salinity stress and the response to temperature in two seed morphs of *Halopyrum mucronatim* (Poaceae). *Aust. J Bot.*, 49: 777-783.

Khan MA, Gull B, and Weber DJ (2002). Improving seed germination of *Salicornia rubra* (Chenopodiaceae) under saline conditions using germination-regulating chemicals Western North *American Naturalist,* 62(1):101–105.

Khurana E and Singh JS (2001). Ecology of tree seed and seedlings: Implications for tropical forest conservation and restoration. *Curr. Sci.*, 80(6): 748-757.

Kigel J (1995). Seed germination in arid and semiarid regions. *In*: Seed Development and Germination, pp. 645–699. Kigel, J., Galili, G. (Eds), New York: Marcel Dekker. 853 pp.

Kim TH, Ito H, Hatanao T, Takayasu J, Tokuda H, Nishino H, Machiguchi T and Yoshida T (2006). New antitumor sesquiterpenoids from *Santalum album* of Indian origin. *Tetrahedron* 62:6981-6989.

Kirtikar KR and Basu BD (1999). Indian medicinal plants Vol. I, Naya Prakashan, Kolkata. pp 828-829.

Koller D, Mayer AM, Poljakoff-Mayber A, and Klein S (1962). Seed germination. *Ann. Rev. Plant Physiol.*, 13: 437-464.

Koornneef M, Bentsink L, Hilhorst H (2002). Seed dormancy and germination. *Current Opinion in Plant Biology,* 5: 33-36.

Koyuncu F (2005). Breaking seed dormancy in black mulberry (*Morus nigra* L.) by cold stratification and exogenous application of gibberellic acid. *Acta Biologic Cracoviensia Series Botanica* , 47/2: 23–26.

Kucera B, Cohn MA and Leubner-Metzger G. (2005). Plant hormone interactions during seed dormancy release and germination. *Seed Science Research,* 15: 281–307.

Kuijt (1969). *In* The Biology of Parasitic Flowering Plants. University of California Press, Berkeley

Kumar P, Purkayastha BK (1972). Note on germination of the seeds of lac hosts. *Indian Journal of Agricultural Sciences,* 42(5): 430-431.

Lakshmi S, Sreenatha KS and Sujata SG (1992). Plantlet production from shoot tip cultures of red sandalwood (*Pterocarpus santalinus*). *Curr. Sci.*, 62: 532-535.

Laude HM (1949). Delayed Germination of California Oatgrass, *Danthonia californica. Agronomy Journal*, 41(9): 404-408.

Leadem CL (1987). The role of plant growth regulators in the germination of forest tree seeds. *In*: Hormonal control of tree growth. S.V. Kossuth and S.D. Ross (editors), Martinus Nijhoff Publishers, Dordrecht., Chap. 4, pp. 61-93.

Lecat S, Corbineau F, and Còme D (1992). Effects of gibberellic acid on the germination of dormant oat (*Avena sativa* L.) seeds as related to temperature, oxygen and energy metabolism. *Seed Science and Technology*, 20: 421–433.

Leprince O, Hendry AF and McKersie B (1993). The mechanisms of desiccation tolerance in developing seeds. *Seed Sci. Res.*, 3(2): 231–246.

Leubner Metzger JD (1983). Promotion of germination of dormant weed seeds by substituted phtalimides and gibberellic acid. *Weed Science*, 31, 285–289.

Leubner-Metzger G, Frundt C, Meins F Jr (1996). Effects of gibberellins, darkness and osmotica on endosperm rupture and class I b-1,3 glucanase induction in tobacco seed germination. *Planta*, 199: 282–288.

Li B, and Foley ME. (1997) Genetic and molecular control of seed dormancy. *Trends in Plant Science*, 2:384-389.

Lipe JA, Morgan PW, Storey JB. 1969. Growth substances and fruit shedding in the pecan, *Carya illinoensis*. Journal of the Society for *Horticultural Science* 94: 668–671.

Liu NY, Khatamian H, Fretz TA. (1981). Seed coat structure of three woody legume species after chemical and physical treatments to increase seed germination. *Journal of the American Society for Horticultural Science*, 106: 691-694.

Logan DC and Stewart GR (1991). Role of Ethylene in the Germination of the *Hemiparasite Striga hermonthica. Plant Physiol.*, 97: 1435-1438.

Lowry OH, Rosenbrough NJ, Farr AL and Randall RJ. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.

MacMillan J (2002). Occurrence of gibberellins in vascular plants, fungi and bacteria. *J. Plant Growth Regul.*, 20: 387-442.

Maguire JD (1977) Seed quality and germination. Pp 219-235 *In* Khan AA (ed). The physiology and biochemistry of seed dormancy and germination. North Holland publication corporation,Amsterdam.

Maideen SK, Selvaraj JA and Vinaya Ray RS (1990). Presowing chemical treatments to hasten germination of *Casuarina equisetifolia. The International Tree Crops Journal*, 6: 173- 181.

Malavasi UC and Malavasi MM (2004). Dormancy Breaking and Germination of *Enterolobium contortisiliquum* (Vell.) Morong seed. *Brazilian Archives of Biology and Technology*, 6:851-854.

Manohar J, Lokesh S, Rai VR (1999). Effect of pre-treatment on the germination of *Santalum album* L. seeds. *Journal of Tropical Forestry*, 15(4): 265-268.

Manonmani V and Vanangamudi K (2002). Effect of seed source and size on seed germination and seedling vigour of sandal (*Santalum album*). *Journal of Tropical Forest Science*, 14(1): 150-155.

Manonmani V and Vanangamudi K (2001). Influence of degree of fruit maturity on seed germination, seedling vigour and storability of sandal (*Santalum album*). *Journal of Tropical Forest Science*, 13(3) : 415-422.

Manzano P, Malo JE, and Peco B (2005) Sheep gut passage and survival of Mediterranean shrub seeds. Seed Science Research 15: 21-28.

Teketay D. (1996) Germination ecology of 12 indigenous and 8 exotic multipurpose leguminous species from Ethiopia. *Forest Ecology and Management*, 80: 209-223.

Martin AC (1946). The comparative internal morphology of seeds. *Am. Midll. Nat.*, 36: 513-660.

Maruyama' A, Yoshiyama M, Adachi' Y, Tani A, Hasegawa R and Esashi Y (1996). Promotion of Cocklebur Seed Germination by Allyl, Sulfur and Cyanogenic Compounds. *Plant Cell Physiol.*, 37(8): 1054-1058.

Masano (1986). Germinative capacity of sandalwood seed from two different resources.*Buletin-Penelitian-Hutan,-PusatPenelitian-dan-engembangan-Hutan. No.* 473: 40-51

Matus-Cadiz MA, and Hucl P. (2005). Rapid and Effective Germination Methods for Overcoming Seed Dormancy in Annual Canarygrass. *Crop Sci.*, 45:1696–1703.

Mayer AM (1974). Control of seed germination. *Ann. Rev. Plant Physiol.*, 25:167-193.

Mayer AM and Poljakoff-Mayber (1982). *In* The germination of seeds. (3ed.) Pergamon, Oxford.

Mayer AM and Poljakoff-Mayber A (1963). *In* The germination of seeds. Macmillan Co., New York. pp 236.

Mayer AM and Poljakoff-Mayber A (1975). The germination of seeds. *In* International Series of Monographs on Pure and Applied Biology, Plant Physiology Div., 2e, Pergamon Press.

Mayer AM and Shain Y (1974). Control of seed germination. *Ann. Rev. Plant Physiol.*, 25:167-93.

McCarty R (1995). Genetic control and integration of maturation and germination pathways in seed development. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, 46: 71–93.

McCourt P (1999). *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, 50: 219–243.

McIntyre GI, Cessna AJ and Hsiao AI. (1996). Seed dormancy in *Avena fatua*: Interacting effects of nitrate, water and seed coat injury. *Physiol. Plant.*, 97: 291–302.

Michalski L. (1969). Content of plant growth regulators in the developing seeds of oak (*Quercus robur* L.): 2. Auxin-like substances. *Acta Societatis Botanicorum Poloniae* 38: 157–163.

Mikulík J and Vladimír Vinter (2002). Evaluation of factors affecting germination of *Dianthus superbus* L. Subsp. *Superbus.*, *Acta Univ. Palacki. Olomuc. Fac. rer. nat. Biol.* 39–40: 13–18.

Mitter V. (1993). *In* Conservation and Management of Plants Genetic Resources (Eds. Rana, R. S. et al.), NBPGR, New Delhi, pp. 236–245.

Miyoshi K and Mii M (1995). Phytohormone pretreatment for the enhancement of seed germination and protocorm formation by the terrestrial orchid, *Calanthe discolor* (Orchidaceae), in asymbiotic culture. *Scientia Horticulturae,* 63: 263-267.

Mohr H and Schopfer P (1995). Ripening and germination of reproductive and distributive organs. *In* Plant Physiology, pp. 409-422. Springer-Verlag, Berlin.

Montgomery DC (2001a). Experiments with a single factor: The analysis of variance. *In*: Design and analysis of experiments. 5e, John Wiley & Sons (Asia) Pt. Ltd. pp 103.

Montgomery DC (2001b). Critical values for Dunnett's Test for comparing treatments with control. *In*: Design And Analysis Of Experiments. 5e, John Wiley & Sons (Asia) Pt. Ltd. pp 658.

Morris, EC, Tieu A, and Dixon K (2000) Seed coat dormancy in two species of *Grevillea* (Proteaceae). *Annals of Botany* 86: 771-775.

Msanga, H.P. (1998). Seed germination of indigenous trees in Tanzania. Edmonton, Canada: Canadian Forest Service, Northern Forestry Centre. 292 p.

Muhammad S and Amusa NA (2003). Effects of sulphuric acid and hot water treatments on seed germination of tamarind (*Tamarindus indica* L). *African Journal of Biotechnology,* 2(9): 276-279.

Murashige T and Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.,* 15: 473-479.

Murthy BNS and Reddy YN (1989). Temperature dependence of seed germination and seedling growth in ber (*Ziziphus mauritiana* Lam.) and their modification by pre-sowing treatments. *Seed Sci. and Technol. 18: 621-627.*

Musselman LJ and Mann WF Jr. (1979). Notes on seed germination and parasitism of seedlings of *Buckleya distichophylla* (Santalaceae). *Castanea* 44:108-113.

Mwasha AJ, Ellis RH and Hong TD (1996) The effect of desiccation on the subsequent survival of seeds of cashew (*Anacardium occid*entale L.). *Seed Science and Technology* 25:115-122.

Nagao MA, K Kanegawa and WS Sakai (1980). Accelerating palm seed germination with gibberellic acid, scarification, and bottom heat. *Hort. Science,* 15: 200-201.

Nagarajaiah C and Rao NS (1993). A successful method of establishing sandal tree wildings. *Current-Research-University-of-Agricultural-Sciences,* (Bangalore) 22: 3-5; 51-52.

Nagaveni HC and Ananthapadmanabha HS (1986). Seed polymorphism and germination in *Santalum album. Van-Vigyan.*, 24(1-2): 25-28.

Nagaveni HC and Srimathi RA (1981). Studies on germination of sandal (*Santalum album* Linn.) Pre-treatment of sandal seeds. *Indian Forester,* 107(6): 348-354.

Nagaveni HC and Srimathi RA (1985). Germination capacity of floating and sinking seeds. Indian Forester, 111: 264-268.

Nagaveni HC, Ananthapadmanabha HS, and Rai-SN (1989).Effect of different chemicals on germination of sandal seeds (*Santalum album* Linn.). *Myforest,* 25(4): 311-313.

Nagaveni HC, Srimathi RA (1981). Studies on germination of sandal (*Santalum album* Linn.). Pre-treatment of sandal seeds. *Indian Forester,* 107(6): 348-354.

Naidu CV (2001) Improvement of seed germination in red sanders (*Pterocarpus santalinus* Linn.) by plant growth regulators. Indian-Journal-of-Plant-Physiology. 6: 2, 205-207.

Naidu CV and Rajendrudu G (2001). Influence of kinetin and nitrogenous salts on seed germination of red sanders (*Pterocarpus santalinus* Linn) *Seed Science and Technology,* 29(3): 669-672

Naidu CV, Mastan-M (2001).Seed pretreatment methods to improve germination in *Pterocarpus santalinus* Linn. *Indian Journal of Forestry,* 24: 3, 342-343.

Natarajan N and Rai RSV (1988). Studies to maximization of germination in *Acacia auriculiformis. Indian Journal of Forestry,* 11(4): 304-306.

Nedeva D and Nikolova A (1997). Desiccation tolerance in developing seeds. *Bulg. J. Plant Physiol.,* 23(3–4):100–113.

Nerson H and Govers A. (1986). Salt priming of muskmelon seeds for low-temperature germination. *Sci. Hort,.* (Amsterdam) 28:85–91.

Netzly DH, Riopel JL, Ejeta G, Butler LG (1988). Germination stimulants of witchweed (*Striga asiatica*) from hydrophobic root exudate of sorghum (*Sorghum bicolor*). *Weed Sci.,* 36: 441-446

Nicolás C, Nicolás G, and Rodriguez D (1996). Antagonistic effects of abscisic acid and gibberellic acid on the breaking of dormancy of *Fagus sylvatica* seeds. *Physiologia Plantarum,* 96: 244–250.

Nicolás G, Nicolás C. & Rodríguez D (1997). Molecular approach to the role of ABA and GA_3 in the dormancy of Fagus sylvatica seeds. In: Ellis, R. H., Black, M., Murdoch A. J., Hong, T.D. (eds.) Basic and applied aspects of seed biology. Current Plant Science and Biotechnology in Agriculture No. 30; Kluwer Academic Publishers, 323-333.

Nikolaeva MG (1977). Factors controlling the seed dormancy pattern. *In* The physiology and biochemistry of seed dormancy, A. A. Khan [ed.], 51–74. North Holland, Amsterdam.

Nowakowska J and Rakowski K (2002). Accelerated and natural ageing processes change the properties of plasma membrane in Norway spruce (*Picea abies* [L.] Karst.) seeds during storage. *Dendrobiology,* 47: 79-82.

Nwoboshi, LC (1982). *In* Tropical Silviculture. Ibadan University Press. pp 333.

Ochuodho JO, Modi AT and Beukes M (2005). The Dormancy and Germination of *Cleome gynandra* L. *In*: The Proceedings of the Fourth Workshop on Sustainable Horticultural Production in the Tropics. Wesonga, et al. (Eds), Department of Horticulture, Moi University, Eldoret, Kenya, 24th – 27th November 2004.

Odetola JA (1987). Studies on seed dormancy, viability, and germination in ornamental palms. *Principes,* 31: 24-30.

Ouédraogo AS, Thomsen K, Engels JMM and Engelmann F (1999) Challenges and opportunities for the enhanced use of recalcitrant and intermediate tropical forest tree seeds through improved handling and storage. Pp 227-234. *In* Marzalina M, Khoo KC, Jayanthi N, Tsan FY and Krishnapillay B (eds): IUFRO Seed Symposium 1998 "Recalcitrant Seeds": Proceedings of the conference. Forest Research Institute, Kuala Lumpur, Malaysia.

Pair JC and Khatamian H (1982). Propagation and growing of the Chinese pistachio. *Can. Proc. Plant Prop. Seed,* 32: 497-503.

Palani M, Dasrhagir MG and Kumaran K (1995). Effect of pre-sowing chemical treatment on germination and seedling growth in *Acacia nilotica. International Tree Crops Journal,* 8:189-192.

Pedroza-Manrique J, Fernandez-Lizarazo C and Suarez-Silva A (2005). Evaluation of the effect of three growth regulators in the germination of *Comparettia falcate* seeds under *in vitro* conditions. *In Vitro Cell. Dev. Biol.-Plant,* 41:838-843.

Phartyal SS, Baskin JM, Baskin CC and Thapliyal RC (2005) Physical dormancy in seeds of *Dodonaea viscosa* (Sapindaceae) from India. *Seed Science Research* 15: 59-61.

Phartyal SS, Thapliyal RC, Koedam N and Godefroid S (2002) Ex situ conservation of rare and valuable forest tree species through seed-gene bank. *Current Science,* 83 (11) :1351-1357.

Piehl MA (1965). The natural history and taxonomy of *Comandra* (Santalaceae). *Mem. Torrey Bot. Club,* 22: 1-80.

Pitel JA and Wang, BSP (1988). Improving germination of basswood (*Tilia americana* L.) seeds with gibberellic acid. *Seed Science and Technology*, 16: 273–280.

Porter R H, Durell M and Room HJ (1947). *Plant Physiol.*, 22: 149– 159.

Priestley DA and Leopold AC (1983). Lipids changes during natural ageing of soybean seeds. *Physiologia Plantarum* 59: 467-470.

Pritchard HW (1991) Water potential and embryonic axis viability in recalcitrant seeds of *Quercus rubra*. *Annals of Botany*, 67(1): 43-49.

Probert RJ, Longley PL (1989). Recalcitrant seed storage physiology in three aquatic grasses (*Zizania palustris*, *Spartina anglica* and *Portesia coarctata*). *Annals of Botany*, 63(1): 53-63.

Rai SN; Nagaveni HC; Padmanabha HSA (1986). Germination and viability of some tree seeds. *Van-Vigyan,* 24: 1-2, 8-12.

Rama Rao M (1911). Host plants of the sandal tree. *Indian Forest Records* 2(4):159-207.

Ramanandane T and Ponnuswamy AS (2004). Aging and anatomical influence on seed storability in rice (*Oryza sativa* L.) and hybrids and parental lines. *Tropical Agricultural research* 16: 37-50.

Ramaswamy MN (1949) Pre-treatment of seeds with coconut water to hasten germination. *Indian Forester,* 75(12): 500.

Rame Gowda, (1992). Studies on seed senescence and seed vigour in some genotypes of rice (Oryza sativa L.) Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore.

Rana U, Nautiyal AR (1989). Coat imposed dormancy of *Acacia farnesiana* seeds. *Seed Research,* 17(2): 122-127.

Rangaswamy NS (1967). Morphogenesis of seed germination in angiosperms. *Phytomorphology,* 17: 477–487.

Rangaswamy NS and Rao PS (1963). Experimental studies on *Santalum album* L.: Establishment of tissue culture of endosperm. *Phytomorphology*, 13:450-454.

Rao KVM and Kalpana R (1994). Carbohydrates and the aging process in seeds of pigeon pea (*Cajanas cajan* L. Millsp) cultivars. *Seed Sci. Technol.*, 22: 495-501.

Rao NK, Roberts EH and Ellis RH (1987). Loss of viability in lettuce seeds and the accumulation of chromosome damage under different storage conditions. *Ann. Bot.*, 60:85-96.

Raulston JC (1990). Redbud. *American Nurseryman*, 171(5): 39-51.

Reddy KK and Srivasuki KP (1990). Vegetative propagation of red sanders (*Pterocarpus santalinus* Linn.). *Indian-Forester*, 116: 7, 536-540.

Rees M. (1997) Seed dormancy. pp. 214-238 in Crawley, M. (Ed.) Plant Ecology. London, Blackwell Science.

Ren, J. and Tao, L. (2004) Effects of different pre-sowing seed treatments on the germination of 10 Calligonum species. *Forest Ecology and Management* 195: 291-300.

Rice KJ and Dyer AR (2001). Seed aging, delayed germination and reduced competitive ability in *Bromustectorum*. *Plant Ecol.*, 155: 237-243.

Roberts EH (1972) Cytological, genetical and metabolic changes associated with loss of viability. Pp 253-306 *In* Roberts EH (ed). Viability of seeds. Chapman & Hall, London.

Roberts EH (1973) Predicting the storage life of seeds. *Seed Science and Technology* 1: 499-514.

Roberts E, and Smith RD (1977). Dormancy and the pentose phosphate pathway. *In*: The Physiology and Biochemistry of Seed Dormancy and Germination (ed. Khan, A.), North-Holland Publ. Co, Amsterdam, pp. 385–411.

Roberts EH (1973). *In* Seed Ecology, Heydecker W (ed.) , Penn. State Univ. Press, University Park, pp. 189-218.

Roberts EH, King MW and Ellis RH (1984). Recalcitrant seeds: their recognition and storage. *In:* Crops Genetic Resources: Conservation and Evaluation. Holden, J.H.W. and Williams, J.T. (Eds.). Allen and Unwin, London. pp 38-52.

Robertson KR (1976). *Cercis*: the redbuds. *Arnoldia*, 36(2): 37-49.

Rodriguez DA and Rojo C (1997). Estudio de la semilla del arbusto *Lupinus montanus* H.B.K. (Leguminosae) *Revista Chapingo, Serie: Ciencias Forestales* 3: 39-45.

Rolston MP (1978). Water impermeable seed dormancy. *The Botanical Review* (New York), 44(3): 365-396.

Rosner LS, Harrington JT, Dreesen DR, and Murray L (2005). Effect of gibberellic acid and standard seed treatments on mountain snowberry germination. *Native Plants Journal,* 3(2): 155-162.

Ross EE (1982). Induced genetic changes in seed germplasm during storage. *In*: The Physiology and Biochemistry of Seed Development, Dormancy and Germination, A. Khan (ed). Elsevier Biomedical Press, Amsterdam. pp. 409-434.

Rout GR, Samanantray S, Das P (2000). In vitro manipulation and propagation of medicinal plants. Biotechnol. Ad. 18: 91-120.

Sacandé M, Van Pijlen JG, Hoekstra FA, and Groot SPC (1998). A multifactorial study of conditions influencing longevity of neem (*Azadirachta indica*) seeds. *Seed Science Research,* 8: 473-482.

Salisbury F and Ross C (2000). Fisiologia Vegetal: Desarrollo de las plantas y fisiologia ambiental. Espana: Thomson editores Paraninfo, S.A. pp 573-574.

Sammour RH (1989). Effect of ageing on the major reserve molecules and their related enzyme in natural aged seeds of flax. *Journal of Islamic Academy of Sciences,* 2(4): 247-251.

Sarita P, Bhatnagar SP, Bhojwani SS (1988). Preliminary investigations on micropropagation of a leguminous timber tree: *Pterocarpus santalinus. Phytomorphology*, 38(1): 41-45.

Sautu A, Baskin JM, Baskin CC and Condit R (2006). Studies on the seed biology of 100 native species of trees in a seasonal moist tropical forest, Panama. *Central America Forest Ecology and Management,* 234: 245–263.

Schmidt L (2000). Dormancy and pretreatment. *In*: Guide to handling of tropical and subtropical forest seed. Olsen K (Ed.). Humlebaek: Danida Forest Seed Centre, p.263-303.

Schopfer P and Plachy C (1985). Control of seed germination by abscisic acid. III. Effect of embryo growth potential (minimum turgor pressure) and growth coefficient (cell wall extensibility) in *Brassica napus* L. *Plant Physiol.*, 77:676-686.

Schuurink RC, Sedee NJA, Wang M (1992). Dormancy of the barley grain is correlated with gibberellic acid responsiveness of the isolated aleurone layer. *Plant Physiol.*, 100: 1834–1839.

Scianna JD (2001). Collecting, processing and germinating rocky mountain juniper seed. *Native Plants Journal,* 2(2): 73-78.

Scott D (1974). Some germination requirements of *Celmisia* species. *New Zealand Journal of Botany,* 13: 653-64.

Scott J (1871). Notes of horticulture in Bengal: No.2-Loranthaceae, the mistletoe order, their germination and mode of attachment. *J. Royal Hort. Soc. India,* 2: 287.

Sestak Z, Castky J, Jervis P (1971) Methods of growth analysis. *In:* Junk, W. *ed.* Plant photosynthetic production. Manual of methods. The Hague, N V Publishers. Pp. 363-443.

Shen TY & Odén PC (2002). Relationships between seed vigour and fumarase activity in *Picea abies*, *Pinus contorta*, *Betula pendula* and *Fagus sylvatica. Seed science and technology*, 30: 177-186.

Shiferaw H H, Teketay D, Nemomissa S and Assefa (2004). Some biological characteristics that foster the invasion of *Prosopis juliflora* (Sw.) DC. at Middle Awash Rift Valley Area, north-eastern Ethiopia. *Journal of Arid Environments,* 58: 135–154.

Shipley B and Parent M (1991). Germination responses of 64 wetland species in relation to seed size, minimum time to reproduction and seedling relative growth rate. *Functional Ecology* 5:111-118.

Shivanna KR and Mohan Ram HY (2005). Contributions of Panchanan Maheshwari's school to angiosperm embryology through an integrative approach. *Current Science,* 89(11): 1820-1834.

Shivanna KR and Rangaswamy NS (1976). Seed germination and seedling morphogenesis of the root parasite *Sopubia delphinifolia* G. Don. *Z. Pflanzenphysiol.*, 80: 112–119.

Siddiqui ZS, Shaukat SS and Arif-Uz-Zaman (2006). Alleviation of Salinity-Induced Dormancy by Growth Regulators in Wheat Seeds. *Turk J Bot.,* 30: 321-330

Singh BG and Rao GR (1993). Effect of chemical soaking of sunflower (*Helianthus annuus*) seed on vigour index. *Indian J. Agric. Sci.*, 63:232–233.

Singh U, Wadhawani AM and Johri BM (1965). Dictionary of economic plants in India (Indian Council of Agriculture and Research, New Delhi). pp 176- 184.

Sivritepe HO and Dourado AM (1998). The effect of storage environment on seed survival and the accumulation of chromosomal aberrations in pea landraces and cultivars (*Pisum sativum* L.). *Tr. J. Bot.*, 22: 223-232.

Sixtus CR, Hill GD and Scott RR (2003). The effect of temperature and scarification method on gorse (*Ulex europaeus* L.) seed germination. *New Zealand Plant Protection,* 56: 201-205.

Smiderle OJ, Souza RCP (2003). Dormência em sementes de paricarana (*Bowdichia virgiloides*, Kunth, Fabaceae –Papilionoideae). *Revista Brasileira de Sementes, Londrina*, 25(1): 72-75.

Smith MT, Ben S.P. Wang and Msanga HP (1990). Dormancy and germination. *In* Tropical Tree Seed Manual, Vozzo JA (Ed.) United States Department of Agriculture, Forest Service.

Smith MT, Berjak P (1995) Deteriorative changes associated with the loss of viability of stored desiccation tolerant and desiccation-sensitive seeds. *In*: Seed development and germination. Kigel, J. D.; Galili, G. (Eds.) New York: Marcel Dekker Inc., pp. 701-746.

Smith N. (1986). Growing natives: more from the chaparral. Fremontia 14(3): 34-35.

Smith OE, Yen WW, Lyons JM (1968). The effects of kinetin in overcoming high-temperature dormancy in lettuce seeds. *Proc. Amer. Soc. Hort Sci.* , 93: 444-453.

Srimathi RA and Rao PS (1969). Accelerated germination of sandal seed. *Indian Forester,* 95(3): 158-159.

Srinivasan VV, Sivaramakrishnan VR, rangaswamy CR, Ananthpadmanabha HS and Shankaranarayan KH (1992). Sandal (*Santalum album* L.) (Indian Council of forestry Research and Education, Dehra Dun). pp 1.

Stenberg ML and Kane ME (1998) *In vitro* seed germination and greenhouse cultivation of *Encycliaboothiana* var. *erythronioides*, an endangered Florida orchid. *Lindleyana* 13: 101–112.

Stewart GR and Press MC (1990). The physiology and biochemistry of parasitic angiosperms. *Ann. Rev. Plant Physiol.* 41: 127-151.

Stewart RR, and Bewley JD (1980) Lipid peroxidation associated with accelerated ageing in soybean axes. *Plant Physiology* 65: 245-248.

Stokes P (1965). Temperature and seed dormancy. *Encycl. Plant Physiol.*,15: 746-803.

Sundarkumari C, Zhang CL, Martin KP, Slater A, Madhusudan PV(2005).Effects of auxins on indirect *in vitro* morphogenesis and expression of *gusA* transgene in lactenaceous medicinal plant, *Euphorbia nivulia* Buch-Ham. *In vitro Cell Dev. Biol. Plant*, 41: 695-699.

Sung JM and Chiu CC (1995) Lipid peroxidation and peroxide-scavenging enzymes of naturally aged soybean seed. *Plant Science* 110: 45-52.

Surendran C, Parthiban KT, Bhuvaneswaran-C and Murugesh M (1998). Silvicultural strategies for augmentation of sandal regeneration. ACIAR-Proceedings-Series. 1998, No. 84, 69-73.

Susila IWW, Effendi M and Sinaga M (1995). Study of the phenology and seed quality of sandalwood (*Santalum album*) in NTT (East Nusa Tenggara). Santalum. 1995, recd. 1977, No.19, 1-8.

Suwanketnikom R and Julakasewee A (2004). Hard seededness and germination of small white flower morning-glory. *Kasetsart J. (Nat. Sci.),* 38 : 425 - 433

Taiz L and Zeiger E (2006). Abscisic Acid: A seed maturation and anti-stress signal. *In* Plant Physiology (4e), Sinauer Associates Inc., Publishers, Sunderlands, Massachusetts, pp 599-600.

Tammela P, Hopia A, Hiltunen R, Vuorela H and Nygren M (2000) Aging in *Pinus syvestris* L. seeds: changes in viability and lipids. *Biochemical Society Transactions* 28: 878-879.

Tang S, TeKrony DM, Egli DB, Cornelius PL and Rucker M. (1999). Seed physiology, production & technology: Survival Characteristics of Corn Seed during Storage: I: Normal Distribution of Seed Survival. *Crop Sci.*, 39:1394–1400.

Taylor IN, Peters NCB, Adkins SW and Walker SR (2004). Germination response of *Phalaris paradoxa* L. seed to different light qualities. *Weed Research,* 44:254–264.

Taylorson RB and Hendricks S B (1977). Dormancy in seeds. *Ann. Rev. Plant Physiol.* 28: 331-354

Teketay D. and Tigabu M. (1996) The effect of pre-sowing seed treatments, temperature and light on the germination of *Tamarindus indica* L., a multipurpose tree. *Journal of tropical Forestry*12: 73-79.

Thakur AS, Thakur PS and Metha R (2005). Effect of presoaking treatments on seed germination in *Berberis aristata. Indian J. Plant Physiol.*, 10(4): 338-343.

Thomas TH, Palevitch D, Biddington NL, Austing RB (1975). Growth Regulators and the Phytochrom-Mediated Dormancy of Celery Seeds, *Physiol. Plant.*, 35:101-106.

Thompson RC (1940). Germination of lettuce seed at high temperature stimulated by thiourea. *Science*, 100(2589):131.

Thompson RC (1944). Germination of Lettuce Seed at High Temperature Stimulated by Thiourea. *Science*, New Series, 100(2589) :131.

Thompson RC and Kosar WF (1939). *Plant Physiol.*, 14: 567-573

Thomsen KA (1997). The effect of harvest time and drying on dormancy and storability in beechnuts In: Ellis, R. H., Black, M., Murdoch A. J., Hong, T.D. (eds.) Basic and applied aspects of seed biology. Current Plant Science and Biotechnology in Agriculture No. 30; Kluwer Academic Publishers, 45-51.

Tian X, Knapp AD, Moore KJ, Brummer EC, and Bailey TB (2002). Cupule removal and caryopsis scarification improves germination of eastern gamagrass seed. *Crop Sci.*, 42:185–189

Tigabu M and Oden PC (2001). Effect of scarification, gibberellic acid and temperature on seed germination of two multipurpose Albizia species from Ethiopia. *Seed Sci. Technol.* 29:11—20.

Tigabu M, and Odéon PC (2001) Effect of scarification, gibberellic acid and temperature on seed germination of two multipurpose *Albizia* species from Ethiopia. *Seed Science and Technology*, 29: 11-20.

Tiwari ON and Chauhan UK (2007). Seed germination studies in *Rhododendron maddenii* Hook.F. and *Rhododendron niveum* Hook.F. *Indian J. Plant Physiol.*, 12(1): 50-56.

Tiwari PK, Agrawal R, Savita K (1999). Studies on the effect of growth regulators on germination percent and plant percent of some leguminous forest tree species. *Journal of Tropical Forestry* 15: 3, 196-201.

Tiwari S, Shah P and Singh K (2004). In vitro propagation of *Pterocarpus marsupium* Roxb.: An endangered medicinal tree. *Indian Journal of Biotechnology*, 3: 422-425.

Toole EH, Hendricks SB, Borthwick HA, and Toole VK (1956). Physiology of seed germination. *Annu. Rev. Plant Physiol.*, 7:299.

Trawatha SE, Tekrony DM and Hidebrand DF (1995). Relationship of soybean seed quality to fatty acid and C6–Aldehyde levels during storage. *Crop Sci.*, 35: 1415–22.

Tsay HS, Lec CY, Agarwal DC, Basker S (2006). Influence of ventilation closure, gelling agent and explant type on shoot bud proliferation and hyperhydricity in *Scrophularia yoshimuae*- a medicinal plant. *In vitro Cell Dev. Biol. Plant*, 42: 445-449.

Turnbull JW (1975). Forest tree seed testing. *In* Report on FAO/DANIDA Training Course on Forest Seed Collection and Handling, Vol. 2, FAO Rome.

Van Waes JM, and Debergh PC (1986). In vitro germination of some Western European orchids. *Physiologia Plantarum,* 67: 253-261.

Vastano Júnior B, Barbosa AP, Gonçalves AN (1983). Tratamentos pré-germinativos de sementes de espécies florestais amazônicas. I – Angelim pedra (*Dinizia excelsa* Ducke –Leguminosae, Mimosoideae). *Acta Amazonica, Manaus*, 13(2): 413-419.

Vázquez-Yanes C and Orozco-Segovia A (1993). Patterns of seed longevity and germination in tropical rain forest. *Annual Review in Ecology and Systematics,* 24, 69-87.

Veasey EA and Freitas JCT (2002). Breaking seed dormancy in *Sesbania sesban*, *S. rostrata* and *S. virgata. Seed Science and Technology*, 30 (1): 211-217.

Verghese J, Sunny TP, Balkrishnan KV (1990). (Z)-(+)-α-santalol and (Z)-(-)-β-santalol concentrations, a new quality determination of east Indian sandalwood oil. *Flavour and Fragrance Journal,* 5:223-226.

Wareing PF (1969). The Physiology of Plant Growth and Development, ed. M. B. Wilkins, pp 603-44. New York, McGraw.

Wareing PF, Saunders PF (1971). *Ann. Rev. Plant Physiol.*, 22:261-288.

Waters Jr. L and Blanchette BL (1983). Prediction of sweet maize field emergence by conductivity and cold tests. *J. Amer. Soc. Hort. Sci.*, 108: 778-781.

Watkins JT, Cantlife JD (1983). Hormonal control of pepper seed germination. *Hort. Science*, 18(3):.342-343.

Watkins JT, Cantlife JD, Huber DJ, Nell TA (1985). Gibberellic acid stimulated degradation of endosperm in pepper. *J Am Soc Hort. Sci.*, 110:61-65.

Weinberger JW (1969). *Hort. Science,* 4:125-26

Wilson JK (1944). Immersing seeds of species of *Robinia* in boiling water hastens germination. *Journal of Forestry*, 42: 453-454.

Wolf H and Kamondo B (1993). Seed pre-sowing treatment. *In*: Tree seed handbook of Kenya. Albrecht J (Ed.), Nairobi, Kenya: Kenya Forestry Research Institute/Gesellschaft für Technische Zusammenarbeir: 55-62.

Wood CB, Pritchard HW and Amritphale D (2000). Desiccation-induced dormancy in papaya (*Carica papaya* L.) seeds is alleviated by heat shock. *Seed Science Research,* 10:135–145.

Woodall GS (2004) Cracking the woody endocarp of Santalum spicatum nuts by wetting and rapid drying improves germination. *Australian Journal of Botany*, 52(2):163-169.

Woodstock LW (1973). Physiological and biochemical tests for seed vigor. *Seed Sci. & Technol.*, 1: 127–57

Woodstock, LW (1988). Seed imbibition: a critical period for successful germination. *Journal of Seed Technology,* 12:1-15.

Yates RT and Curtis JT (1949). The effect of sucrose and other factors on the shoot-root ratio of orchid seedlings. *Am.J.Bot.*,36: 390-396.

Younsheng C and Sziklai O (1985). Preliminary study on the germination of *Toora sinensis* (A.JUSS). Roem. Seed from eleven Chinese provenances. *For. Ecol. Manage.*, 10: 269-281.

Zar JH (1996) *In* Biostatistical Analysis, 3e. Prentice Hall, Inc., Upper Saddle River, NJ, USA.