STUDIES ON SEED GERMINATION BEHAVIOUR OF *Santalum album* L. UNDER NURSERY CONDITIONS

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE
in
FORESTRY
(Minor Subject: Agronomy)

By

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(L-2018-A-85-M)

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LUDHIANA - 141004

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CERTIFICATE - I

This is to certify that the thesis entitled **"STUDIES ON SEED GERMINATION BEHAVIOUR OF *Santalum album* L. UNDER NURSERY CONDITIONS"** submitted for the degree of **Master of Science** in the subject of **Forestry** (Minor subject: **Agronomy)** of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Rajdeep Singh** (**L-2018-A-85-M**) under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE – II

This is to certify that the thesis entitled **"STUDIES ON SEED GERMINATION BEHAVIOUR OF *Santalum album* L. UNDER NURSERY CONDITIONS"** submitted by **Rajdeep Singh** (**L-2018-A-85-M**) to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Master of Science,** in the subject of **Forestry** (Minor subject: **Agronomy**) has been approved by the Student's Advisory Committee along with External Examiner after an oral examination on the same.

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ABSTRACT

The present investigation entitled, "Studies on seed germination behavior of *Santalum album* L. under nursery conditions" was undertaken to standardize the suitable seed source (KFRI, Kerala and UHF Neri Campus, Hamirpur, Himachal Pradesh), growing environment (open and shade) and to assess the effect of different pre-sowing seed treatments i.e. Cold water (48 hours), GA_3 (0.05 % for 24 hours), GA_3 (0.5 % for 24 hours), H_2SO_4 (10 % for 5 min) and H_2SO_4 (10 % for 1 min) on seed germination, growth and quality of sandalwood seedlings. Observations were recorded for seed germination parameters and then the seedlings were transplanted to polybags along with host plant *Cajanus cajan*. Observations like plant collar diameter, height and number of leaves were recorded after every 15 days for six months after transplantation, whereas the data for other parameters viz. root length (cm), root and shoot fresh weight (g), root and shoot dry weight (g), root:shoot ratio, sturdiness quotient and quality index were taken after six months. The three factor CRD with four replications was used as an experimental design to analyze 20 treatment combinations. ANOVA indicated significant effect of pre-sowing seed treatments on all the parameters studied. While the effect of seed source, growing environment and all interaction effects were found to be non significant. Among the pre-sowing seed treatments, GA_3 0.5 % for 24 hours has shown the best performance for seed germination parameters. Whereas, H_2SO_4 (10% for 5 min) gave better results for seedling growth and quality parameters. Treating the seeds with cold water for 48 hours performed poorly among all pre-sowing seed treatments.

Keywords: Seed source, pre-sowing treatments, growing environment, *Santalum album*

Signature of Major Advisor **Signature of the Student**

ਨਿਬੰਧ ਦਾ ਸਿਰਲੇਖ	:	ਨਰਸਰੀ ਦੀਆਂ ਸਥਿਤੀਆਂ ਅਧੀਨ ਸੈਂਟਲਮ ਐਲਬਮ ਐਲ ਦੇ ਬੀਜ ਉਗਣ ਵਾਲੇ ਵਿਵਹਾਰ ਬਾਰੇ ਅਧਿਐਨ
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ਮੌਜੂਦਾ ਜਾਂਚ ''ਨਰਸਰੀ ਹਾਲਾਤਾਂ ਅਧੀਨ ਸੈਂਟਲਮ ਐਲਬਮ ਐਲ ਦੇ ਬੀਜ ਉਗਣ ਵਾਲੇ ਵਿਵਹਾਰ ਬਾਰੇ ਅਧਿਐਨ'' ਉਚਿਤ ਬੀਜ ਸਰੋਤ (ਕੇ.ਐਫ.ਆਰ.ਆਈ., ਕੇਰਲਾ ਅਤੇ ਯੂ.ਐੱਚ.ਐਫ. ਨੇਰੀ ਕੈਂਪਸ, ਹਮੀਰਪੁਰ, ਹਿਮਾਚਲ ਪ੍ਰਦੇਸ਼), ਵੱਧ ਰਹੇ ਵਾਤਵਰਣ (ਖੁਲੇ ਅਤੇ ਛਾਂ) ਦੇ ਮਾਨਕੀਕਰਨ ਲਈ ਕੀਤੀ ਗਈ ਸੀ ਅਤੇ ਵੱਖ-ਵੱਖ ਬਿਜਾਈ ਬੀਜ ਦੇ ਉਪਚਾਰਾਂ ਦੇ ਪ੍ਰਭਾਵ ਦਾ ਮੁਲਾਂਕਣ ਕਰਨ ਲਈ ਜਿਵੇਂ ਕਿ ਠੰਡੇ ਪਾਣੀ (48 ਘੰਟੇ), ਜੀਏ 3 (0.05% 24 ਘੰਟਿਆਂ ਲਈ), ਜੀਏ 3 (0.5% 24 ਘੰਟਿਆਂ ਲਈ) ਐਚ 2 ਐਸਓ 4 (10% 5 ਮਿੰਟ ਲਈ) ਅਤੇ ਐਚ 2 ਐਸਓ 4 (10% 1 ਮਿੰਟ ਲਈ) ਬੀਜ ਦੇ ਉਗਣ, ਚੰਦਨ ਦੇ ਬੂਟੇ ਦੀ ਵਾਧੇ ਅਤੇ ਗੁਣਵੱਤਾ ਲਈ)। ਬੀਜ ਦੇ ਉਗਣ ਤਦੇ ਮਾਪਦੰਡਾਂ ਲਈ ਨਿਰੀਖਣ ਦਰਜ ਕੀਤੇ ਗਏ ਆਤੇ ਫਿਰ ਪੌਦੇ ਮੇਜਬਾਨ ਪੌਦੇ ਕੇਜਾਨਸ ਕੇਜਾਨ ਦੇ ਨਾਲ ਪੌਲੀਬੈਗਾਂ ਵਿੱਚ ਲਗਾਏ ਗਏ । ਪੌਦੇ ਕਾਲਰ ਵਿਆਸ, ਉਚਾਈ ਅਤੇ ਪਤਿਆਂ ਦੀ ਸੰਖਿਆ ਜਿਵੇਂ ਟ੍ਰਾਂਸਪਲਾਂਟੇਸ਼ਨ ਤੋਂ ਬਾਅਦ ਛੇ ਮਹੀਨਿਆਂ ਲਈ ਹਰ 15 ਦਿਨਾਂ ਬਾਅਦ ਰਿਕਾਰਡ ਕੀਤੇ ਗਏ ਸਨ । ਜਦੋਂ ਕਿ ਹੋਰ ਮਾਪਦੰਡਾਂ ਦੇ ਅੰਕੜੇ, ਰੂਟ ਦੀ ਲੰਬਾਈ (ਸੈ.ਮੀ.) ਰੂਟ ਅਤੇ ਸ਼ੂਟ ਕਰੋ ਤਾਜ਼ਾ ਭਾਰ (ਗ੍ਰਾਮ), ਰੂਟ ਐਂਡ ਸ਼ੂਟ ਡ੍ਰਾਈ ਵੇਟ (ਗ੍ਰਾਮ), ਰੂਟ: ਸ਼ੂਟ ਰੇਸ਼ੋ, ਸਟਾਰਡਨੇਸ ਕੁਆਇੰਟ ਅਤੇ ਕੁਆਲਟੀ ਇੰਡੈਕਸ ਛੇ ਮਹੀਨਿਆਂ ਬਾਅਦ ਲਏ ਗਏ । ਚਾਰ ਪ੍ਰਤੀਕ੍ਰਿਤੀਆਂ ਦੇ ਨਾਲ ਤਿੰਨ ਕਾਰਕ ਸੀਆਰਡੀ ਦੀ ਵਰਤੋਂ 20 ਇਲਾਜ ਦੇ ਸੰਜੋਗਾਂ ਦਾ ਵਿਸ਼ਲੇਸ਼ਣ ਕਰਨ ਲਈ ਇੱਕ ਪ੍ਰਯੋਗਾਤਮਕ ਡਿਜ਼ਾਇਨ ਵਜੋਂ ਕੀਤੀ ਗਈ ਸੀ । ਐਨੋਵਾ ਨੇ ਅਧਿਐਨ ਕੀਤੇ ਸਾਰੇ ਮਾਪਦੰਡਾਂ ਤੇ ਬਿਜਾਈ ਤੋਂ ਪਹਿਲਾਂ ਬੀਜ ਦੇ ਮਹੱਤਵਪੂਰਣ ਪ੍ਰਭਾਵ ਦਾ ਸੰਕੇਤ ਦਿੱਤਾ ਜਦੋਂ ਕਿ ਬੀਜ ਸਰੋਤ ਵੱਧ ਰਹੇ ਵਾਤਾਵਰਣ ਅਤੇ ਸਾਰੇ ਪਰਸਪਰ ਪ੍ਰਭਾਵ ਦੇ ਪ੍ਰਭਾਵ ਮਹੱਤਵਪੂਰਣ ਨਹੀਂ ਪਾਏ ਗਏ । ਬਿਜਾਈ ਤੋਂ ਪਹਿਲਾਂ ਬੀਜ ਦੇ ਇਲਾਜ ਵਿੱਚ 24 ਘੰਟਿਆਂ ਲਈ ਜੀਏ3 0.5% ਨੇ ਬੀਜ ਦੇ ਉਗਣ ਦੇ ਮਾਪਦੰਡਾਂ ਲਈ ਸਭ ਤੋਂ ਵਧੀਆ ਪ੍ਰਦਰਸ਼ਨ ਦਿਖਾਇਆ ਜਦੋਂ ਕਿ ਐਚ 2 ਐਸ ਓ 4 (10% 5 ਮਿੰਟ ਲਈ) ਨੇ ਬਿਜਾਈ ਦੇ ਵਾਧੇ ਅਤੇ ਗੁਣਵੱਤਾ ਦੇ ਮਾਪਦੰਡਾਂ ਲਈ ਵਧੀਆ ਨਤੀਜੇ ਦਿੱਤੇ । ਬੀਜਾਂ ਦਾ ਠੰਡੇ ਪਾਣੀ ਨਾਲ 48 ਘੰਟਿਆਂ ਤੱਕ ਇਲਾਜ ਬਿਜਾਈ ਕਰਨ ਵਾਲੇ ਸਾਰੇ ਬੀਜ ਇਲਾਜਾਂ ਵਿੱਚ ਮਾੜਾ ਪ੍ਰਦਰਸ਼ਨ ਕੀਤਾ ਗਿਆ ।

ਮੁੱਖ ਸ਼ਬਦ: ਬੀਜ ਸਰੋਤ, ਬਿਜਾਈ ਤੋਂ ਪਹਿਲਾਂ ਦੇ ਇਲਾਜ, ਵੱਧ ਰਹੇ ਵਾਤਾਵਰਣ, ਸੈਂਟਲਮ ਐਲਬਮ ।

ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ **ਵਿਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

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LIST OF ABBREVIATIONS AND SYMBOLS

%	:	Per cent
0	:	Degree
Min	:	Minute
b.s.	:	Broad sense
C	:	Celsius
CD	:	Critical Difference
Cm	:	centimetre(s)
CV	:	Coefficient of Variance
d.f.	:	Degree of freedom
et al	:	and others
etc.	:	etcetera (and so on)
Fig.	:	Figure
G	:	gram
i.e.	:	That is
M	:	meter(s)
Mm	:	millimetre(s)
Mg	:	milligram
Ml	:	millilitre
NS	:	Non-significant
CRD	:	Complete Randomized Design
spp.	:	Species
T	:	treatment
viz.,	:	Videlicet (Namely)

CHAPTER-I

INTRODUCTION

Punjab, "the land of five rivers" with only 1.57 per cent (50, 362 sq. km) of the country's total geographical area is primarily an agrarian state with 84 per cent of its area is under agriculture with very high cropping intensity and has around 6.83 per cent of the its area under forest cover and tree cover. As major proportion of land is under agriculture hence, there is limited scope to increase the area under the forest cover except bringing the non forest lands under forest cover. Right from the enunciation of National Forest Policy of 1952 followed by another in 1988, various measures are being taken to improve the forest cover in the country. Under National Forest Policy of 1988, there was strong enforcement to bring 20 per cent area in major agricultural plains under forest cover. To meet the objectives of increasing the area under forest and tree cover in Punjab as well as to meet the ever increasing demand for wood products, planting of fast growing tree species outside the conventional forest area is the need of time. Integration of tress with commercial importance like poplar, eucalyptus, dek, sandalwood, etc in the existing land use system can contribute towards additional income generation and one of the potential strategies towards diversification of farming system in the state.

The *Santalum album* belongs to family *'Santalaceae'* which is represented by1000 species throughout the world (Jeeva *et al* 1998). The genus *Santalum* consists of 18 species, the out of which Indian sandalwood (*Santalum album*) and Australian sandalwood (*Santalum spicatum*) are of commercial importance (Shea *et al* 1998 and Ansari *et al* 2007). *S. album* commonly known as chandan naturally occurs between 30˚N to 40˚S from India in the west to Juan Fernandez Island in the east and from Hawaiian Archepelago in the north to Newzealand in the south (George 1984 and Srinivasan *et al* 1992). It is found in the countries like Australia, Indonesia, Japan, Belgium, China, Cambodia, Madagaskar, Germany, Holland, Norway, Russia, Switzerland and the United States. In India, it is naturally distributed over 9600 km^2 in the deciduous forests of peninsular India (Srinivasan *et al* 1992 and Radomiljac *et al* 1998). In India, about 90 per cent of area of chandan occurs in two south states i.e. Karnataka and Tamil Naidu. Other states which also constitute major sandal tracts are Andhra Pradesh, Maharashtra, Orissa and 8Madhya Pradesh (Jeeva *et al* 1998). Though *S. album* is indigenous to Deccan Plateaus, it has been introduced and naturalized in other parts of India (Sandeep *et al* 2016).

Santalum album is an evergreen plant, attaining 10-15 m height and 1-2 m girth at the stage of maturity i.e. 60-80 years (Ghosh *et al* 1985). The leaves are opposite, thin and ovate to lanceolate in shape. Flowering and fruiting occurs twice in a year from March to May and September to December. Flowering starts appearing at an age of 7 to 8 years and fruiting after

10 years. The colour of bark is reddish to dark brown in the young tree. Under natural conditions, the increment of heartwood is about 1kg per year and the girth of 1 cm per year. It is a conventionally propagated through seeds by the process of endozoochory and is highly polymorphous in nature. Significant variations have been reported in leaf width and length, oil content and in heartwood colour (Kushalappa 1983; Bagchi and Veerendra 1985 and Kulkarni 1995). Genotypic variations are also common in *S. album* for various anatomical characters like xylem cell diameter, cortex width, number of vascular bundles and epidermal thickness (Veerendra and Bagchi 1986).

Sandal can grow on various types of soil like sandy, clayey, red, laterite, loamy and even on black cotton soils. Trees which grow on stony and gravelly soil perform better and have more scented wood (Srimathi *et al* 1980). Rainfall between 600 to1600 mm is found to be optimum for its growth. Attitudinally, it can grow up to height of 1800 m from the sea level.

Santalum album is semi-root parasitic plant and depends upon other plants for its nutrition. Scot (1871) was first person to report the parasitic mode of nutrition in *S. album* seedlings. It can parasitize over 300 different species of forest trees but it shows preference to some specific hosts. The order of preference is *Pongamia pinnata*, *Albizia lebbeck* and *Tectona grandis* (Rama 1911). Its host requirement in nursery stage is different from that is in plantation stage. *Cajanus cajan* and *Casuarina equisetifolia* has been proved to be best during nursery and plantation stages respectively (Kulkarni 1995).

It is one of the best plant species and well associated with Indian culture. It is the most valuable species among the forest trees of India and is considered as prize gift to Indian culture and heritage. *S. album* trees are primary source of derived oils and fragrant heartwood, which are highly priced. The heartwood of sandal is highly valued for its fragrance wood, which yields oil and sandal oil is preferred in cosmetic, perfumes and medicines (Sanjaya *et al* 1998). Oil is present in the roots and heartwood, thus for extracting oil, the tree is always harvested by uprooting. Upon steam distillation, *S. album* yields on an average 5-7 per cent of high grade perfumery valued essential oil known as east Indian *Santalum album* oil. Essential oils are the complex compounds, which deposited in the cell organelles, heartwood and in excretory cavities or canals and are called as "Liquid Gold". Sandal's oil contains 90 per cent santalol, 2.5 to 5 per cent santalyl acetate and 1.5 to 3 per cent santalences. Santalol are of two types α santalol and β santalol and β santalol is responsible for 90 per cent of its characteristic odoriferous nature. The value of *S. album* oil is high where the β-santalol content is high in the oil. (Arun Kumar *et al* 2012**).** The essential oil of *S. album* is used for skin problems like pimples, prickly heat, skin eruptions, itching, swelling and rashes.

The *S. album* woodproduction was 3176 tons per year during 1960-65 and regularly decline as 1500 tons per year in 1997-98, 500 tons per year in 2007 to less than 300 tons (Jain

et al 2003 and Gairola *et al* 2007). The oil production also decreased from 60 tons 1981 to 40-50 tons in 1999-2000 (Ananthapadmanabha 2000). During mid 90's India exported around 2000 tones of wood and about 100 tones of oil to different countries annually (Rai and Sharma 1990). As the annual production of chandan is declining, the export of these sandal products has also shown a declining trend. Sandal heartwood prices have increased from Rs. 365 per ton to Rs. 6.5 lakhs per ton in the last century (1900-2000). However, it rose from Rs. 37 lakhs per ton in 2007 to Rs. 56.5 lakhs per ton in 2012. In spite of that our country has faced a considerable decline in the wood production of *S album.*

One of the major reasons for declining of sandalwood populations in India are over-harvesting and illegal poaching of native stands (Loneragan 1990; Rai and Sharma 1990; Srinivasan *et al* 1992 and Meera *et al* 2000). Since almost all of the wood extraction is from natural populations, the pressure on the existing populations has been tremendous. This over exploitation has led to a steady decrease in the availability of *S. album* in India (Radomiljac *et al* 1998 and Nageswara 2004). Along with rapid over exploitation, there are many other factors which lead to rapid decline in sandal plantations. These are as natural regeneration of *S. album* 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). Seed germination of this species under nursery conditions is also very poor and non-predictable. The 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 per cent germination (Nagaveni and Shrimath 1981).

After understanding the economic and social value of the sandal plantations, various rule and regulations has been set up the government to increase the area under the sandal plantations like *Santalum album* Advisory Committee formed by the Government of India to take necessary steps. The rules vary from state to state for the possession and storing the sandal, like in Karnataka according to section 83 of Karnataka Forest (Amendment) Act 2001, every sandal tree in the state even on public or private land will be the property of the state. Apart from these rules and regulations steps have also been taken to increase the area under sandal plantation by introducing the sandal in non-traditional areas like north India.

According to e IUCN Red List of Threatened Species the demand for this species exceeds the rate of supply. The natural population of Indian sandalwood had been continuously under threat from illegal harvesting and over exploitation for many years, if not decades. With the reduced availability of wood and the high value of the wood and oil in the international market, extensive smuggling of the wood is encouraged. With the population dwindling in its natural habitat states i.e., Karnataka and Tamil Nadu, it can be safely assumed that economically viable trees (>30 cm girth at breast height) were nearly absent by the end of 20th century. In India the species is threatened by 'spike disease' which leads to

mortality of trees due to changes in the physiology of the species (Thomson *et al* 2018). There are also more minor threats from a decline in habitat quality from over grazing and fire which also puts the species at risk.

The heartwood is described as astringent, bitter, moderately hard, heavy, durable, yellow or brown in appearance, with an oily texture, and is an exquisite material for carving intricate designs. The images of gods and mythological figures that are carved from the wood have a huge demand in the Indian market (Arunkumar *et al* 2012) and other carved objects have a great cultural significance in different parts of the world (Baldovini *et al* 2010). Some of the other popular articles that are made from the wood include boxes, cabinet panels, jewel cases, combs, picture frames, hand fans, pen holders, card cases, letter openers and bookmarks (Arunkumar *et al* 2012).

In the Karnataka Government owned Cauvery Handicrafts Emporium, presently one-kilogram sandalwood costs ~US$300, while one kilogram of sandalwood oil costs ~US$6,000. But these prices can be much more in global illegal markets. There has been a marked decline in the occurrence of the species on the market due to the decline in the number of commercially harvestable and available trees. The annual global demand for sandalwood heartwood for handicrafts has been estimated to be approximately 5,000–6,000 tonnes, with the main markets in China, Singapore, Korea and Japan but also Europe and the USA (Thomson *et al* 2018). India produces 85% of the worlds sandalwood oil, 80 tonnes per year are used domestically and the remaining 40 + million tonnes are exported. Indonesia produces 10% of the world's supply, with the remaining 10% from multiple sources (Thomson *et al* 2018).

Keeping in view the economic potential of the species as well as to diversify the choice of tree species for the farmers of Punjab, the study has been planned to study the germination behavior of *Santalum album* seed under Punjab conditions. No systematic study has been conducted with respect to seed and seedling behavior of *Santalum album*, an economically important tree species under Punjab conditions.

Objectives

a. To evaluate the effect of different pre-sowing seed treatments on germination of *Santalum album*
b. To evaluate the seedling growth performance of *Santalum album* under nursery conditions

CHAPTER-II

REVIEW OF LITERATURE

The *Santalum album* belongs to family *'Santalaceae'* which is represented by 1000 species throughout the world (Jeeva *et al* 1998). The genus *Santalum* consists of 18 species, out of which the Indian sandalwood (*Santalum album*) and Australian sandalwood (*Santalum spicatum*) are of commercial importance. *S. album* is one of the best plant species, well associated with Indian culture and is considered as prize gift to Indian culture and heritage. *S. album* is important source of high-grade perfumery value essential oil and also used for skin problems like pimples, prickly heat, skin eruptions, itching, swelling and rashes. The economic and social value of sandal led to its over exploitation, over-harvesting and illegal poaching from natural populations which leds to declining of sandalwood populations in India. In order to increase the area under sandal plantations, various afforestation programmes were carried out in different parts of the country. Introducing the sandal in non-traditional areas like north India is another way for increasing sandal plantation.

Keeping in view the economic potential of the species as well as to diversify the choice of tree species for the farmers of Punjab, the present study entitled, "Studies on seed germination behavior of *Santalum album* L. under nursery conditions" was carried out at PAU, Ludhiana. So in this chapter attempt has been made by way of reviewing the literature pertaining to the influence of seed source and pre-sowing seed treatment on seed germination and growth performance. Though, the information on these aspects in *Santalum album* is very less. It was found that little work has been done on these aspects with respect to this species. So, the relevant literature pertaining to the studies on of seed sources and pre-sowing seed treatments of some other tree species has also been included and has been reviewed under following headings:

2.1 ORIGIN AND DISTRIBUTION

Although there are about 18 sandalwood species belonging to the genus *Santalum* which are*; S. freycinetianum, S. haleakalae, S. ellipticum, S. peniculatum, S. pyrularium, S.*

involutum, S. boninese, S. insulare, S. austrocaledonicum, S. yasi, S. macgreg-orii, S. accuminatum, S. murrayanum, S. obtusifolium, S. lanceolatum, S. fernandezianum, S. salicifolium and S. spicatum (Fox 2000) Among all these, *S. album* is the most important commercial tree species. There are doubts about the nativity of *S. album*. Gode (1961) advocated that it is an indigenous to India. While others believed that it is introduced to India from Timor in Indonesia (Fischer 1938 and Thirawat 1955). Thirawat agreed Fischer's opion that sandal is exotic to India as many species belonging to genus *Santalum* are naturally found in the islands of Malaysia and Australia and only one species *Santalum album* is found in India. Two other researchers Boyce (1959) and Rajgopalshetty (1977) validated Thirawat and Fischer theory by providing two evidences. First is Sandal is not found to invade into all the eco-climatic zones of India. Second is the occurrence of spike disease in India only not in Timor, indicating acclimatization of sandal in the new environment.

Chandan naturally occurs between 30˚N to 40˚S from India in the west to Juan Fernandez Island in the east and from Hawaiian Archepelago in the north to New Zealand in the south (George 1984 and Srinivasan *et al* 1992). It is distributed in the countries like Australia,1ndonesia, Japan, Belgium, China, Cambodia, Madagaskar, Germany, Holland, Norway, Russia, Switzerland and the United States. *Santalum austrocaledonicum* and *S. yasi* have outside of its native range except in Australia it was introduced for trial purposes (Thomson 2006). *S. austrocaledonicum* mostly occur in Fiji. *Santalum ellipticum, S. freycinetianum, S. haleakalae and S. paniculatum* have primarily been planted inside of their natural range for economic and preservation purposes (Merlin *et al* 2006) whereas *S. acuminatum* is widely spread in all Australian mainland states (George 1984).

In India, sandalwood is generally occurs in the dry deciduous forests of Deccan Plateau at the edge of the Western Ghats Range. The total extent of its distribution is around 9000 km^2, of which 8200 km^2 is in the states of Karnataka and Tamil Naidu. Apart from these two states sandal plantations also found in other states like Andhra Pradesh, Kerala, Madhya Pradesh, Orissa, Maharashtra, Rajasthan, Uttar Pradesh, Bihar and Manipur (Venkatesan and Srimathi 1981 and Srinivasan *et al* 1992). Naturally, it is distributed in peninsular India, but now it is also been introduced in other parts too.

Sandal being state tree of Karnataka has highest area under the sandal plantations which accounts 5245 km^2 that is more than 50 per cent of the total country. Southern part of the state has more sandal plantations as compare to northern parts and abundantly found in Chickamagalur, Shimoga, Coorg, Mysore, Dharward, Hassan, Kannada, Tumkar and Bellary areas. Various abiotic and biotic stresses interfere with the natural regeneration of sandal (Swaminathan *et al* 1997).

In Tamil Naidu the sandalwood plantations are distributed over 3040 km^2 mainly in north Arcot (Javadi's and Yelagiri hills), Periyar, Salem, Vellore and Coimbatore districts and

sparsely in Madurai, Nilgiris and Trichy. In Andhra Pradesh, about 175 km^2 of area is covered by sandalwood trees and are mainly distributed in Chittoor, Tirumala hills, Kadapa, Hyderlabad, Kurnool and Arakku valley. In Kerala, it is distributed in Marayoor, Wynad and Thenmalai and which accounts over 15 km^2. The sandalwood area of Marayoor reserve of Anjanad Valleys is dense and gives superior heartwood. The sandalwood population of Orissa has an area of 35 km^2 which is distributed in Kalahandi, Rayagada, Jeypore and Parlekmandi districts. Whereas in Madhya Pradesh 33 km^2 area is under sandalwood plantations which is scattered in the forest of Sehora, Sagar and Seoni.

Most of the sandal species are evergreen, small trees or large shrubs, can grow up to the height of about 5–20 m or more and girth of 1–2.5 m with slender drooping or erect branching. It is slow growing semi root parasites which depends upon the host plants for water, minerals, and nutrients (Stemmermann 1977). Sandal seedlings are capable of growing up to an elevation of 1800 m from the sea level and thrive best on various type of soils except alkaline, saline and swampy situations. Trees which grow on stony and gravelly soil perform better and have more quality scented wood (Srimathi *et al* 1980).

S. album is highly polymorphic as it possesses significant genetic variability for the characters like; seed size and seed weight (Bagchi and Sharma 1989 and Ramaswamy, 1972). The leaves of sandal are opposite and decussate and sometimes show whorled arrangement. Six different morphological types of leaves like ovate, lanceolate, linear, elliptical, big and small are reported and out of these, ovate leaves are very common, while lanceolate leaves occur to small extent and elliptic or linear leaves occur at a low frequency. The colour of leaves is bluish/greenish yellow to green (Srimathi *et al* 1983).

During initial stages of growth and development of this species, the stem is green and tender, but as the plants get matured, its stems turns brownish in colour and become hard. The colour of bark of matured sandal tree varies from reddish brown to dark brown. Stem in young trees is smooth but as the plant matures it turns rough and develops vertical cracks. Sapwood of sandal is white and scentless, while heartwood is brown and scented (Srimathi *et al* 1983).

Sandalwood seedlings have small delicate roots which contains scented oil. During early growth of plants roots have nodules which are the sign of haustorium formation in later stages of growth. While small number of sandalwood plant too exist without these haustorial nodules upto age of 2 years (Nagaveni and Srimathi 1985).

Sandal starts flowering at an early age of two to three years and flowers twice in a year i.e. March to May and September to December. Flowers are purplish brown in colour, unscented and born in axillary or terminal cymose panicles (Ananthapadmanabha *et al* 1991 and Srinivasan *et al* 1992). Petals are tetra or pentamerous and rarely hexamerous (Hewson and George 1984). Fruit is a drupe, globose, 1.25 cm diameter, purplish-black, with hard-

ribbed endocarp (Luna 1996). The ovary is semi-inferior yields one single-seeded drupe. Seeds are naked i.e. lack testa.

2.2 DORMANCY STUDIES IN SANDALWOOD

The effect of time on viability of sandalwood was studied by Nagaveni and Ananthapadmanabha (1989). The viability of sandal seeds was 80-85 per cent upto 8 to 9 months from the date of collection and it further decreases considerably after 12 and 24 months of storage. They reported that reduction in seed viability and germination is due to decreased enzyme activity and reduced protein content. The depletion of food reserve upon prolonged storage is another cause for poor seed viability and germination (Ananthapadmanabha *et al* 1989).

Srimathi and Nagaveni (1995) studied the effects of storage on viability and germination of sandalwood seed and reported that sandal seeds remain dormant for 2 months after collection i.e. seeds collected during September–October and sown during April–May shows maximum germination. But the seeds collected from some single trees exhibited less dormancy and started germination within 30 days of collection and reached 50 to 55 per cent in 90 days. The same seeds when stored and germinated after 4 to 5 months, germination reached 75-80 per cent in 60-70 days. The seeds should be de-pulped and dried soon after the collection for better germination and viability.

Another study carried out by Chauvin and Ehrhart (1998) on sandalwood trees and reported that it bear flowers and fruits two times in a year, i.e. in September/October and in March/April. The seeds of both the seasons perform alike and due to those reasons, storage of seeds becomes a necessity. They further studied the variation in germination of two different provenances of *Santalum austrocaledonicum* and reported the presence of variation in germination after storage of 16–24 month period. In the progeny trial of *Santalum austrocaledonicum* during the germination phase, unexpected and important differences were noticed in the per cent germination between two provenances. The previous experimentations and routine germination on the ‘lle des Pins’ provenance was 55 per cent after two years storage where as the unstudied 'Mare' provenance failed completely with one lot had germination rate 19 per cent only, after 15 months storage in identical conditions.

In another similar study done by Baskin and Baskin (1998) reported that the seeds of sandal have physiological dormancy (PD) or perhaps morpho-physiological dormancy (MPD) i.e. seeds have a minute embryo. They concluded that seeds of sandal required warm stratification for breaking dormancy**.**

African sandalwood (*Osyris lanceolate*) is among the woody plants collectively known as sandalwood. The best seed conditions and environment in which seeds of *O. syrislanceolata* could be stored to prolong their viability span were investigated at Iringa Tree Seed Centre, Tanzania by storing the sandal seeds at varying storage moisture contents and

storage temperatures. It was observed that seeds stored at 3-5°C after being dried to moisture content of 20 per cent retained viability longer than those stored at other conditions. By the end of the 36th week of storage, the viability was 60 per cent and rate of viability loss per week was 0.5 per cent. It was observed that temperatures below 3°C and over 13°C rapidly decreased the viability of seeds. Moisture content below 15 per cent and over 25 per cent were also noted to be lethal. Thus, seeds of *O. lanceolata* could be stored at least for short-term supply, although their viability generally remained short, suggesting the need for further research to find out other better storage conditions (Mwangingo *et al* 2004).

Clarke and Doran (2012) have stated that no exact cause has been identified for the dormancy of *Santalum album* and concluded that the seeds of this species might have an exogenous kind of dormancy. Similar type of dormancy present in *S. album* seeds was identified by Dileepa *et al* (2015). Ripened fruits of *S. album* were collected from trees in Peradeniya, Sri Lanka. The collected seeds were given 3 different pre-sowing treatments (1) imbibition of non-scarified vs scarified seeds to test for physical dormancy (PD), (2) the effects of gibberellic acid (GA_3) on germination of seeds to assess for PD and (3) embryo elongation prior to radicle emergence to examine morphological dormancy. Seeds will have combinational dormancy if PY is present in conjunction with PD and MPD would occur if the seeds have PD + MD. It was observed that seeds of *S. album* have MPD as germination was increased with GA_3 indicated a physiological component to dormancy and increased E:S ratio prior to radicle emergence revealed an additional morphological component.

An experiment was conducted by Subasinghe (2014).to identify the relationship between the seed storage time and the germination. Seeds were collected from about 15 years old healthy *S. album* trees. Collected seeds were then stored starting from a storage period of 3 weeks. 100 seeds were taken from the stored seeds and treated with 0.05 per cent GA_3 for 12 hours and treated seeds were sown in sand beds. This process was continued at one week intervals up to the 12th week and then it was continued at two week intervals up to 32 weeks. Water was used as a control for soaking at four week intervals. It was observed that after 3rd week of storage, the seeds showed 80 per cent germination when soaked with 0.05 per cent GA_3 and it was less than 40 per cent when soaked with water. After 7th week of seed storage, germination was 50 per cent when soaked with 0.05 per cent GA_3 while after 28 weeks of storage under the same treatment, 0 per cent germination was recorded. However, on the 12th week, both treatments produced approximately similar result.

2.3 SEED GERMINATION STUDIES

2.3.1 Seed Source and Provenance

Jain *et al* (1998) conduct a survey which covers important sandal-bearing areas in the states of Karnataka, Andhra Pradesh, Tamil Nadu, Kerala, Orissa and Madhya Pradesh. The

main purpose of this survey was the identification of provenances of sandalwood. In this study, eight potential provenances of sandalwood were identified *viz* Bangalore, Thangji, Mandagadde, Shimoga, Chitteri, Javadis, Marayoor, Koraput and Seoni. These eight sandal-bearing areas have been identified as potential provenances based upon the population density, phenotypic characteristics, latitude, longitude and ecoclimate. Comparison of present survey with that of 1970 indicated that the sandal population has declined considerably due to various biotic and abiotic factors. Thus, the present situation demands various initiatives for protection and propagation of this valuable tree species.

Ramalakshmi and Rangaswamy (1998) conducted a study to determine effect of physiological and morphological variation on the germination and the viability of seeds. The seeds were collected from various provenances: Mandagadde, Thangli, Marayoor and Chitteri. The study indicated that positive correlation exists between seed weight, viability and germination%. They reported that seed weight could be used to determine the seed quality. Among the seeds of these four provenances, seeds collected from Mandagadde provenance was of best quality and shows better germination , more viability and have more weight. The seed quality of other provenances, in order of merit, was Thangli > Marayoor > Chitter L.

Sindhuveerendra *et al* (1998) conducted a study to determine the variation presents in the seeds of various provenances of sandal for various seed characteristics. Seeds from Mulbagal, Mysore, Shimoga, Honagal, Mandagadde and Marayoor provenance were collected. It was observed that the seeds from various seed sources showed significant variations for morphological and physiological traits. Seeds of Mulbagal provenance has highest range of seed length (10.27-6.25 mm) whereas, the lowest in Mysore provenance (8.92-7.45 mm). Seed width was maximum in Shimoga provenance (9.29-6.19 mm) and minimum in Mandagadde provenance (7.79-6.09 mm). Seed weight was highest in Honagal provenance (0.34-0.11 mm) and lowest in Marayoor provenance (0.02-0.08 mm). These observations suggest that the source of seeds causes the significant variations for various traits.

Chauvin and Ehrhart (1998) conducted an experimental trail to determine the differences between germination rates of seeds of two provenances of *Santalum austrocaledonicum* - ne des Pins provenance and Mare provenance. Collected seeds were kept in storage for two years before sowing. The seeds of ne des Pins provenance showed routine germination (55% after two years storage) but the Mare provenance showed only 19 per cent germination rate and nothing after 15 months storage under identical conditions.

The general recommendations and information for choosing good provenances was summarized by Lauridsen and Kjaer (2002). The experiments were conducted on *Gmelina*

*arborea*and the results revealed that the progenies of different landraces performed differently for adaptability and production. Progenies of natural sources were inferior to that of landraces. Light inbreeding depression was found to be associated with family structures in natural population and this favours the selection of progenies from natural plantations than mixed provenances, which might have problems in adaptation. Among the natural populations, provenances from eastern Assam and Tripura in north-east India were seemed to be the safe and best choices in respect of adaptability and survival.

Nursery experiments were conducted to study the effect of seed source and seed collection time of *Santalum album* L. on germination parameters by Jagadish *et al* (2008). Seeds were collected from four contrasting locations in Karnataka. Significant variations were observed among seeds sources. Seeds from Mysore source performed better than all other sources. Seeds collection time was also studied in which seeds were collected at three times viz. early, mid and late collections at 15 days interval. The study showed that from the three collection timings, mid and late collections performed significantly superior than early seed collection for most of the parameters. So, they concluded that seeds having high germination ability are determined by the seed source and proper collection time of seeds.

Hidayan and Yuliah (2017) conducted an experiment to study the growth performance of sandal plants in Gunung Kidul Regency. Seeds were collected from five provenances i.e. Sumba, Timor, Belu, Rote and Imogiri. Observations were made on the total height of the tree, bole length and the stem diameter. It was documented that considerable variations were present among the five provenance studied for two traits – total height of tree and stem diameter. But no variation was observed for the bole length. Among the five provenance studied, Rote provenance showed the best performance for various growth parameters (height was 6.07 m and diameter of stem was 5.08 cm) and for adaptation ability Rote (92.19%) was best.

Tate and Page (2018) conducted an experiment on propagation of sandalwood by leafy stem cuttings. They studied the effect of genotype, cutting source, cutting size, and propagation medium on germination of 15 genotypes from two island provenances (Erromango and Tanna). Cuttings of two different sizes (1-node/400 mm^2 and 2-node/800 mm^2 leaf area) were propagated on propagation potting media. It was reported that cutting size had no significant effect on rooting percentage, number or root growth. But a significant variation was observed in provenance for rooting capacity. Genotypes from the island of provenance Erromango showed better adventitious root induction and rooting success as compared to Tanna.

Setiawan *et al* (2019) conducted a research to determine the variations for survival rate and several growth parameters in four sandalwood provenances conserved at Block A:

Sumba, TTU, Belu and Rote. Collected seeds were sown in Randomized Complete Block Design (RCBD). Observations were made for various growth parameters like stem diameter, height, and bole length and crown diameter. Significant variations were observed among provenances for the traits like survival rate, diameter of stem and diameter of crown, while for bole length, all the provenances perform uniformly. Rote provenance was the best provenance in the terms of survival rate.

2.3.2 Seed Size

Srimathi *et al* (1977); Srimathi and Kulkarni (1982) and Manonmani *et al* (1996) studied the effects of seed size on the seed germination and reported that bigger sized seeds recorded better survival and establishment than medium sized and small sized seeds. They concluded that the better germination from heavy and large sized seeds could be assigned to their chemical composition and large reserve of nutritional matter in them. The reserves translocation proceeded differently in large and small sized seeds from endosperm to embryo. The transform of nitrogen from endosperm to the embryo is more in larger and better filled, seeds after sowing as compared to small seeds.

Nagaveni and Ananthapadmanabha (1986) conducted an experiment to determine the relation between seed polymorphism and germination in *Santalum album.* The seeds were collected from two sources i.e. Bangalore and Coimbatore. Collected seeds were grouped into three categories on the basis of seed weight i.e. small seeds (up to 0.1g), medium seeds (0.1 to 0.2g) and large seeds (>0.2g). Seeds were first stored for two months to overcome the dormant period. It was observed that the seeds from small seed start germinating after 15 days of sowing, medium sized seed started germinating after 30 days of sowing and large sized seeds started germinating after 90 days of sowing. Seedling growth and survival varies proportional with the seed weight. Seedlings raised from small seeds showed 50-60% survival rate, medium seed showed 70-75% survival rate and large sized seeds showed 90-95% survival rate and they recommended the use of large seeds for better germination and seedling growth parameters.

On similar lines, Bagchi and Sharma (1989) performed biometrical studies on seed characters of *Santalum album* and concluded that there was significant genetic variability in seed characters like; weight of seed and seed size in the selected tree seed sources of *S. album.* They observed that seed characteristics of *S. album* showed significant correlations in seed germination. In contrast to above study, El-Kassaby *et al* (1992) observed that there was no relationship in seed size and seed weight on germination capacity and speed in *Pseudotsuga menziesii* seeds of 19 seed orchard trees. They concluded that clone or genotype had a greater influence on seed germination and size of seed had no relationship with percentage of germination rate, germination value and initial growth of seedling over the

years.

The effect of seed size on seed viability, germination and plant growth parameters were studied by Agboola (1996) in three tropical tree species i.e. *Terminalia superba, T. ivorensis and Gmelina arborea.* No significant variations were recorded for the effect of seed size on germination. But seed size has significant effect on dry weight of seedlings. Rate of germination and relative growth rate was more in seedlings of *Gmelina arborea* which were raised from small sized seeds. Total dry weight of *G. arborea* seedlings raised from small seeds was 2-3 times less as compared with seedlings raised from large seeds. Similar work was done by Chacon *et al* (1998) and they studied the effect of seed size on germination and growth of *Cryptocarya alba* under controlled conditions. Seeds were divided into three size classes based on seed length as large, medium and small seeds. It was noticed that the germination rate was more in larger seeds as compared to small sized seeds. Seedlings raised from larger seeds were more vigorous as they produce large number of shoots and leaves in comparison to those seedlings which were raised from medium and small sized seeds. Seedlings raised from large sized seeds recorded higher leaf allocation but seedlings raised from small sized seeds showed higher root allocation.

Effect of seed size on emergence, germination, growth rate and survival rates of the seedlings of *Senna occidentalis* was examined by Saeed and Shaukat (2000). They accomplished that germination per cent was depended on seed size. Large sized seeds showed higher germination, produced longer and more number of roots and shoots as compared to small sized seeds. Further, rate of emergence was also higher in larger sized seeds as they were able to emerge more rapidly than smaller ones. So, it was concluded that seed size has significant effect on germination, growth rate and survival of seedling.

Manonmani and Vanangamudi (2002) conducted an experiment to determine the effect of seed size and seed source on germination in sandalwood. Seeds were collected from four different sources (Harur, Siruvani, Coimbatore and Mettupalayam) and graded into two classes based upon their seed size i.e. large seeds (72mm) and small seeds (68mm). It was observed that the effect of seed source was significant for all morpho-physiological traits related to seeds. Seeds collected from Coimbatore performed better than seeds collected other three sources. While large sized seeds of all the four sources recorded better germination, survival and seedling growth as compared to small sized seeds.

Cicek and Tilki (2007) studied variation in germination, survival and growth of *Castanea sativa* produced by the effect of seed size. On the basis of seed weight, the seeds were divided into three categories i.e. small (<5 g), medium (5-8 g) and large (>8 g). Germination parameters were significantly affected by seed weight as the large sized seeds recorded early and better germination than small sized seeds. Further, survival rate of

seedlings was recorded at the end of first growing season and it was noticed that seeds size significantly affected the various growth parameters like seedlings emergence, survival, root collar diameter and dry weight. All these parameters varied proportionally with the seed size. Larger the seeds more will be the seedling diameter, height and dry weight. But no effect of seed size was observed on shoot-root ratio and number of roots.

Leishangthem and Rana (2017) carried out a study to determine the effect of seed size and pre-sowing seed treatments on germination of some tropical and sub-tropical trees. Various growth parameters like germination per cent, seed vigour, and emergence rate, initial seedling growth were found to be affected by the seed size. In general, seed size and pre-sowing seed treatments have significant effect as large sized seeds gave maximum germination in comparison to small sized seeds and pre-sowing seed treatments helped to overcome seed dormancy and led to rapid germination of seeds.

Attri *et al* (2018) conducted an experiment to determine the effect of seed size and different pre-sowing seed treatments on the seed germination in many tree species. It was observed that both seed size and pre-sowing seed treatments have a significant effect on the germination and seedling growth parameters. Large sized seeds germinate rapidly and produced more vigorous and superior seedlings as compared with the seedling raised from small seeds. Pre-sowing seed treatments helped to break seed dormancy in many species and enhances seed germination, germination rate and seedlings growth under normal and stress conditions. Similarly, Karki *et al* (2018) determines the effect of different seed sizes on the germination and seedling growth of a temperate tree species. The collected seeds were divided into different classes on the basis of seed length and weight. Large sized seeds showed better germination per cent and mean daily germination than small sized seeds. It was also reported that large sized seeds show better germination because levels of starch and other food storage materials were more in large sized seeds than the small sized seeds.

2.3.3 Pre-Sowing Seed Treatments

Different types of pre-sowing seed treatments i.e. chemical or physical can be used to break the dormancy in sandalwood. Effect of different chemical stimulant on seed germination of sandalwood was studied by Nagavani and Srimathi (1980). They treated the seed with KNO_3, NH_4NO_3, $NaNO_2$, KNO_2 and GA_3 and observed that GA_3 increased the seed germination percentage and rate. While nitrates and nitrites seed treatments were non effective. Optimal treatment was 0.05 per cent of GA_3 for 16 hours, as it gave 80-85 per cent success in seed germination. Further in 1981 they studied the effect of scarification on germination of sandal seeds. Scarification was done with ethanol, sulphuric acid, soaking in water (cold and 80℃) and removal of seed coat. Out of all these treatments, ethanol and soaking of seeds in water was found to be least effective. While sulphuric acid gave 45-60 per

cent of seed germination in 60 days as compared to untreated seeds. (Nagavani and Srimathi 1981).

Ananthapadmanabha *et al* (1988) reported that dormancy of sandalwood plants can be removed by the treatments with dilute hydrochloric acid or dilute sodium hydroxide or gibberellic acid. Mature seeds were collected and soaked in 50 ml of 5 solutions: hot water (10 min); cold water (time unknown); 0.1 HCL or 0.25 per cent NaOH for 4 hours and 0.05 per cent gibberellic acid for 16 hours. Germination was carried out on moist cotton wool and counts were made. NaOH, HCl and GA_3 were found to promote the germination. While in hot water and cold water seed germination was almost the same as the control values. Sequence for the effectivity was HCl > GA_3> NaOH > hot water > cold water. Likewise Nagaveni and and Ananthapadmanabha(1989) studied the effects of 11 chemicals on seed germination of sandalwood. Mature seeds were collected and treated with 1 per cent $ZnCl_2$, 0.5 per cent NaOH, 100 ppm IBA, 5 per cent cytozyme, 0.5 per cent thiourea, 100 ppm IAA, 0.5 per cent HCL, methanolic extract of new sandal leaves, 1 per cent H_2O_2 for 2 hr, 10 ppm kinetin for 3 hrs. All these treatments speeds up the germination and first germinants appeared after 15-45 days of sowing. Germination percentage was also increased for all the treatments expect for NaOH, IBA per cent and kinetin. Best results were obtained in case of H_2O_2 (75%), methanolic extract (70%) and thiourea (68%).

Nikam and Barmukh (2009) conducted a study to check the effect of gibberellic acid on germination of *Santalum album.* The mature black-coloured drupes of *S. album* were collected from a naturally growing sandal trees on the campus of university of Pune, India in the month of May, 2006. The endocarp was removed under aseptic conditions and the seeds pre-treated with 2, 4, 6 and 8 mm gibberellic acid (GA_3) for 12 h, then inoculated on Murashige and Skoog's (S) medium. Imbibition of seeds in 2, 4, 6 and 8 mm GA_3 resulted in improved seed germination. Out of all these doses, 4 mm GA_3 treatment was superior in enhancing *in vitro* germination. It not only improved final germination but also reduced mean germination time. While delayed germination was observed in the seeds treated with 6 and 8 mm GA_3 for 12 h.

Vijayan and Rahees (2015) studied the effect of light on seed germination of *S. album.* Mature seeds were collected and treated with pre-sowing seed treatments with gibberllic acid, water and covered with red, black and blue clear plastic papers. Highest germination percentage was obtained under full light condition (50.3%) when treated with GA_3 followed by GA_3 treated seeds covered with transparent light or clear plastic paper (49.3%). Lowest germination percentage was notice under red and blue light treatment. Jayawardena *et al* (2015) conducted an experiment in which, two pre-germination seed treatments, firstly, samples of non-scarified seeds and scarified seeds were placed on tissue

papers moistened with 100 ppm and 500 ppm. Secondly, GA_3 imbibition treatment where samples of scarified and non-scarified seeds were placed on moist tissue papers in petri dishes treatments. Samples of both treatments were positioned on a bench in alternating light/dark. The results of imbibitions showed that non-scarified seeds showed 20– 22 per cent germination, where as manually scarified seeds increased 27– 29 per cent germination. Treatment with GA_3, especially with 500 ppm give better germination whereas, 100ppm GA_3 treatment was less effective for both non-scarified and scarified seeds.

Sutheesh *et al* (2016) conducted a study to determine the effect of various organic and inorganic pre-sowing seed treatements on germination in sandal. The mature seeds were collected from Marayoor Nachivayal forest of Idukki district, Kerala. The seeds were subjected to 16 pre-sowing seed treatments, including soaking in tap and boiling water, acid scarification and soaking in GA^3, cow dung slurry and cow urine in different concentration and duration. The pretreated seeds were then sown in plastic trays filled with vermiculite as germination medium and watered regularly. Germination percentage was recorded on regular intervals and it was observed that the highest germination percentage (74.33%) was noticed in treatment eighth i.e. soaking of seeds in 500 mg l-1 GA_3 for 24 hours and lowest (44.33%) in treatment fifth i.e. soaking of seeds in H_2SO_4 for 5 minutes. Acid scarification and boiling water treatments decreased the germination percentage of the seeds.

Roshan *et al* (2018) conducted an experiment to examine the effect of different pre-sowing treatments on germination of sandalwood in nursery. Seeds of *Santalum album* L. were collected from0 Hirbundhmouza of Hirbundh Range and Institute of Wood Science & Technology, Bangalore. Selected seeds were given four different treatments pretreatment by soaking in water, pretreatment by boiling water, pretreatment by alternate wetting and drying and pretreatment by gibberellic acid. Sandalwood seeds were soaked in water for 24 hours before sowing. Water soaked seeds started germination after 60 days and in 61 to 100 days time period, only 3-4 per cent germination was obtained. By seed treatment with boiling water, germination started after 50 days and in 51 to 100 days, hardly 8% germination was obtained. By alternate wetting and drying seed treatment, germination started after 40 days and in 41 to 100 days only 8 to 9 per cent germination was recorded. The best results were obtained by treating seeds with gibberellic acid as the germination was started after only 24 days of seed sowing.

Pamungkas and Nichol (2019) conducted a study to obtain information on the influence of scarification techniques and the use of VAM on germination media of sandalwood seed. Five scarification treatments (control, soaked in water for 24 and 48 hours, GA_3 300 and 500 ppm for 17 hours) and two VAM application (with and without VAM) was carried out. The results showed that the cumulative germination was higher on the seeds

treated with scarification of soaked in GA_3 solution 300 and 500 ppm than control, soaked in water 24 hours and 48 hours. The use of VAM did not significantly affect the cumulative germination. It was observed that seed sown on media with VAM tended to germinate slower than media without VAM.

Polaiah *et al* (2020) perform an experiment at ICAR-Directorate of Medicinal and Aromatic Plants Research, Anand. The mature seeds of sandalwood were collected from the mother plant. The collected seeds were then subjected to 11 different pre-sowing seed treatments (T1-control, T2-water soaking, T3-cow urine, T4-cow dung slurry, T5-HNO_3, T6-KNO_3, T7-thiourea, T8-GA_3 500 ppm, T9-GA_3 1000 ppm, T10-GA_3 1500 ppm and T11-conc H_2SO_4.) before sowing. The effect of these treatments were recorded for germination percentage and seedling growth parameters i.e. collar diameter, fresh weight of root, fresh weight of shoot, fresh weight of foliage, fresh weight of plant, root length, shoot length ,dry weight of root, dry weight of shoot, dry weight of foliage and number of leaves of the sandalwood. Maximum seed germination percentage (41%) was recorded for seeds treated with GA_3 1500 ppm as compared to the untreated seeds. GA_3 1500 ppm not only improves the germination but also had positive effect on seedling growth parameters.

2.4 HOST PLANT AND GROWTH STUDIES

Sandal is semi-root parasite plant. The parasitic mode of nutrition of sandalwood seedling was first time reported by Scot (1871). The importance of this concept was not realized over the years as no scientific investigation was conducted on this aspect.

Barber (1903) conducted a detailed investigation on parasitic behavior of sandalwood seedlings and gave two reports. In first report, root system of sandalwood seedling grown under different environment conditions was examined. It was observed that the sandalwood parasite over different host plants by the formation of haustoria on the roots of host plants. In second report structure of haustoria and mode of attachment to roots of host plants was fully described. Haustorial connections take place in two steps. In first the haustoria is formed and penetrate towards the roots of host plants and in second step, channel of communication between the haustoria and roots of host plants is formed.

Selective nature of sandalwood haustoria was reported by Rama (1910). Sandalwood haustorium has selective power to attach extensively with good host plants and loosely or partially with bad hosts. This intensity of attachment can be used to classify the host plants as good, moderately well or the bad host plants. Rama (1911) also reported that sandalwood seedlings cannot survive without the host plant beyond one year. It may attack the roots of almost all the plants but it shows preference to some specific hosts. The order of preference is *Pomgamia pinnata*, *Albizia lebbeck* and *Tectona grandis*.

Venkato (1938) opined that different host plants can be classified as good, moderately

good and bad host only after growing the individual host plant with sandalwood. On the basis of pot experiment, 108 host plants were classified into three groups:

Class A : Very good or good hosts. Sandalwood seedlings grow vigorously over these hosts.

Class B : Moderately good hosts. Sandalwood seedling grows over these hosts but showed reduced rate of growth.

Class C : Poor, bad to toxic hosts. Sandalwood seedling cannot survive over these hosts and these inhibit the growth of seedling.

He aslo reported that short living plants such as shrubs and those with shady crowns would serve as good host.

Iyengar (1965) considered the parasitism as to be the condition for normal life and importance for an individual for its nourishment at the cost of another individual. On the basis of various pot experiments with different host plants, Sreenivaso (1933) concluded that the osmotic pressure of tissuses of sandalwood seedling is higher than the osmostic pressure in tissuse of host plant. This high osmotic pressure in sandal roots ensures the unidirectional flow of nutriants from host plants to sandal and absorbs calcium and iron from host plant. Iyengar (1960) reported that sandal draws nitrogen and phosphorus from the host plant and calcium and potassium from soil. According to Ramaih *et al* (1962), sandal draws K, Ca, Mg, Zn, Cu and Fe from the host plant.

Parthasarathi *et al* (1974) reported that absorption of nutrients from the soil directly by sandalwood roots is also very common. This occur as the sandal roots have almost similar or compare cation exchange capacity (CEC) to many of its host plants. On the basis of differential response of CEC of roots of host plant on parasitisation by sandal, three groups of host plants can be made. First group of those host plants whose CEC tends to increase on parasitisation by sandal. These plants are reffered as good hosts. Second group includes those host plants whose CEC remains unchanged on parasitisation by sandal. These hosts are called as medium good hosts. Third group includes those plants whose CEC values decreased upon parasitisation by sandal. These hosts are called as bad hosts. CEC values of sandal roots are influenced by CEC values of host's roots. CEC values of sandalwood's roots proportionately increased from poor to good host (Kamala and Angadi 1992).

Ananthpadmanabha *et al* (1984) through their studies on host-sandal association concluded that even though sandal can survive without host for a short period but for healthy development sandalwood requires host plant i.e. pot hosts (initial or primary)-the primary host is planted into a container having *S. album* seedling during nursery propagation, (2) intermediate hosts (bridging nursery and field) and (3) long-term (secondary) hosts. The influence of host plant on the physiological attributes of field-grown sandal tree was

examined by Rocha *et al* 2014. The six years old sandal tree were collected and field grown experiments were carried out to understand the influence of *Casuarina* (host plant) on carbon assimilation, plant water potential and nutrient uptake. It was observed that carbon assimilation rate was higher (17.66 µmol cm^{-2} s^{-1}) than sandal trees growing without host (15.13 µmol cm^{-2} s^{-1}). Sandal tree growing with host plant also showed better water potential (-0.85 MPa) than sandal tree without host plant (-1.27 MPa). Leaf nutrient contents were higher in sandal tree with host plant (N = 2.65%, K = 0.24%, P = 2.31%) compared with sandal tree without host plant (N = 2.48%, P = 0.16%, K = 1.68%).

2.5 MISCELLANEOUS STUDIES RELATED WITH *SANTALUM ALBUM*

Failure to establish new stock and deforestation of existing stands of *Santalum album* describes that over the next decade mature trees are likely to be in short supply. Also, sandal is subjected to a few diseases, of which most destructive is the spike disease. Since the inadequate natural regeneration and not being planted on a large scale, there is need to develop a reliable method of *in-vitro* propagation. Micropropagation offers a quick means of multiplying its germplasm for aforestation and conserving best and exceptional germplasm (Winton 1978 and Bonga and Durzan 1987). There are several studies available indicating information of indirect regeneration of *S. album* on juvenile or mature explants (Lakshmi 1986 and Rao and Bapat 1992). However, plantlet regeneration from mature trees of sandal is still uncommon (Rao and Bapat 1992).

Dayal (1986) suggested the use of bush sowing technique for the propagation of sandal. Seeds of *Santalum album* were taken and treated with chemicals to protect them from rodents and birds. Treated seeds were then sown on thorny *Lantana camara* and *Azadirachta indica* bushes of one meter height. Germination percentage was recorded and further the natural regeneration of plants was recorded after 17 years of sowing. It was observed that germination started after 11 days of sowing and continued upto 2 months. It was observed that total 177 seedlings were germinated. Out of these 177 seedlings, 75 per cent seedlings have regenerated through root suckers and all were healthy after 11 years. In another similar study Rajan (1995) reported that bush sowing technique could help the sandal to regenerate profusely. It was observed that under the shade of already established sandal and at 5-7cms thick mat of *Casuarina* needles and bamboo leaf litter sandal grew very well. Suggestions were also made to use of *Lantana* as bush mat for growing sandal. Attributes of *Lantana* as a nurse for sandal and as a weed were observed in detail and recommendations were made to grow sandal after establishment of *Lantana* in semi-arid tracts.

Nagarjaiah and Rao (1993) reported that various factors like low germination (10-20%), rummaging of germinated seeds by squirrels and rodents, browsing or trampling of young seedlings by cattle leads to poor natural regeneration of sandalwood. Various tests

were carried to check the natural regeneration of seedlings. Seedlings were collected from the forests with sandal rich reserves at University of Agricultural Science, Bangalore and Karnataka. Seedlings were divided into three groups on the basis of their root lengths. Group 1st with root length less than 5 cms, group 2nd with root length of 5-10 cms and third group with root lengths more than 10 cms. All these seedlings were planted in polythene bags and kept under shade. The fourth group consists of seedlings of mixed root length was taken and planted on pots which have *Cassia siamea* as host plants. Observations were made after 8 months for the traits like root and shoot lengths. It was observed that the seedlings of group third have maximum survival rates (60%) and seedlings of group 1st have lowest survival rates (35%). While the seedlings grown with host plants also showed the same survival rate of 60 per cent as that of group third.

Vegetation propagation by air layering or through root suckers/cuttings could be used for the propagation of Sandalwood. In air layering method, branches of 2 cm diameter were selected for layering during months of June-July when there are frequent rainfalls. A ring of bark of 1 cm in width is removed from selected branches. The exposed part of branches were dusted with seradix B and kept moist. After 35-49 days roots emerged. When the roots become 8 cm in length, these branches were cut off from the mother plant and sown in the pot. For better development and high survival rate host plants could also be sown with the sandal plant in the pot (Rao and Srimathi 1977 and Vijayakumar *et al* 1981). While the vegetative propagation of sandal by mean of rooting cuttings was demonstrated by Uniyal *et al* (1985). Secondary roots of 5cms were taken out during the month of April and treated with Seradix. The treated roots were planted horizontally in nursery beds and kept moist by frequent irrigation. Roots started sprouting in about 30-40 days after sowing.

2.6 Conservation Action

In view of the vanishing population of sandalwood, Governments of Karnataka and Tamil Nadu relaxed the earlier stringent policies on sandalwood cultivation and harvest. This has encouraged sandalwood cultivation by the farmers, entrepreneurs and NGOs. With the huge demand for wood and oil in the international market, in the last decade, extensive private plantations are now being established across India which, in a way, has paved the way for reviving the past glory of sandalwood in India. Similarly, large areas are being brought into sandalwood cultivation in other countries such as Australia and China. Some of the measures needed for its conservation and sustained utilisation are as follows:

An important factor that needs immediate attention from India's sandalwood cultivation perspective is that there should be uniform rules and regulations across the country that encourages hassle free harvesting and marketing of the Sandalwood and its products which would also support in its better utilisation and conservation.

Establishing regional level seedling/clonal seed orchard of superior genotypes for obtaining quality seed material. Proper assessment of sandalwood population at the country level especially in the case of India. Re initiating tree improvement strategies using plantation grown sandalwood as the source/base population. Encouraging mass production of seedlings and distribution by various Forest Departments within the country so that sandalwood cultivation is extensively encouraged

Developing proper package and practises for sandalwood cultivation that would enable in bringing financial gains to the farmers which can also help in conservation of the population. Role of genetic and environment on the heartwood and oil quality needs to be extensively studied. (Arunkumar *et al* 2012)

CHAPTER-III

MATERIALS AND METHODS

The present studies entitled, "Studies on seed germination behavior of *Santalum album* under nursery conditions" was conducted in the experimental area of Department of Forestry and Natural Resources, Punjab Agricultural University, Ludhiana during the year 2019 with objectives to determine the: effect of different pre-sowing seed treatments on seed germination and seedling growth performance of *Santalum album* under nursery conditions. The details of experimental site, methodologies adopted and materials used in the present investigations are described in the chapter under the following sub-headings:

3.1 EXPERIMENTAL SITE
3.2 METHODOLOGY
3.3 TREATMENT DETAIL
3.4 OBSERVATIONS RECORDED
3.5 STATISTICAL ANALYSIS

3.1 EXPERIMENTAL SITE:

3.1.1 Location: The experiment was undertaken at the Teaching Area of the Department (Forestry and Natural Resources), College of Horticulture and Forestry, PAU main campus Ludhiana at an altitude of 247 meters above mean sea level and the geographical coordinates are 30°54´N latitude and 75°40´E longitude. The experimental area is a representative of central agro-climatic region of Punjab.

3.1.2 Climatic and Edaphic Factors: The experimental area falls under the broad region having tropical to sub-tropical climate with a prolong dry season, which begins in mid-September and lasts till late June. May-June are considered as the hottest months with intense evapo-transpiration during which hot desiccating winds blow throughout the day. The rainy season occurs from mid July to first fortnight of September when the region receives majority share of annual rainfall. December-January are the coldest months when occasional ground frost occurs in the plains. Generally, from October to June dry conditions prevail in the region of the experimental site. A few light showers during the winters may be received from the Northwestern depressions arising in the Mediterranean Sea. The site receives on an average 500-750 mm of rainfall throughout the year which is unevenly distributed and near about 75-80 % of which is showered between the months of July to September.

The texture of the soil is sandy loam to clayey with normal reaction. Generally, the soil of the Central zone of Punjab state is identified as alluvial which has a slight problem of alkalinity and salinity. The soil of the experimental zone has evolved under semi-arid conditions.

Table 3.1: Average Climate data during the year-2019

Month	Mean Air Temp.(^{o}C)	Mean Rainfall (mm)	Mean RH (%)
January	12.3	66.0	71.0
February	14.7	95.6	75.0
March	18.6	7.4	68.0
April	27.3	41.6	50.0
May	30.0	20.0	37.0
June	33.6	29.9	42.0
July	30.3	218.4	71.0
August	30.3	331.4	76.0
September	29.3	264.8	77.1
October	24.5	0.0	68.2
November	19.5	35.2	68.2
December	11.7	46.8	79.0

3.2 METHODOLOGY:

Well-matured seeds were procured from KFRI, Kerala and UHF, Neri campus Hamirpur, Himachal Pradesh and seeds were graded based on seed size. After then seeds were given different pre-sowing seed treatments and then sown in nursery beds. Observations were recorded for germination parameters and then the seedlings were transplanted to polybags along with host plant *Cajanus cajan*. Observations for plant diameter, height and number of leaves were recorded after every 15 days for six months after transplantation, whereas the data for other seedling parameters i.e. shoot fresh biomass (g), root fresh biomass (g), length of tap root (cm), root and shoot dry biomass (g), sturdiness quotient, root shoot ratio, and quality index of the study were taken after six months.

3.3 TREATMENT DETAIL

a) Seed Sources – Two

KFRI : KFRI, Kerala

UHF : UHF Neri campus Hamirpur, Himachal Pradesh

b) Pre Sowing Seed Treatments – Five

P_1 : Cold water (48 hours),

P_2 : GA_3 (0.05 % for 24 hours)

P_3 : GA_3 (0.5 % for 24 hours)

P_4 : H_2SO_4 (10 % for 5 min)

P_5 : H_2SO_4 (10 % for 1 min)

c) Growing Environment– Two

OE : Open

SE : Shade

Total number of treatments : 2x5x2 = 20

Plot size : 1 square meter (20 seeds)

Potting media : 1 [Sand, Soil and FYM (2:1:1)]

Replication : 4

Statistical design : Factorial experiment in Completely Randomized Design (CRD)

3.4 OBSERVATIONS TO BE RECORDED:

3.4.1 Germination Studies

3.4.1.1 Days taken for complete germination

Twenty seeds were randomly taken from each treatment in four replications and sown in media [Sand, Soil and FYM (2:1:1)]. The total number of days counted for complete germination of each treatment. Emergence of plumule was considered as a criterion for seed germination.

3.4.1.2 Germination per cent

Germination per cent of the seeds was worked out as number of total sown seeds and number of actually germinated seeds after elapse of germination period.

$$\text{Germination;(\%)} = \frac{\text{Number of total seeds germinated}}{\text{Number of total seeds sown}} \times 100$$

Table 3.2: Description of 20 treatment combinations

T_1	UHF Cold water (48 hours)Shade
T_2	UHF GA_3 (0.05 % for 24 hours)Shade
T_3	UHF GA_3 (0.5 % for 24 hours)Shade
T_4	UHF H_2SO_4 (10 % for 1 min)Shade
T_5	UHF H_2SO_4 (10 % for 5 min)Shade
T_6	KFRI Cold water (48 hours)Shade
T_7	KFRI GA_3 (0.05 % for 24 hours)Shade
T_8	KFRI GA_3 (0.5 % for 24 hours)Shade
T_9	KFRI H_2SO_4 (10 % for 1 min)Shade
T_{10}	KFRI H_2SO_4 (10 % for 5 min)Shade
T_{11}	UHF Cold water (48 hours)Open
T_{12}	UHF GA_3 (0.05 % for 24 hours)Open
T_{13}	UHF GA_3 (0.5 % for 24 hours)Open
T_{14}	UHF H_2SO_4 (10 % for 1 min)Open
T_{15}	UHF H_2SO_4 (10 % for 5 min)Open
T_{16}	KFRI Cold water (48 hours)Open
T_{17}	KFRI GA_3 (0.05 % for 24 hours)Open
T_{18}	KFRI GA_3 (0.5 % for 24 hours)Open
T_{19}	KFRI H_2SO_4 (10 % for 1 min) Open
T_{20}	KFRI H_2SO_4 (10 % for 5 min)Open

Table 3.3: Layout plan for nursery

T1R1		T1R2		T1R3		T1R4
T2R1		T2R2		T2R3		T2R4
T3R1		T3R2		T3R3		T3R4
T4R1		T4R2		T4R3		T4R4
T5R1		T5R2		T5R3		T5R4
T6R1		T6R2		T6R3		T6R4
T7R1		T7R2		T7R3		T7R4
T8R1		T8R2		T8R3		T8R4
T9R1		T9R2		T9R3		T9R4
T10R1		T10R2		T10R3		T10R4
T11R1		T11R2		T11R3		T11R4
T12R1		T12R2		T12R3		T12R4
T13R1		T13R2		T13R3		T13R4
T14R1		T14R2		T14R3		T14R4
T15R1		T15R2		T15R3		T15R4
T16R1		T16R2		T16R3		T16R4
T17R1		T17R2		T17R3		T17R4
T18R1		T18R2		T18R3		T18R4
T19R1		T19R2		T19R3		T19R4
T20R1		T20R2		T20R3		T20R4

3.4.1.3 Germination energy (GE)

The per cent germination energy was worked as per the formula given below i'e' number of total seeds those had germinated when the peak of germination is achieved that means number of highest seed germinated in a period of 24 hours.

$$GE\ (\%) = \frac{\text{Total number of seeds that has germinated up to the time of peak germination achieved}}{\text{Number of total seeds sown}} \times 100$$

Or

$$GE\ (\%) = \frac{A_1}{N_1} + \frac{A_2}{N_2} + \frac{A_3}{N_3} + \frac{A_4}{N_4} + \ldots . \frac{A_n}{N_n}$$

Where A1, A2, A3 ..,An are the number of seeds newly germinated on N1, N2, N3 ……, Nn days, respectively.

3.4.1.4 Germination value (GV)

It is a measure of speed and completeness of germination with a single figure. Seed germination countin was done every day and the germination value was deliberated by the formula given by Czabator in 1962 for germination value.

Germination value (GV) = Peak Value of seed germination (PV) × Mean Daily Germination (MDG)

Where;

GV = Germination value

PV = Peak value of seed germination is the highest value of the cumulative seed germination per cent divided by the number of days since the beginning of the experiment.

Or

$$PV = \frac{\text{Cumulative germination \%}}{\text{Days since sowing}}$$

MDG=Mean daily germination

Or

$$MDG = \frac{\text{Cumulative germination per cent at the end of the test}}{\text{Days since sowing to the end of test}}$$

3.4. Growth and Biomass Traits of Seedlings (in nursery)

For seedlings growth and biomass studies, eight randomly selected plants from each treatment (two from each replication) were selected. The following attributes were taken.

3.4.2.1 Seedling height (cm)

The height of the seedling was recorded with the help of graduated rod from the upper surface of soil filled in polythene bag to the shoot tip of the seedling.

3.4.2.2 Seedling collar diameter (mm)

The seedling diameter at collar region of randomly selected seedlings was measure from the collar region and expressed in millimeters.

3.4.2.3 Number of leaves

Leaves fully opened were considered as matured leaves and were counted visually in each seedling before uprooting.

3.4.2.4 Length of tap root (cm)

The main tap root length i.e. from the cut base from where shoot and root were separated to the tip of the tap root was recorded with a measuring scale.

3.4.2.5 Fresh biomass of shoot and root (g)

The randomly selected eight seedlings were cut at the collar region with the help of secateur to separate root and shoot portion for taking fresh biomass of each component. The fresh shoot and root biomass was measured in grams immediately after uprooting the seedlings.

3.4.2.6 Dry biomass of shoot and shoot (g) .

After measuring the fresh biomass of shoot and roots, separately both these components i.e. shoot and roots were dried in oven maintained at 50 ℃ till constant dry weight of the shoot and roots was achieved.

3.4.2.7 Root and shoot ratio

The root and shoot ratio of the randomly selected plants of each treatment was calculated, separately as per the formula given:

$$\text{Root-shoot ratio} = \frac{\text{Dry weight of root}}{\text{Dry weight of shoot}}$$

3.4.2.8 Sturdiness quotient (SQ) of seedlings

The seedling sturdiness quotient was worked out by dividing height (cm) of seedlings with diameter (mm) of seedlings at collar portion according to the formula given by Roller (1977). Smaller the values of sturdiness quotient indicates sturdy the plant having higher chances of the survival under field conditions.

3.4.2.9 Quality index (QI)

The seedling's Quality index (QI) was worked out as per the the formula given by Dickson *et al* (1960) for seedling's quality index and the formula is:

$$\text{Quality Index (QI)} = \frac{\text{Seedling dry weight (g)}}{\frac{\text{Seedling height (cm)}}{\text{Collar diameter (mm)}} + \frac{\text{Shoot dry weight (g)}}{\text{Root dry weight (g)}}}$$

3.4 STATISTICAL ANALYSIS:

The statistical data on seed germination and seedling growth parameters were analyzed as per the procedure used in CRD (Completely Randomized Design) by Gomez and Gomez (1984). Critical difference (CD), analysis of variance and variance components were worked out for the understanding and interpretation of the results of the present study following online CPCS1, PAU software. The statistical analysis for each trait was performed on the table and mean values for the analysis of variance (ANOVA) were arranged as following:

ANOVA TABLE

Source of variation	Degree of freedom	Mean of squares	Fcal
Total	rabc-1		M_t/ M_e
A	a-1	MSA	F_A
B	b-1	MSB	F_B
AB	(a-1) (b-1)	MSAB	F_{AB}
C	c-1	MSC	F_C
AC	(a-1) (c-1)	MSAC	F_{AC}
BC	(b-1) (c-1)	MSBC	F_{BC}
Error	(r-1) (abc-1)	MSE	

Where,

A = Seed source

B = Pre-sowing seed treatments

C = Growing environments

MSA= Mean sum of squares due to Seed source

MSB= Mean sum of squares due to Pre-sowing seed treatments

MSC= Mean sum of squares due to Growing environments

MSE = Mean sum of squares due to error.

3.2.2 Critical difference (CD)

The critical difference was estimated as follows:

$CD = SE_d \, Xt_{0.05}$ error d.f.

Where,

SE_d= Standard error of difference estimated as:

SE_d for A= $\frac{\sqrt{MSE}}{rbc}$ SE_d for B = $\frac{\sqrt{MSE}}{rac}$ SE_d for C = $\frac{\sqrt{MSE}}{rab}$

$t_{0.05}$error degree of freedom = t value at 5 per cent level of significance.

3.2.3 Variability parameters

Coefficient of variability was estimated as under:

$$CV\ (\%) = \left[\frac{\sqrt{SD}}{\overline{X}}\right] \times 100$$

Where,

SD = Standard deviation

$\overline{X}$ = Population mean

Coefficient of variability was worked out as recommended by Burton and Devane (1953) and Pillai and Sinha (1968)

$$PCV\ (\%) = \left[\frac{\sqrt{Vp}}{\overline{X}}\right] \times 100$$

$$GCV\ (\%) = \left[\frac{\sqrt{Vg}}{\overline{X}}\right] \times 100$$

$$ECV\ (\%) = \left[\frac{\sqrt{Ve}}{\overline{X}}\right] \times 100$$

Where,

Vp = Phenotypic variance

Vg = Genotypic variance

Ve = Environmental variance

PCV = Phenotypic Coefficient of Variability

GCV = Genotypic Coefficient of.Variability

ECV = Environmental Coefficient of Variability

$\overline{X}$ = Population mean

CHAPTER-IV

RESULTS AND DISCUSSION

The present investigation entitled, Studies on seed germination behavior of *Santalum album* L. under nursery conditions was undertaken to standardize the suitable seed source, growing environment and to assess the effect of pre-sowing chemical seed treatments on seed germination, quality and growth of sandalwood seedlings. The results of present investigation have been described in this chapter under the following headings;

4.1 EFFECT OF SEED SOURCE

4.2 EFFECT OF PRE-SOWING SEED TREATMENTS

4.3 EFFECT OF GROWING ENVIRONMENT

4.4 INTERACTION EFFECTS

4.1 EFFECT OF SEED SOURCE

4.1.1 Germination Parameters

The data presented in table 4.1 depict the result obtained on various seed germination parameters viz. days taken to complete germination, germination value, germination (%) and germination energy. It is evident from the data that all the germination parameters like days taken to complete germination, germination (%), germination value and energy were statistically non significant among tested seed sources. Data on days taken to complete germination revealed that the seeds of both sources i.e. UHF (54.77) and KFRI (54.78) germinate in almost in same duration. Data for germination was recorded at each 10 days interval after the germination starts. During initial period i.e. 30 days after sowing germination percentage was more in KFRI source (0.59) than UHF source (0.51). Later at 40 and 50 days after sowing germination percentage was more in UHF seed source as compared with the seeds of KFRI source. Then at 60 days after sowing, both the seed sources i.e. UHF (18.95) and KFRI (18.52) showed almost similar germination percentage. Both seed sources i.e. UHF and KFRI shows similar performance for germination energy and germination value. No significant differences ware observed among the statistical values of these traits. But it is noticed that sandal seeds attained maximum germination at 60 days after sowing.

In almost all the germination parameters, similar values i.e. no significant differences were obtained in both seed sources. This may occur due to batter adaptations of both the seed sources under Punjab conditions. The occurrence of the species in a large geographical range consist of great diversity of edapho-climatic conditions of its habitats are reflected in the genetic constitution of its diverse populations (Gera *et al* 1999). The locality factors like longitude, latitude, precipitation and altitude have no association with racial variations among the populations of diverse origin (Shekar *et al* 2002). Since, the sandal seeds were collected

from different locations and from the trees of approximately similar; therefore, variations seen in seed parameters can be contributed by genetic architecture or can be the result of adaptation to diverse environmental conditions (Mathur *et al* 1984). In addition to this exposure and genotype of mother tree, vigour crown, climate and soil of place of seed origin can be the factors affecting the seed traits (Salzar and Quesada 1987).

Solan Seed KFRI Seed

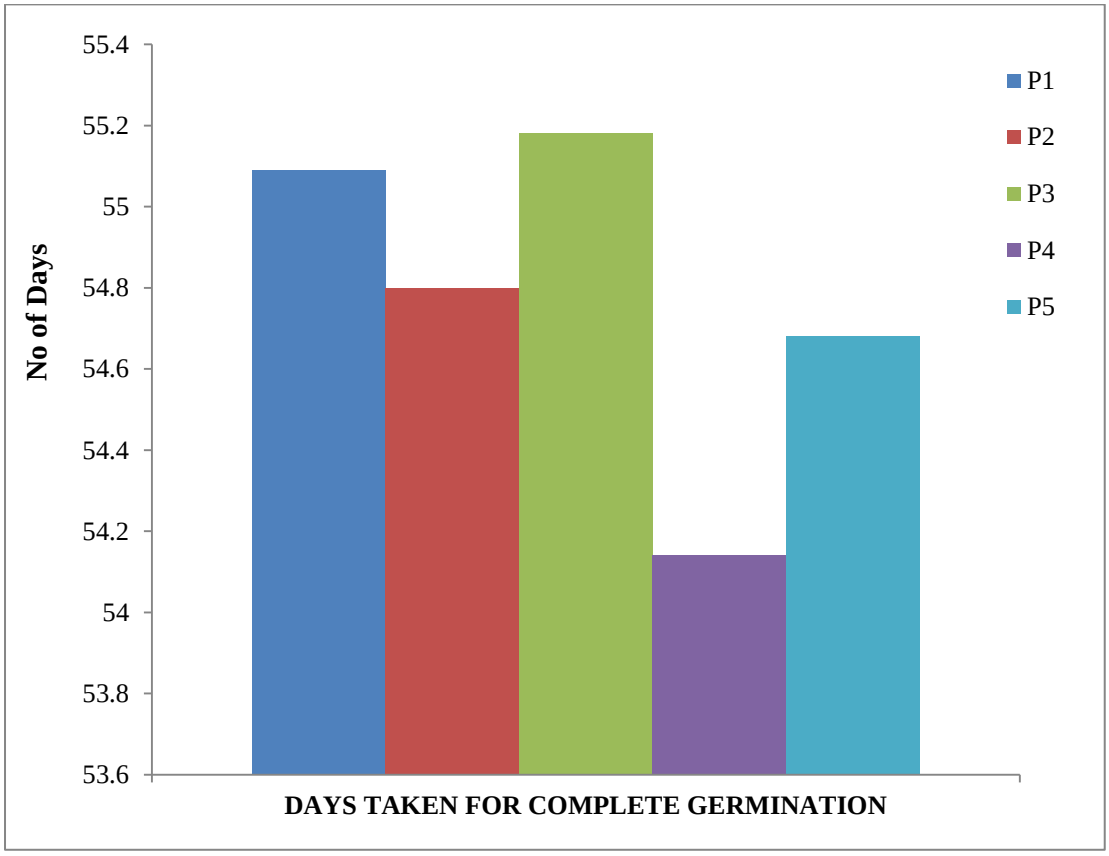

Figure 4.1: Days taken for complete germination by seeds primed with different pre-sowing seed treatments

Table 4.1: Effect of seed source, pre-sowing seed treatments and growing environment on germination parameters of *Santalum album*

Treatments	Days taken to complete germination	Germination (%)				Germination energy (%)	Germination value
		30 DAS	40 DAS	50 DAS	60 DAS		
Seed source							
UHF	54.77	0.51	9.49	13.71	18.95	31.58	0.17
KFRI	54.78	0.59	8.75	13.06	18.52	30.87	0.16
$SE_m\pm$	0.10	0.05	0.38	0.29	0.40	0.67	0.02
C D at 5%	NS	NS	NS	NS	NS	NS	NS
Pre-sowing seed treatments							
P_1 (Cold water 48 hours)	55.09	0.41	8.23	13.50	18.66	31.09	0.13
P_2 (GA_3 0.05 % for 24 hours)	54.80	0.62	10.75	13.27	20.74	34.56	0.17
P_3 (GA_3 0.5 % for 24 hours)	55.18	0.45	8.35	12.81	17.20	28.66	0.29
P_4 (H_2SO_4 10 % for 5 min)	54.14	0.91	10.66	15.06	19.99	33.32	0.14
P_5 (H_2SO_4 10 % for 1 min)	54.68	0.37	7.62	12.27	17.09	28.49	0.11
$SE_m\pm$	0.16	0.09	0.59	0.46	0.63	106	0.03
C D at 5%	0.54	0.29	1.96	1.54	2.11	3.51	0.11
Growing environment							
SE	54.73	0.58	9.27	13.49	18.80	31.33	0.17
OE	54.82	0.51	8.97	13.28	18.67	31.12	0.16
$SE_m\pm$	0.10	0.05	0.38	0.29	0.40	0.67	0.02
C D at 5%	NS	NS	NS	NS	NS	NS	NS
Interactions between seed source and pre-sowing seed treatment							
C D at 5%	NS	NS	NS	NS	NS	NS	NS
Interactions between seed source and growing environment							
C D at 5%	NS	NS	NS	NS	NS	NS	NS
Interactions between pre-sowing seed treatment and growing environment							
C D at 5%	NS	NS	NS	NS	NS	NS	NS

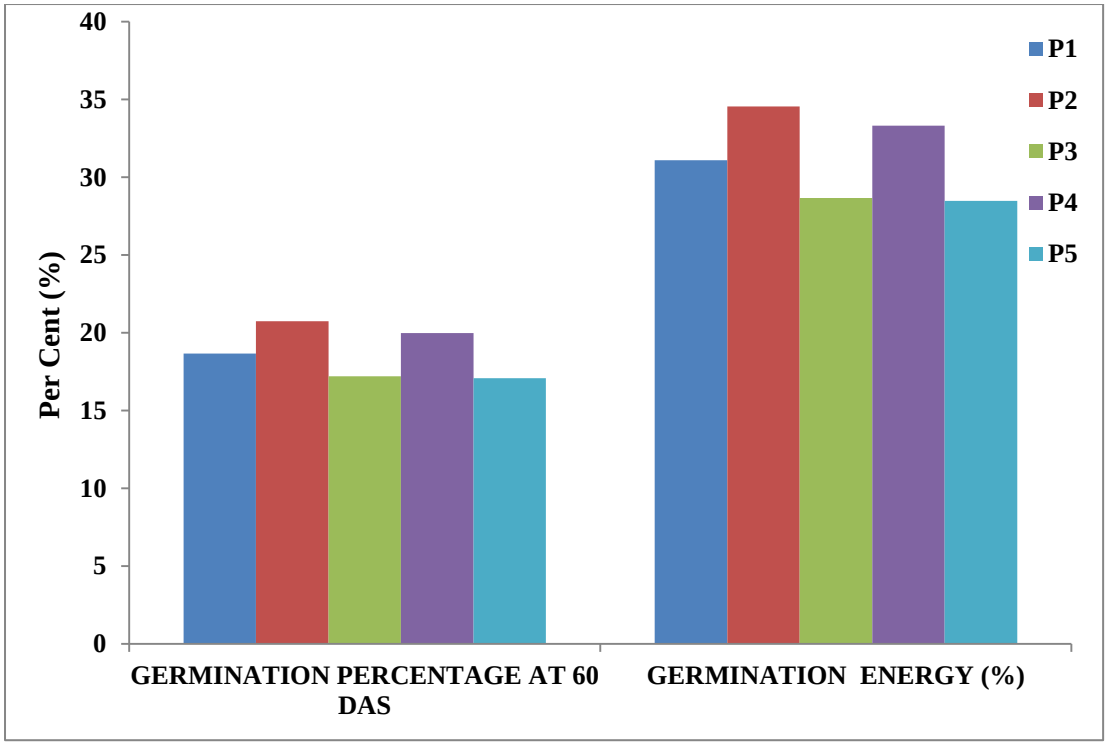

Figure 4.2: Germination percentage and energy obtained by seeds primed with different pre-sowing seed treatments

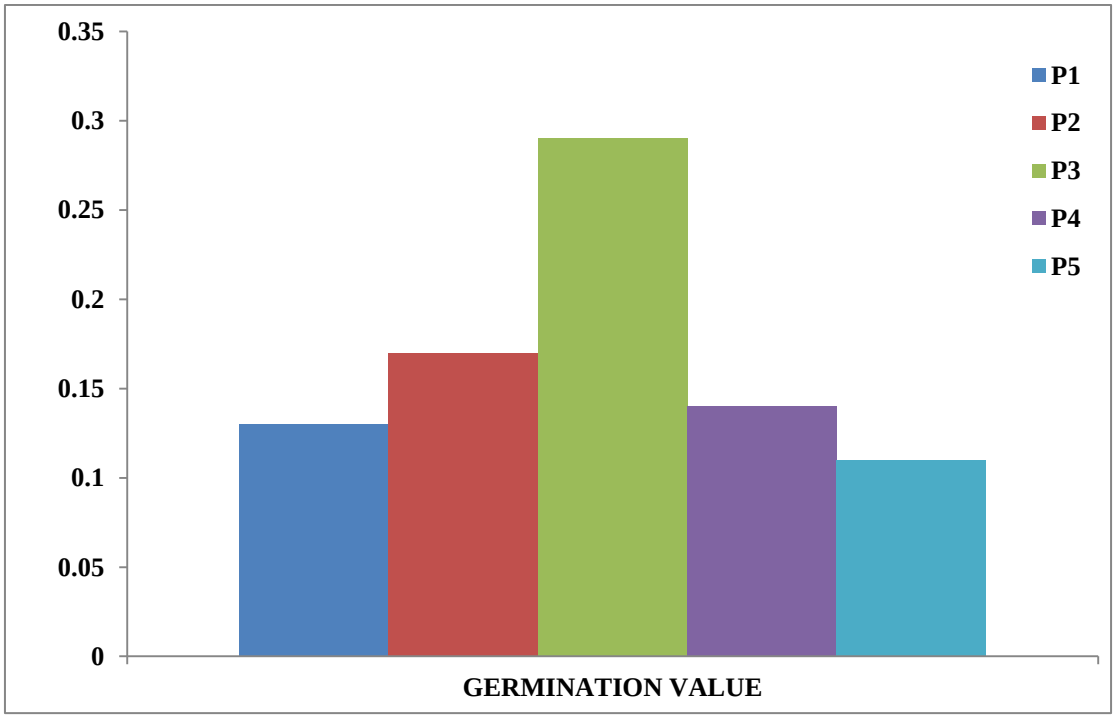

Figure 4.3: Germination value from seeds primed with different pre-sowing seed treatments

4.1.2 Growth Parameters

The findings of investigations presented in tables 4.2, 4.3, 4.4 and 4.5 indicated that seed source had significant influence on seedling growth parameters in *Santalum album* only

on number of leaves. Whereas, for other growth parameters like plant height, collar diameter,, root and shoot fresh weight, root length and root and shoot dry weight, statistically non-significant results were obtained.

Data for plant height, no. of leaves and collar diameter was taken after every 15 days. Data presented in the table 4.2 revealed that almost similar plant height was obtained in both seed sources during initial stage of their growth i.e. 30 days after transplanting. Though, the seed sources i.e. UHF (8.27 cm) and KFRI (8.04 cm) showed significant difference at 45 days after transplanting. But there after plant height of both seed sources remained statistically alike and seed sources UHF (23.64 cm) and KFRI (23.77 cm) obtained almost similar values at 180 days after transplanting.

Analysis of the data further revealed that effect of seed sources for collar diameter was statistically non significant during each stage of growth. At 180 days after transplanting, collar diameter in seeds of KFRI source was 4.36 mm and in UHF source, it was 4.28 mm. UHF seed source showed better performance for number of leaves at each stage i.e. from 30 days up to 180 days growing period. Maximum number of leaves at 180 days after transplanting was obtained in seeds from UHF (18.05) and minimum number of leaves (17.10) was obtained in seeds from KFRI source. The variation present among both seed sources for root length was found to be non significant. The values of root length in UHF source was14.89 cm whereas in seeds of KFRI source, the root length was 14.99 cm. Interpretations of data of root and shoot fresh weight indicated that higher value (2.02 g) was noticed in seeds of UHF source and lower of 1.98 g in the seeds of KFRI source. But as such the differences in values of root and shoot fresh weight of both the sources were statistically non significant. Data presented in table 4.5 also described that root and shoot dry weight also recorded almost statistically similar values in both seed sources i.e. UHF (0.55 g) and KFRI (0.54 g).

Similar findings were also conform by Tate and Page (2018). They examined 15 genotypes from two provenances Erromango and Tanna to determine the effect of seed sources on seed germination and growth parameters in *santalum album* and they reported that no significant effects were observed for plant height, rooting percentage, number or root growth. But a significant variation was noticed in provenances for rooting capacity by them. The present findings are also in conformity with the findings of Hidayan and Yuliah (2017) who evaluated the seeds collected from five provenances to see the performance of sandal for total height of the tree, bole length and the stem diameter. Significant variations were observed for total variable height of tree and stem diameter. But no variation was observed for the bole length.

Table 4.2: Effect of seed source, pre-sowing seed treatments and growing environment on plant height of *Santalum album*

Treatments	Plant height (cm)											
	15 DAT	30 DAT	45 DAT	60 DAT	75 DAT	90 DAT	105 DAT	120 DAT	135 DAT	150 DAT	165 DAT	180 DAT
Seed source												
UHF	7.38	7.56	8.27	8.67	9.96	11.26	12.77	14.12	15.92	16.90	19.97	23.64
KFRI	7.37	7.55	8.04	8.60	9.89	11.11	12.58	14.03	15.80	16.73	20.52	23.77
$SE_m\pm$	0.01	0.01	0.04	0.03	0.07	0.10	0.14	0.16	0.21	0.25	0.28	0.28
C D at 5 %	NS	NS	0.15	NS	NS	NS	NS	NS	NS	NS	NS	NS
Pre sowing treatments												
P_1 (Cold water 48 hours)	7.37	7.55	8.16	8.56	9.87	11.08	12.42	13.66	15.00	16.09	19.23	22.97
P_2 (GA_3 0.05 % for 24 hours)	7.38	7.60	8.38	8.80	10.33	11.69	13.37	14.89	14.49	17.93	21.25	24.74
P_3 (GA_3 0.5 % for 24 hours)	7.33	7.51	8.07	8.47	9.60	10.65	11.97	13.43	16.52	16.00	19.26	22.63
P_4 (H_2SO_4 10 % for 5 min)	7.43	7.59	8.09	8.78	10.10	11.46	13.20	14.66	16.24	17.93	21.62	25.13

P_5 (H_2SO_4 10 % for 1 min)	7.35	7.53	8.07	8.57	9.73	11.05	12.41	13.73	15.03	16.13	19.87	23.05
SE_m±	0.02	0.02	0.07	0.05	0.11	0.16	0.23	0.25	0.33	0.39	0.45	0.45
C D at 5 %	0.05	0.05	NS	0.19	0.38	0.53	0.78	0.84	1.10	1.30	1.50	1.48
Growing environment												
SE	7.38	7.56	8.20	8.64	9.92	11.15	12.70	14.09	15.88	16.85	20.14	23.55
OE	7.37	7.55	8.12	8.63	9.94	11.21	12.65	14.06	15.83	16.79	20.35	23.85
SE_m±	0.01	0.01	0.04	0.03	0.07	0.10	0.14	0.16	0.21	0.25	0.28	0.28
C D at 5 %	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interactions between seed source and pre-sowing seed treatment												
C D at 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interactions between seed source and growing environment												
C D at 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interactions between pre-sowing seed treatment and growing environment												
C D at 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 4.3: Effect of seed source, pre-sowing seed treatments and growing environment on collardiameter of *Santalum album*

Treatmens	Collar diameter (cm)											
	15 DAT	30 DAT	45 DAT	60 DAT	75 DAT	90 DAT	105 DAT	120 DAT	135 DAT	150 DAT	165 DAT	180 DAT
Seed source												
UHF	1.22	1.55	2.12	2.58	2.90	3.31	3.53	3.78	3.97	4.13	4.23	4.28
KFRI	1.21	1.53	2.10	2.54	2.94	3.36	3.61	3.85	4.04	4.20	4.31	4.36
$SE_m\pm$	0.01	0.01	0.01	0.01	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
C D at 5 %	0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Pre sowing seed treatments												
P_1 (Cold water 48 hours)	1.22	1.55	2.09	2.51	2.85	3.28	3.52	3.76	3.93	4.09	4.19	4.25
P_2 (GA_3 0.05 % for 24 hours)	1.22	1.54	2.13	2.58	2.96	3.39	3.64	3.88	4.07	4.23	4.35	4.40
P_3 (GA_3 0.5 % for 24 hours)	1.20	1.50	2.04	2.46	2.78	3.16	3.39	3.62	3.80	3.96	4.06	4.12
P_4 (H_2SO_4 10 % for 5 min)	1.23	1.56	2.16	2.62	3.01	3.42	3.67	3.91	4.10	4.28	4.36	4.43

P_5 (H_2SO_4 10 % for 1 min)	1.20	1.54	2.13	2.59	3.00	3.40	3.64	3.90	4.12	4.28	4.38	4.42
SE_m±	0.01	0.01	0.02	0.02	0.04	0.04	0.05	0.05	0.06	0.06	0.06	0.06
C D at 5 %	0.02	0.03	0.06	0.09	0.13	0.15	0.16	0.17	0.18	0.18	0.19	0.18
Growing environment												
SE	1.21	1.54	2.12	2.54	2.92	3.32	3.56	3.80	4.00	4.16	4.26	4.32
OE	1.22	1.53	2.10	2.56	2.92	3.34	3.58	3.82	4.01	4.17	4.28	4.32
SE_m±	0.01	0.01	0.01	0.01	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
C D at 5 %	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interactions between seed source and pre-sowing seed treatment												
C D at 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interactions between seed source and growing environment												
C D at 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interactions between pre-sowing seed treatment and growing environment												
C D at 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

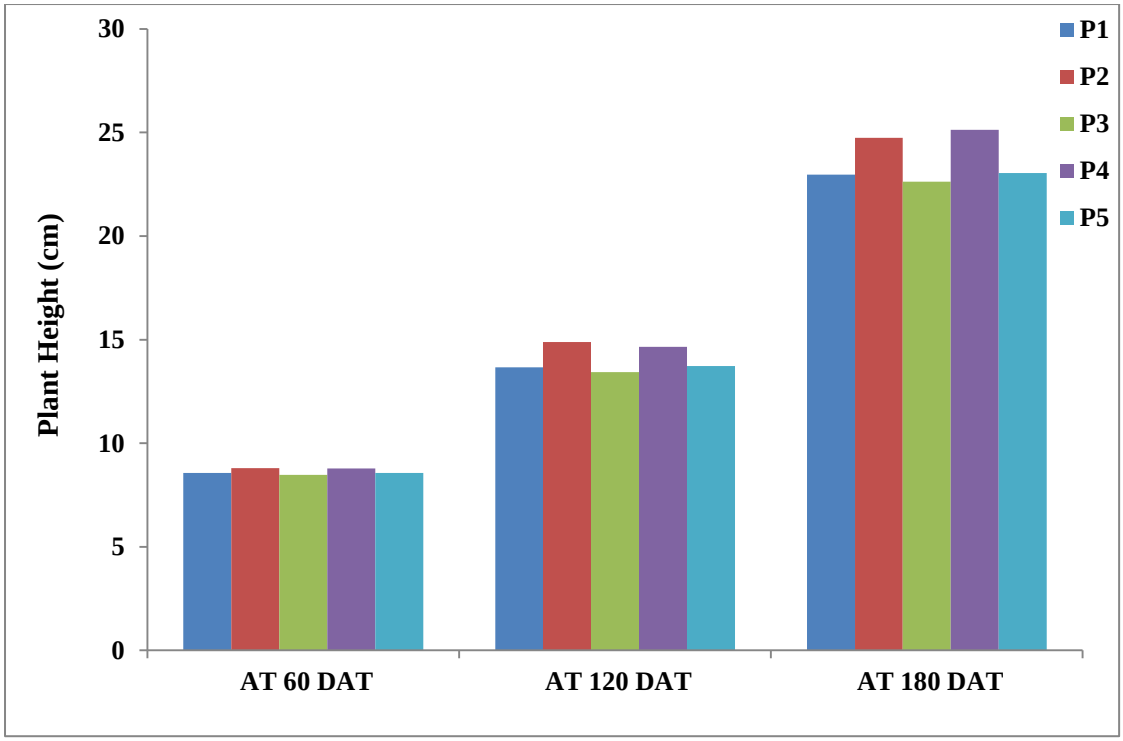

Figure 4.4: Plant height at 60, 120 and 180 DAT in seeds primed with different pre-sowing seed treatments

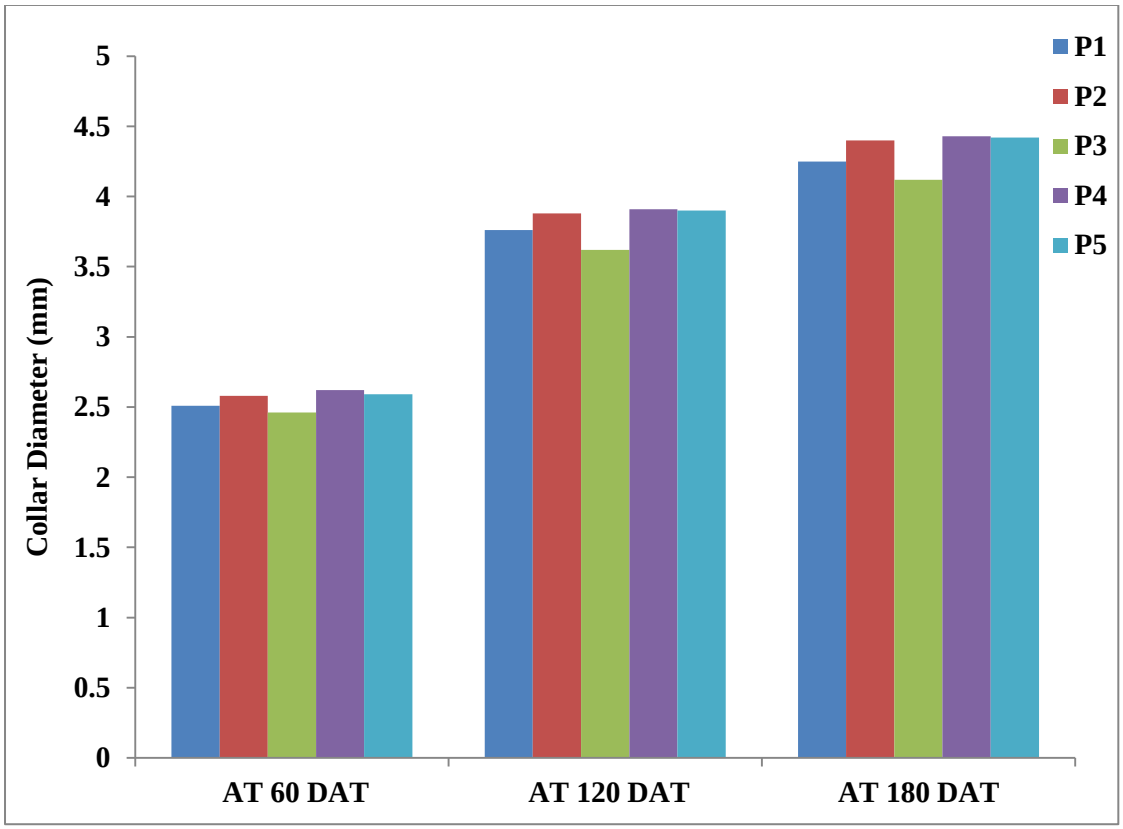

Figure 4.5: Collar diameter at 60, 120 and 180 DAT in seeds primed with different pre-sowing seed treatments

Table 4.4: Effect of seed source, pre-sowing seed treatments and growing environment on number of leaves of *Santalum album*

Treatments	Number of leaves (cm)											
	15 DAT	30 DAT	45 DAT	60 DAT	75 DAT	90 DAT	105 DAT	120 DAT	135 DAT	150 DAT	165 DAT	180 DAT
Seed source												
UHF	4.35	5.79	6.97	8.20	9.84	11.14	12.07	13.56	14.37	15.64	16.50	18.05
KFRI	4.53	5.59	6.77	7.98	9.42	10.61	11.58	12.76	13.63	14.84	15.62	17.10
$SE_m\pm$	0.04	0.05	0.04	0.11	0.11	0.13	0.11	0.15	0.16	0.19	0.20	0.22
C D at 5 %	0.16	0.19	0.15	NS	0.39	0.44	0.39	0.51	0.55	0.65	0.67	0.74
Pre sowing seed treatments												
P_1 (Cold water 48 hours)	4.36	5.55	6.83	8.12	9.76	10.89	11.90	13.03	13.68	14.80	15.54	16.97
P_2 (GA_3 0.05 % for 24 hours)	4.70	6.02	7.06	8.48	10.11	11.49	12.23	13.76	14.58	15.80	16.64	18.23
P_3 (GA_3 0.5 % for 24 hours)	4.38	5.58	6.74	7.65	9.16	10.51	11.46	12.79	13.74	14.99	15.71	17.21
P_4 (H_2SO_4 10 % for 5 min)	4.47	5.77	6.97	8.30	9.73	10.92	11.98	13.36	14.34	15.77	16.78	18.43

P_5 (H_2SO_4 10 % for 1 min)	4.31	5.53	6.75	7.90	9.40	10.57	11.58	12.85	13.66	14.86	15.65	17.04
$SE_m\pm$	0.07	0.09	0.07	0.18	0.18	0.21	0.18	0.24	0.26	0.31	0.32	0.35
C D at 5 %	0.25	0.30	0.23	NS	0.62	NS	NS	NS	NS	NS	NS	1.17
Growing environment												
SE	4.39	5.69	6.84	7.92	9.44	10.67	11.61	12.98	13.70	14.85	15.79	17.33
OE	4.50	5.69	6.90	8.26	9.82	11.08	12.05	13.34	14.30	15.64	16.34	17.82
$SE_m\pm$	0.04	0.05	0.04	0.11	0.11	0.13	0.11	0.15	0.16	0.19	0.20	0.22
C D at 5 %	NS	NS	NS	NS	NS	NS	0.39	NS	0.55	0.65	NS	NS
Interactions between seed source and pre-sowing seed treatment												
C D at 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interactions between seed source and growing environment												
C D at 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interactions between pre-sowing seed treatment and growing environment												
C D at 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

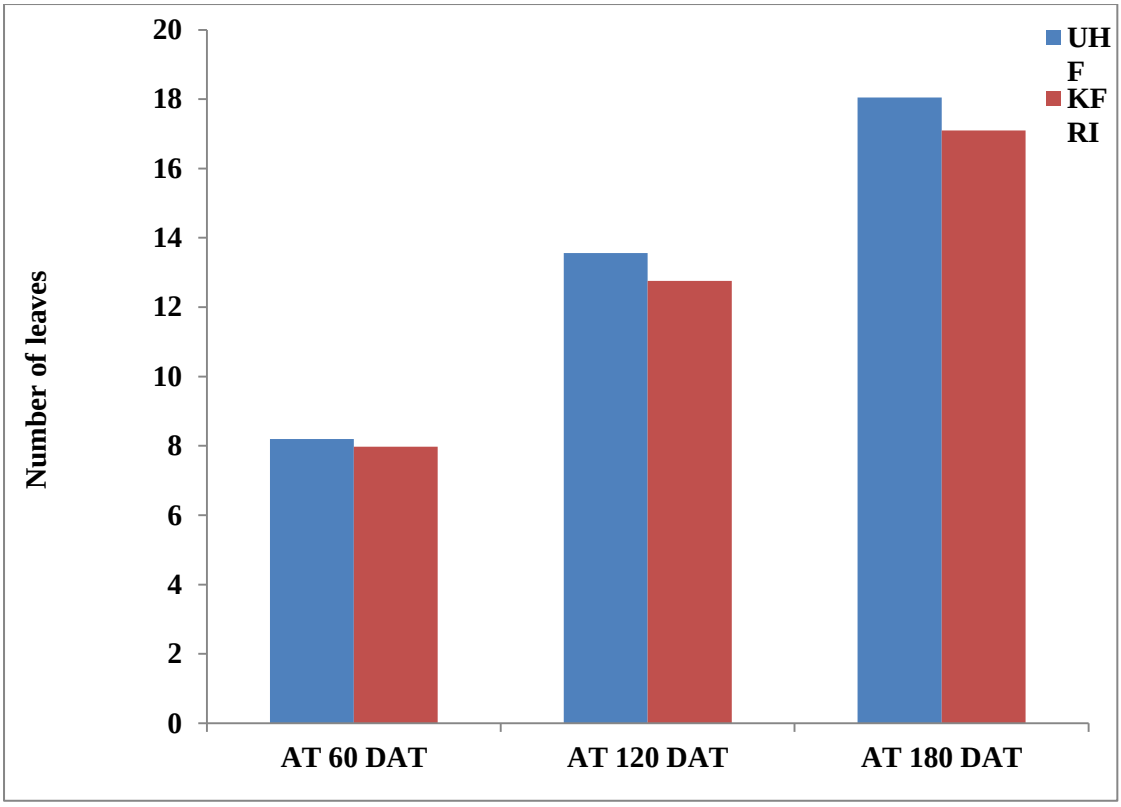

Figure 4.6: Number of leaves at 60, 120 and 180 DAT obtained under two seed sources

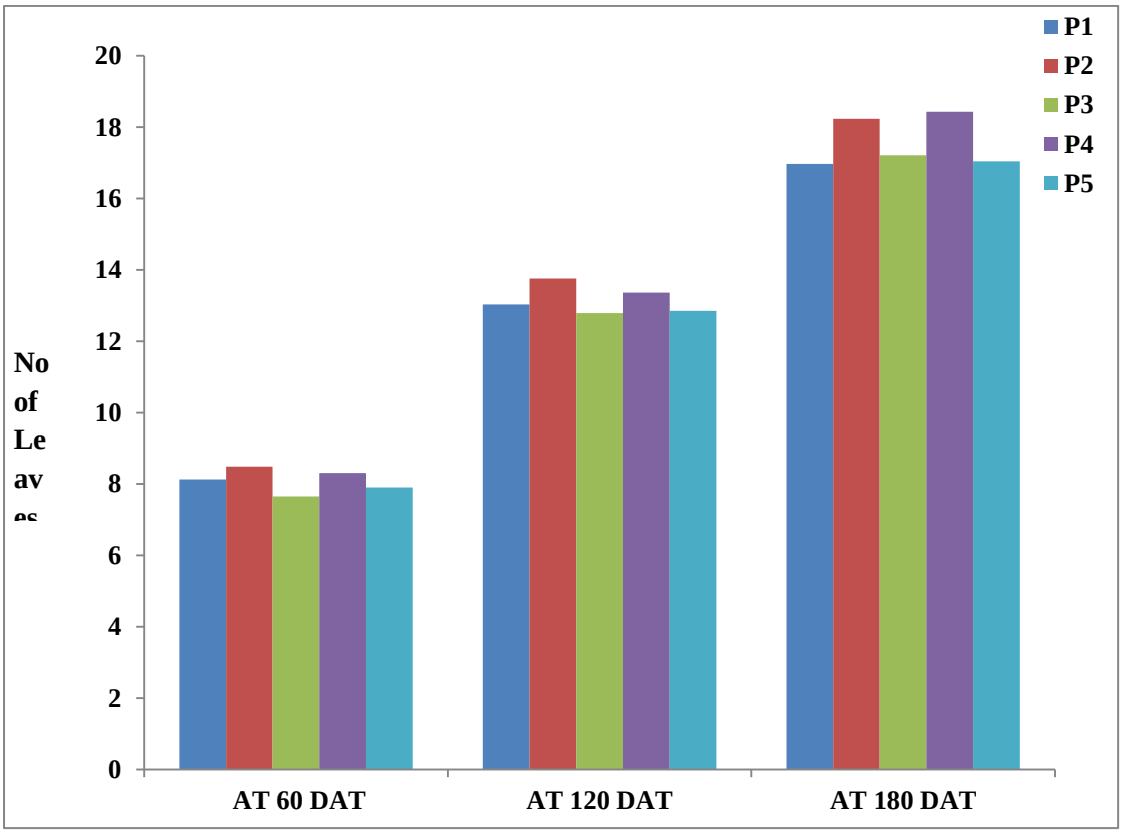

Figure 4.7: Number of leaves at 60, 120 and 180 DAT in seeds primed with different pre-sowing seed treatments

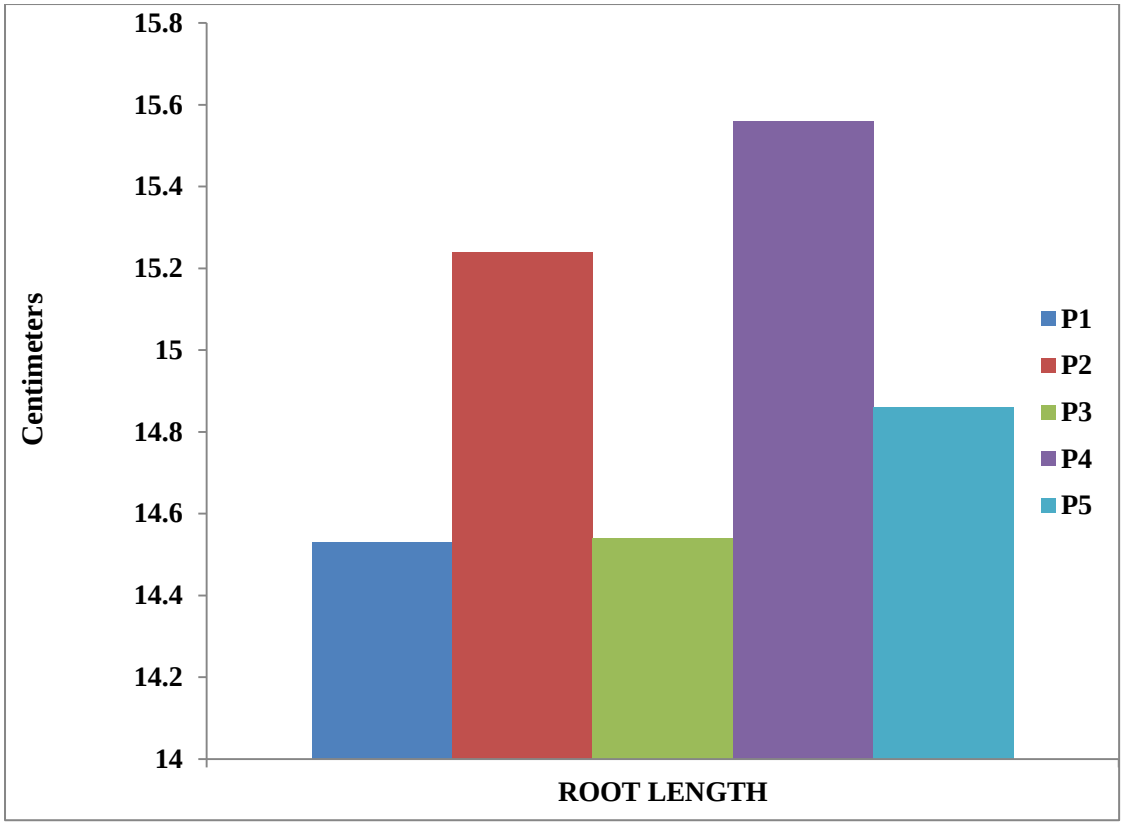

Figure 4.8: Root length from seeds primed with different pre-sowing seed treatments

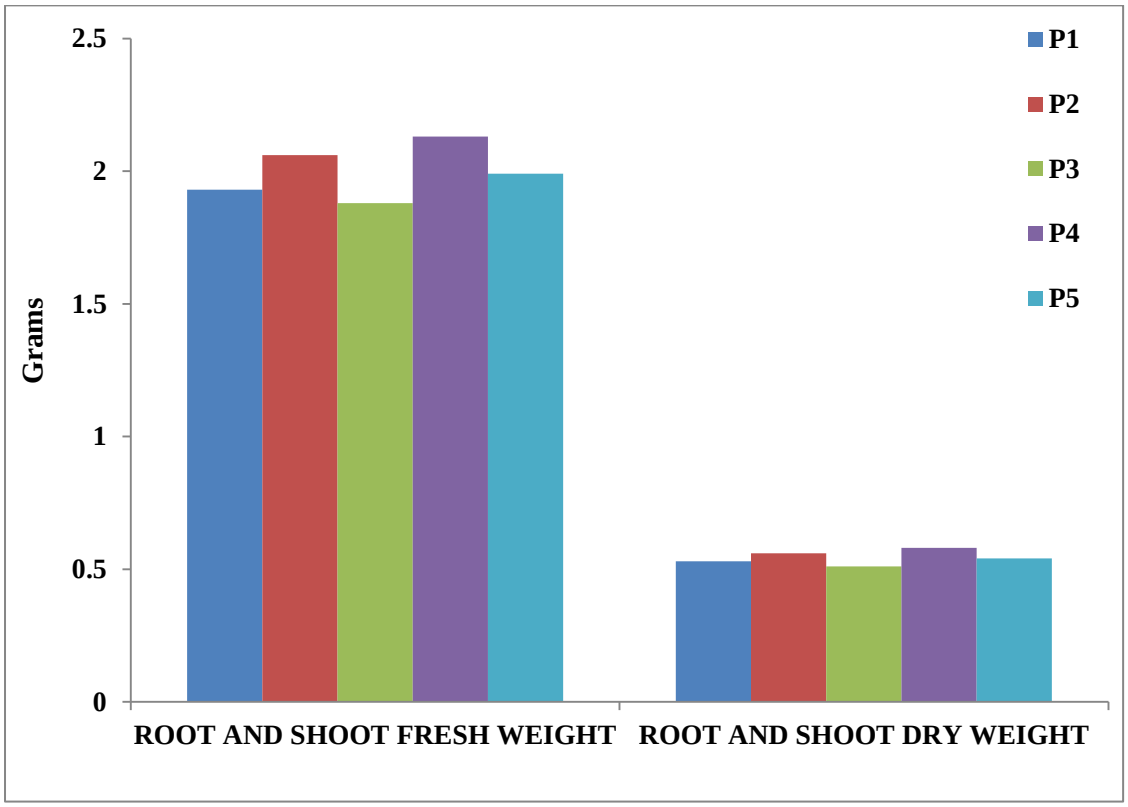

Figure 4.9: Root and shoot fresh and dry weight obtained in seeds primed with different pre-sowing seed treatments

Table 4.5: Effect of seed source, pre-sowing seed treatments and growing environment on growth parameters of *Santalum album*

Treatments	Root length (cm)	Root and shoot fresh weight (g)	Root and shoot dry weight (g)
Seed source			
UHF	14.89	2.02	0.55
KFRI	14.99	1.98	0.54
$SE_m\pm$	0.11	0.03	0.01
C D at 5 %	NS	NS	NS
Pre sowing seed treatments			
P_1 (Cold water 48 hours)	14.53	1.93	0.53
P_2 (GA_3 0.05 % for 24 hours)	15.24	2.06	0.56
P_3 (GA_3 0.5 % for 24 hours)	14.54	1.88	0.51
P_4 (H_2SO_4 10 % for 5 min)	15.56	2.13	0.58
P_5 (H_2SO_4 10 % for 1 min)	14.86	1.99	0.54
$SE_m\pm$	0.17	0.04	0.01
C D at 5%	0.58	0.13	0.04
Growing environment			
SE	14.92	2.00	0.55
OE	14.97	2.00	0.54
$SE_m\pm$	0.11	0.03	0.01
C D at 5%	NS	NS	NS
Interactions between seed source and pre-sowing seed treatment			
C D at 5%	NS	NS	NS
Interactions between seed source and growing environment			
C D at 5%	NS	NS	NS
Interactions between pre-sowing seed treatment and growing environment			
C D at 5%	NS	NS	NS

4.1.3 Quality Parameters

The data presented in table 4.6 indicates that seed source does not exerted significant influence on seedling quality parameters in *Santalum album* viz. quality index and sturdiness quotient. Whereas, seed source has significant effects on root:shoot ratio.

Further analysis of the data revealed that maximum value for root:shoot ratio (0.74) was obtained in seeds collected from UHF source and minimum value for root shoot ratio (0.72) was obtained in seeds of KFRI source and this difference was statistically significant. Variation in the values of sturdiness quotient was also non significant and higher value i.e. 5.62 was obtained in seeds of UHF source and lower value of 5.52 was recorded in seeds from KFRI source. As for as the quality index is concerned, both seed sources were statistically similar i.e. UHF (0.07) and KFRI (0.07)

It is clearly evident from the results that both the seed sources showed similar performance for various seedling quality parameters. Various quality parameters, rather than growth parameters can be used for comparsion of seedlings. Traditionally, certain morphological characters such as quality index, sturdiness quotient and root:shoot ratio are used for seed quality specifications (Cleary *et al* 1978). The high root:shoot ratio results in more amount of root production, which is important for establishment of seedlings and more absorption of nutrients (Hossain *et al* 1998). Setiawan *et al* (2019) also reported that seed source determine the survival rate and quality index in sandalwood provenances.

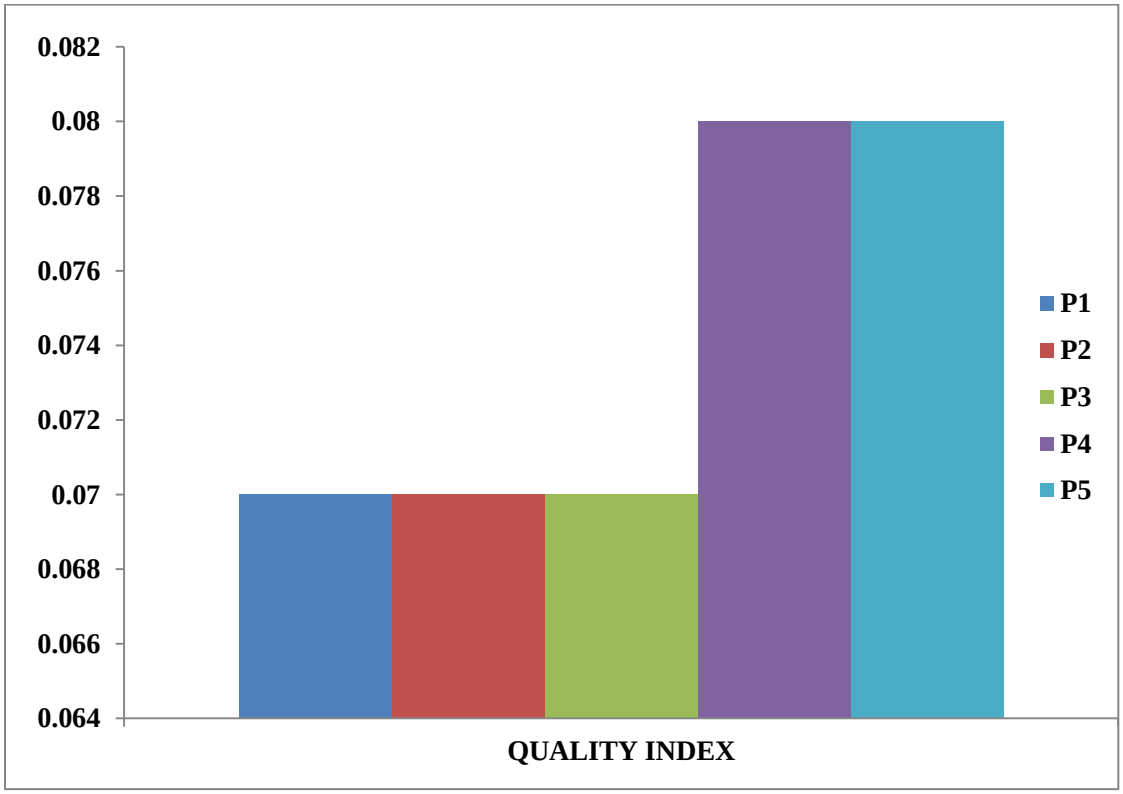

Figure 4.10: Quality index obtained in seeds primed with different pre-sowing seed treatments

Table 4.6: Effect of seed source, pre-sowing seed treatments and growing environment on squality parameters of *Santalum album*

Treatments	Root shoot ratio	Sturdiness quotient (SQ)	Quality index (QI)
Seed source			
UHF	0.74	5.62	0.07
KFRI	0.72	5.52	0.07
$SE_m\pm$	0.01	0.05	0.01
C D at 5 %	0.02	NS	NS
Pre sowing seed treatments			
P_1 (Cold water 48 hours)	0.72	5.45	0.07
P_2 (GA_3 0.05 % for 24 hours)	0.74	5.69	0.07
P_3 (GA_3 0.5 % for 24 hours)	0.75	5.69	0.07
P_4 (H_2SO_4 10 % for 5 min)	0.70	5.73	0.08
P_5 (H_2SO_4 10 % for 1 min)	0.74	5.28	0.08
$SE_m\pm$	0.01	0.08	0.01
C D at 5 %	0.03	0.27	0.01
Growing environment			
SE	0.73	5.51	0.07
OE	0.73	5.63	0.07
$SE_m\pm$	0.01	0.05	0.01
C D at 5 %	NS	NS	NS
Interactions between seed source and pre-sowing seed treatment			
C D at 5%	NS	NS	NS
Interactions between seed source and growing environment			
C D at 5%	NS	NS	NS
Interactions between pre-sowing seed treatment and growing environment			
C D at 5%	NS	NS	NS

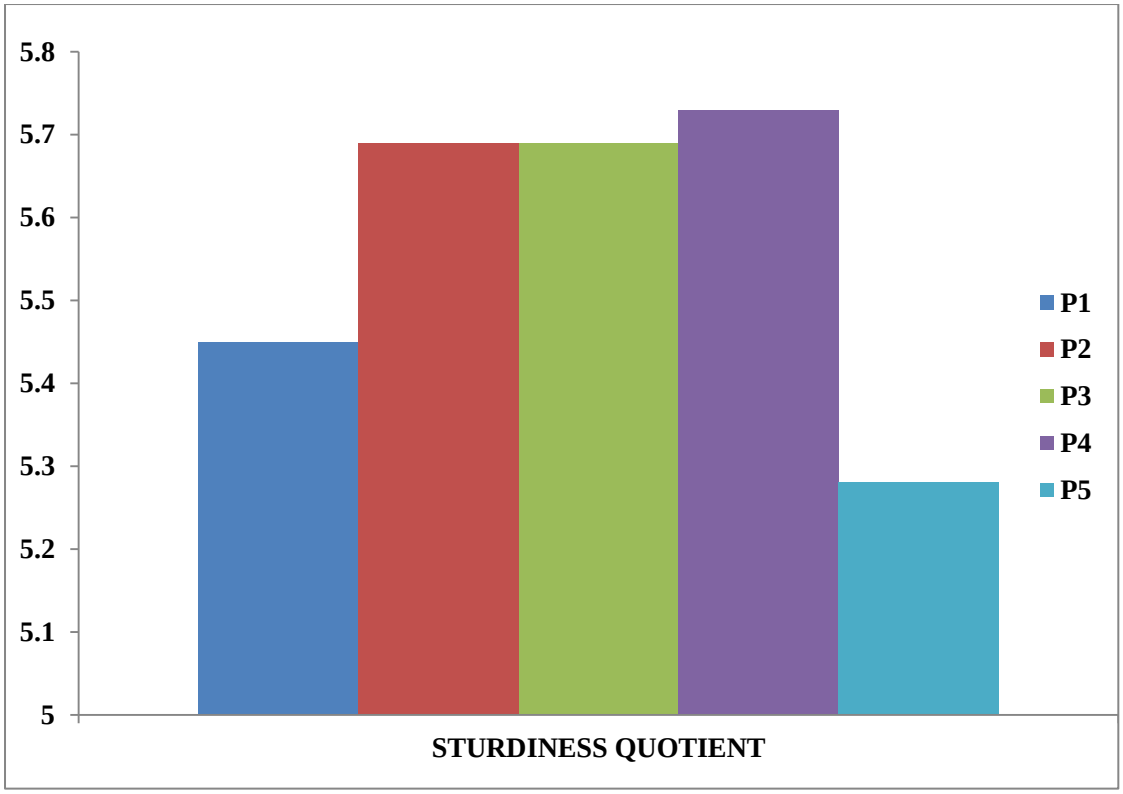

Figure 4.11: Sturdiness quotient obtained in seeds primed with different pre-sowing seed treatments

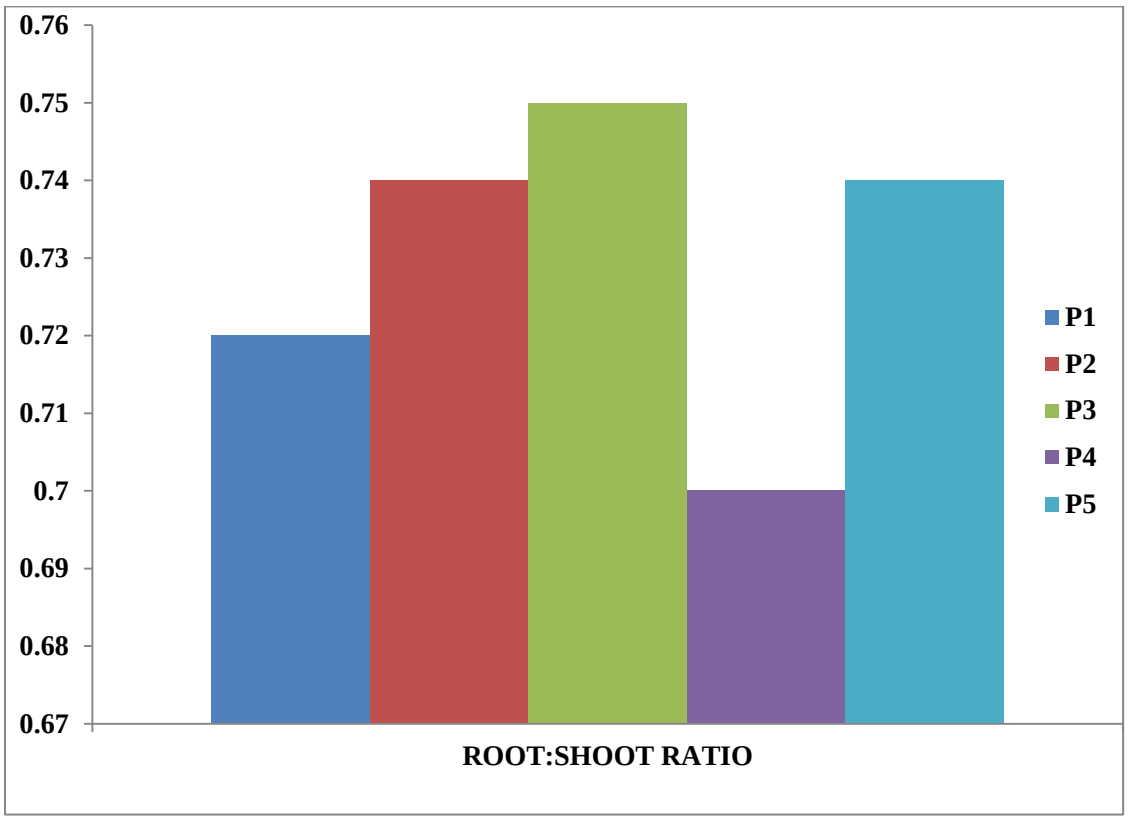

Figure 4.12: Root:shoot ratio obtained in seeds primed with different pre sowing seed treatments

4.2 EFFECT OF PRE-SOWING SEED TREATMENTS

4.2.1 Germination Parameters

It was observed from the data provided in the table 4.1 that different pre sowing seed treatments had significant effect on all germination parameters viz. germination per cent, days taken to complete germination, germination energy and value.

Data on days taken for complete germination revealed that minimum period of 54.14 days was taken for complete germination by the seeds of treatment P_4 (H_2SO_4 10% for 1 min) and maximum days (55.18) were taken by the seeds of treatment P_3 (GA_3 0.5 % for 24 hours). The germination per cent was increased with germination period. The seed treated with GA_3 0.05 % for 24 hours recorded higher germination percentage at 40 days after sowing (10.75%), while at 60 days after sowing it was significantly higher (20.74 %). Minimum germination percentage was observed in seed treated with H_2SO_4 10% for 1 min i.e. 17.09 per cent. Data pertaining to germination energy suggests that maximum germination energy (34.56 %) was obtained in seeds treated with GA_3 0.05 % for 24 hours. The minimum germination energy (28.49 %) was recorded in seed treated with H_2SO_4 10% for 1 min. Meanwhile, maximum germination value of 0.29 was observed in seeds treated with GA_3 0.5 % for 24 hours and minimum germination value (0.11) was noticed in seeds treated with H_2SO_4 10% for 1 min.

As mentioned above, the better results were obtained in the seed treated with gibberllic acid followed by sulphuric acid. These results are in coherent with the findings of Norton (1986) and he revealed that the seeds when treated with GA_3 resulted in the increase in germination percentage because the burning of hard seed coat results in the imbibitions of optimum amount of water where imbibitions is the first place of germination process and it has also the ability to break the dormancy. Acid scarification has also been reported to disintegrate the leathery seed coat testa in sandalwood seed (Norton 1986). Scarification of sandal seeds to break dormancy was also advocated by Nagavani and Srimathi (1981) as sulphuric acid gave better (45-60%) germination after 60 days of seed sowing as compared to untreated seeds.

4.2.2 Growth Parameters

Observations on different seedling growth parameters viz. seedling height, collar diameter, root length, number of leaves, root and shoot fresh and dry weight are influenced significantly by pre-sowing seed treatments. Data for seedling height, collar diameter, number of leaves were recorded at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165 and 180 days after transplanting (DAT) and the results obtained are presented in tables 4.2, 4.3 and 4.4. The table 4.5 presents the data related to other seedling growth parameters like root and shoot fresh weight, root length and root and shoot dry weight.

Statistically, superior plant height of 25.13 cm was recorded in seeds treated with

H_2SO_4 (10% for 5 min) at 180 days after transplanting. Minimum plant height i.e. 22.63 cm was observed in seeds treated with GA_3 0.5 per cent for 24 hours. The data provided in the table 4.3, it was noticed that different seed treatments had significant effect on seedling collar diameter. The data for collar diameter was recorded after every 15 days interval after transplanting. At 180 days after transplanting, higher collar diameter (4.43 mm) was observed in seeds treated with H_2SO_4 (10% for 5 min) and statistically minimum collar diameter of 4.12 mm was observed in seeds treated with GA_3 0.5 % for 24 hours. At 30 days after transplanting, maximum number of leaves were observed in seeds primed with GA_3 0.05 % for 24 hours with the value of 6.02 and statistically minimum number of leaves (5.53) were recorded in seeds primed with H_2SO_4 (10% for 1 min). At 90 days after transplanting, maximum number of leaves (11.49) were seen in seeds treated with GA_3 0.05 % for 24 hours and statistically minimum number of leaves (10.51) were observed in seed treated withGA_3 0.5 % for 24 hours. At 180 days after transplanting, maximum number of leaves (18.43) were witnessed in seed primed with H_2SO_4 (10% for 5 min) and statistically minimum number of leaves 16.97 were noticed in treatment P_1 i.e. cold water seed treatment for 48 hours.

A critical analysis of the data present in table 4.5 for seedling root length revealed that the influence of different sowing treatments varied significantly. Maximum seedling root length of 15.56 cm was found in seeds primed with H_2SO_4 (10% for 5 min) and the lowest values i.e. 14.53 cm for root length was recorded in seeds treated with cold water for 48 hours. Maximum root and shoot fresh weight (2.13 g) was observed in seeds primed with H_2SO_4 (10% for 5 min). The minimum root and shoot fresh weight (1.88 g) was found in

seeds primed with GA_3 0.5 % for 24 hours. Maximum root and shoot dry weight (0.58 g) was found in seeds primed with H_2SO_4 (10% for 5 min). Whereas, minimum root and shoot dry weight i.e. 0.51 g was recorded in seeds primed with GA_3 0.5 % for 24 hours.

The the results thus obtained are similar with the conclusions given by Ananthapadmanabha *et al* (1988) for different pre-sowing seed treatments. The acid scarification helps in breaking the seed dormancy and led to better germination. Good germination further results in good growth and developments, scarified seeds produced the seedling with maximum plant height and more dry weight. Norton (1986) also noticed the acid scarified seeds showed quick germination and produced vigour seedlings. He also concluded that treated Seed's seedlings produced more plant height, dry weight and collar diameter as compared with seedling raised from untreated seeds.

4.2.3 Quality Parameters

It is evident from the data given in table 4.6 that root:shoot ratio, sturdiness quotient and quality index were found to be statistically significant among the different seeds treatments. Seeds treated with H_2SO_4 (10% for 5 min) has minimum root:shoot ratio i.e. 0.70 and maximum root:shoot ratio of 0.75 was recorded in seeds which were treated with GA_3 0.5 % for 24 hours. Maximum sturdiness quotient (5.73) was noticed in seed primed with H_2SO_4 (10% for 5 min) and minimum sturdiness quotient (5.28) was seen in seed treated with H_2SO_4 (10% for 1 min). Statistically superior quality index of 0.08 was noticed in both treatments i.e. P_4 and P_5 as compared to all other pre-sowing seed treatments.

Traditionally, quality of seedlings have been measured in the form of certain morphological characters such root:shoot ratio, sturdiness quotient, quality index and some other features (Cleary *et al* 1978). Various studies have shown that these of quality parameters, rather than growth parameters can be judged the performance of seedlings.

The root:shoot ratio represents the volume of root and shoot produced. Higher the value of root:shoot ratio, the production of roots will be more and this is also a obligatory for seedling establishment through anchorage and more absorption of nutrients (Hossain *et al* 1998). The sturdiness quotient refers to ratio of the height of the seedling to root or collar diameter and express vigour and robustness of the seedlings. Smaller the quotient value, higher the survival chance of seedlings (Gebretsadik 2018). Similarly, Chauhan and Sharma (1997) also confirm that the low sturdiness quotient value helps in promoting vigorous early growth of seedlings in plantations.

Seedling quality index is also a promising measure of morphological traits and is supposed to be a good indicator of quality seedlings. Its value measure robustness and biomass distribution while considering several other important parameters (Johnson and Cline 1991). Sturdiness quotient is less vigorous and non-destructive index which is used to compare the plant height with collar diameter. Sturdiness quotient varied inversely with

survival rate of seedlings. The smaller quotient value indicates higher expected chance of survival and growth of seedlings, especially on windy or dry sites.

4.3 EFFECT OF GROWING ENVIRONMENT

The data presented in tables 4.1, 4.2, 4.3, 4.4, 4.5 and 4.6 depict the result obtained on seed germination, growth and quality parameters in the seedlings of *Santalum album* viz. days taken to complete germination, germination percentage, germination energy, germination value, plant height, collar diameter, number of leaves, root length, root and shoot fresh weight, root and shoot dry weight, root:shoot ratio, sturdiness quotient and quality index. Overall, it was noticed that growing environment did not produced any significant effect on all the studied parameters in the present study.

4.3.1 Germination Parameters

Data on days taken to complete germination revealed that minimum period i.e. 54.73 days were taken by the seeds to complete their germination under shade condition in comparison to open conditions i.e. 54.82 days. But as such there were no significant differences in readings of both growing environments. At 60 days after sowing germination of 18.80 per cent was recorded under shade and 18.67 per cent in seeds grown in open conditions. Data pertaining to germination values for energy depicts that germination energy i.e. 31.33 per cent in shade and germination energy (31.12%) in open conditions were almost same and showed non significant differences. It was also apparent from the data that higher germination values (0.17) in shade conditions and minimum germination value (0.16) obtained under open growing conditions were statistically same. Almost similar non significant results have obtained for all the germination parameters.

The present results are in contrast to the findings of Devagiri (1997) and Gera *et al* (1999), who repoprted that the variations in the germination parameters could be the results of variation in the microclimate and environmental conditions. The expression of genetic constitution of the species for the different traits can change due environmental conditions of particular region resulting in geographically distinct clines (Kumar *et al* 2004). The findings of Vijayan and Rahees (2015) are also in contrast with the present study and maximum germination was reported by them under open conditions.

4.3.2 Growth Parameters

It was also seen from the data present in tables 4.2, 4.3, 4.4 and 4.5 that growing environment did not produced any significant effect on seedling growth parameters and it was seen that maximum plant height i.e. 23.85 cm was recorded under open conditions and minimum plant height of 23.55 cm in the seeds grown in shade conditions at 180 days after transplanting. Similar values for collar diameter were obtained in both open (4.32 mm) and shade (4.32 mm) conditions at 180 days after transplanting. Hence the effect was non significant in both growing environments. Number of leaves were more (17.82) in plants grown in open conditions from the number of leaves (17.33) in plants grown in shade conditions but as such the results were non significant.

Similarly, root length (14.97 cm) in open and (14.92 cm) in shade showed the non significant differences in both growing environmental conditions. In case of root and shoot fresh weight, similar values were obtained under both growing conditions i.e. shade (2.00 g) and open (2.00 g). Interpretations of data of root and shoot dry weight also showed non significant results i.e. shade (0.55 g) and open (0.54 g).

4.3.3 Quality Parameters

Quality of seedlings is extensively used a term in forestry and agroforestry, which has

received significant attention in afforestation and plantation practices. Quality of seedlings is important because very little or no care is given to afforested seedlings as compared to that is given to individual decorative and fruit trees. Once the seedlings are planted, they have to survive at their own without any appropriate artificial application of irrigation or fertilizers. The similar thing often happens in the case of tropical or sub-tropical small holder agroforestry or plantation sites. The results of various studies have also shown that field survival, fitness, growth and productivity of afforested seedlings are related to the quality of seedlings used in plantation and afforestation programs (Evans 1982).

The data presented in table 4.6 indicates that growing environments did not exerted significant influence on root:shoot ratio, sturdiness quotient and quality index.Analysis of the data revealed that the same root:shoot ratio (0.73) was observed in both growing environments. Similarly, the values of sturdiness quotient in open conditions (5.63) and shade conditions (5.51) were statistically non significant. The quality index's value was similar in both open and shade (0.07). Hence the effect of growing environmental conditions on quality index was also found to be statistically non significant.

4.4 INTERACTION EFFECT

Interaction effects between the seed source and pre-sowing seed treatments; seed source and growing environment and pre-sowing seed treatment and growing environment were calculated to determine which seed source with which pre-sowing seed treatment performs better under which environment.

Data from table 4.1 specify that the interaction effect between the seed source and pre sowing seed treatments, seed source and growing environment and pre-sowing seed treatment and growing environment were found to be non significant for the all germination parameter viz. days taken to complete germination, germination percentage, germination energy and germination value.

It was also seen from the data presented in tables 4.2, 4.3, 4.4 and 4.5 that interaction between the seed source and pre-sowing seed treatments, seed source and growing environment and pre-sowing seed treatment and growing environment were found to be non significant for the all growth parameter viz. plant height, collar diameter, root length, number of leaves, root and shoot fresh weight and root and shoot dry weight.

Similarly, the data from table 4.6 verified that interaction between the seed source and pre-sowing seed treatments, seed source and growing environment and pre-sowing seed treatment and growing environment were statistically non significant for the all quality parameters viz. root:shoot ratio, sturdiness quotient and quality index of the seedlings of *Santalum album.*

CHAPTER-V

SUMMARY

The *Santalum album* belongs to family *'Santalaceae'* that is represented by 1000 species throughout the world. The genus *Santalum* consists of 18 species, out of which Indian sandalwood (*Santalum album*) and Australian sandalwood (*Santalum spicatum*) are of commercial importance. It is commonly known as, dollar earning parasite and queen of essential oils. In India, it is grown extensively in deciduous forests of peninsular region on government and private lands. It is considered as the most valuable and worthy species among the forest trees of India and considered as prize gift to Indian culture and heritage. Sandalwood trees, are primary source of derived oils and fragrant heartwood, which is preferred in cosmetics, perfumes and medicines. For oil extraction, the trees are always harvested by uprooting because oil is present in the roots and heartwood. Management of nursery is important for healthy and successful plantations that helps to propagate vigorous seedling. In advance forestry practices, it is necessary to produce quality seedlings stock by inducing morphological changes in the plants so that they become capable to bear the shock of field and improve their productivity. Early survival and growth of seedlings are significant feature of forest management to ensure rapid establishment of tree species. Application of modern and improved nursery techniques for producing quality seedlings has emphasized in recent years.

Because of ample potential of *Santalum album* L. for afforestation, agroforestry interface, diversification of farming system, to meet the increasing demand of derived oils and fragrant heartwood and at the same time trying to achieve realization of its multiple uses, the seedling performance vis-a-vis growth of seedlings of *S. album* under Punjab conditions is not known. The present study was an attempt to identify the best pre sowing treatment, seed source and growing conditions of *S.album* under nursery conditions as an early indicator before investing into plantations. Therefore, the present investigation entitled, "Studies on seed germination behavior of *Santalum album* L. under nursery conditions" was undertaken to evaluate the different seed germination parameters and seedling growth parameters with different seed sources, pre-sowing seed treatments and growing environments.

To meet the aims of present studies, the present research was conducted in the research area of the Department of Forestry and Natural Resources, Punjab Agricultural University, Ludhiana during the year 2019. The research area is located at 247 m above mean sea level and lies at $75^{o}40`$ E longitude and $30^{o}$ 45` N latitude. The entire study consisted of five pre-sowing treatments viz. cold water 48 hours, GA_3 (0.05 % for 24 hours), GA_3 (0.5 % for 24 hours), H_2SO_4 (10 % for 5 min) and H_2SO_4 (10 % for 1 min) with two seed sources i.e. KFRI Kerala and UHF Neri, Hamirpur, Himachal Pradesh and two growing environments i.e.

open and shade conditions. Completely Randomised Design (CRD) with four replications was used to conduct the experiments. The observations were recorded on germination parameters like days for complete germination, germination per cent, germination energy and germination value; seedling growth parameters like plant height, collar diameter, number of leaves, root length, root and shoot fresh weight and root and shoot dry weight and seedling quality parameters like root:shoot ratio, sturdiness quotient and quality index. The observations for seed germination parameters were documented till the completion of seed germination. Whereas, seedling growth parameters were recorded at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165 and 180 days after transplanting (DAT).

5.1 EFFECT OF SEED SOURCE

It is evident from the data that all the germination parameters like days taken to complete germination, germination percentage, germination energy and germination value were statistically non significant among the tested seed sources. Data on days taken to complete germination revealed that the seeds of both sources i.e. UHF (54.77) and KFRI (54.78) germinate in almost in same duration. Sandal seeds attained maximum germination at 60 days after sowing. At 60 days after sowing both the seed sources i.e. UHF (18.95) and KFRI (18.52) showed similar germination percentage. Both seed sources i.e. UHF and KFRI produced similar performance for germination energy and germination value. No significant differences were observed among the statistical values of these traits.

Both the seed sources i.e. UHF (8.27 cm) and KFRI (8.04 cm) showed significant difference only at 45 days after transplanting. Whereas, plant height of both seed sources i.e. UHF (23.64 cm) and KFRI (23.77 cm) was almost statistically similar at 180 days after transplanting. At 180 days after transplanting, collar diameter in seeds of KFRI source was 4.36 mm and in UHF source was 4.28 mm. Maximum number of leaves at 180 days after transplanting were obtained in seeds from UHF (18.05) and minimum number of leaves (17.10) were recorded in seeds from KFRI source. The variation present among both seed sources for root length was found to be statistically non significant. The value of root length of UHF source was 14.89 cm whereas in the seeds of KFRI source, it was 14.99 cm. For root and shoot fresh weight, higher value i.e. 2.02 g was recorded in the seeds of UHF source in comparison to KFRI source (1.98 g). The root and shoot dry weight produced almost similar values in both seed sources i.e. UHF (0.55 g) and KFRI (0.54 g).

For seedling quality, three parameters were studied. Maximum value for root shoot ratio (0.74) was recorded in seeds from UHF source and minimum value for root shoot ratio (0.72) was noticed in seeds from KFRI source and the difference was statistically significant. Variation in the values of sturdiness quotient was non significant and higher value of 5.62 was obtained in seeds of UHF and lower value (5.52) in seeds from KFRI source. Whereas, the quality index of both seed sources was same i.e. 0.07.

5.2 EFFECT OF PRE-SOWING SEED TREATMENTS

It is evident from the data that different pre sowing treatments had significant effect on all germination parameters viz. days taken to complete germination, germination percentage, germination energy and germination value. Minimum germination period (54.14) days was taken for complete germination by the seeds of treatment P_4 i.e. H_2SO_4 (10% for 1 min) and maximum days (55.18) were taken by the seeds of treatment P_3 i.e. GA_3 (0.5 % for 24 hours). The seed treated with GA_3 0.05 % for 24 hours recorded higher germination percentage at 60 days after sowing i.e. 20.74 per cent whereas, minimum seed germination percentage was recorded in seed treated with H_2SO_4 10% for 1 min i.e. 17.09 per cent. Maximum germination energy i.e. 34.56 per cent was noticed in the seeds treated with GA_3 0.05 % for 24 hours and minimum germination energy (28.49 %) was seen in seed treated with H_2SO_4 (10% for 1 min). Meanwhile maximum germination value (0.29) observed in GA_3 0.5 % for 24 hours and minimum germination value (0.11) was recorded in seed treated with H_2SO_4 (10% for 1 min).

Data of growth parameters revealed that statistically maximum plant height of 25.13 cm was observed in seeds treated with H_2SO_4 (10% for 5 min) at 180 days after transplanting. Minimum plant height (22.63 cm) was observed in seed treated with GA_3 0.5 per cent for 24 hours. Different seed treatments had significant effect on collar diameter. At 180 days after transplanting, maximum collar diameter (4.43 mm) was recorded in seeds treated with H_2SO_4 (10% for 5 min) and statistically minimum collar diameter (4.12 mm) was observed in seed treated with GA_3 0.5 % for 24 hours. Maximum number of leaves (18.43) at 180 days after transplanting were recorded in seeds primed with H_2SO_4 (10% for 5 min) and statistically minimum number of leaves (16.97) were observed in treatment P_1 (cold water 48 hours).

Seedling root length revealed that the influence of different sowing treatments varied significantly. Maximum seedling root length (15.56 cm) was found in seeds primed with H_2SO_4 (10% for 5 min) and the lowest values (14.53 cm) of root length was noticed in seeds treated with cold water for 48 hours. Maximum root and shoot fresh weight (2.13 g) was noticed in the seeds treated with H_2SO_4 (10% for 5 min) and minimum root and shoot fresh weight (1.88 g) was found in seed primed with GA_3 (0.5 % for 24 hours). In the same trend as that of root and shoot fresh weight the values for root and shoot dry weight were noticed i.e. higher in seeds pimed with H_2SO_4 (10% for 5 min) and minimum in seed primed with GA_3 0.5 % for 24 hours.

The values for root:shoot ratio, sturdiness quotient and quality index were found to be significant among the different pre-sowing seed treatments. Seeds treated with H_2SO_4 (10% for 5 min) has minimum root:shoot ratio i.e. (0.70) than in seeds which are primed with GA_3 (0.5 % for 24 hours). Maximum sturdiness quotient (5.73) was noticed in seeds treated with H_2SO_4 (10% for 5 min) and minimum sturdiness quotient (5.28) was observed in seeds primed

with H_2SO_4 (10% for 1 min). Superior quality index (0.08) was noticed in both treatments in which seeds treated with H_2SO_4 as compared to all other priming treatments.

5.3 EFFECT OF GROWING ENVIRONMENT

Growing environments did not have significant influence on different seed germination parameters like days taken to complete germination, germination percentage, germination energy and germination value. Data revealed that 54.73 days were taken for complete germination by the seeds grown under shade conditions and maximum days i.e. 54.82 were taken by the seeds grown in open conditions and there was no statistically significant difference in readings recorded under both growing environments. At 60 days after sowing, the germination of 18.80 per cent was recorded under shade and 18.67 per cent obtained in seeds grown in open and such values were almost similar. In same trend, germination energy (31.33%) in shade and (31.12%) in open conditions did not varied significantly. It was also apparent from the data that higher germination values were noticed under shade conditions than open growing conditions but with non significant differences.

Maximum plant height i.e. 23.85 cm was recorded in open conditions and minimum plant height 23.55 cm in the seeds grown under shade conditions at 180 days after transplanting. For collar diameter, similar values were obtained in both open and shade conditions at 180 days after transplanting i.e. 4.32 mm. Number of leaves were more (17.82) in plants grown in open conditions from the number of leaves (17.33) in plants grown under shade conditions but the difference was statistically non significant. Root length 14.97 cm in open and 14.92 cm in shade also showed non significant differences under both growing environmental conditions. Similarly, for root and shoot fresh weight, statistically similar values were obtained under both growing conditions. Data of root and shoot dry weight also did not varied significantly i.e. shade 0.55 g and open 0.54 g.

Growing environments did not exert any significant influence on root:shoot ratio, sturdiness quotient and quality index in the present study. Root:shoot ratio i.e. 0.73 was recorded in both growing environments. Similarly, the values of sturdiness quotient in open conditions (5.63) and shade conditions (5.51) were found to be non significant. While the values of quality index were similar under both open and shade i.e. 0.07.

5.4 INTERACTION EFFECTS

Interactions between the seed source and pre-sowing seed treatments; seed source and growing environment and pre-sowing seed treatment and growing environment were calculated to determine which seed source with which pre sowing seed treatment performs better in which environment.

The interaction effect of seed source and pre-sowing seed treatments; seed sources and growing environment and pre-sowing seed treatments and growing environment were found to be statistically non significant for the all germination parameters viz. days taken to

complete germination, germination percentage, germination energy and germination value.

Similarly, the interactions between the seed source and pre-sowing seed treatments; seed source and growing environment and pre-sowing seed treatments and growing environment were found to be statistically non significant for the all growth parameters viz. plant height, collar diameter, number of leaves, root length, root and shoot fresh weight and root and shoot dry weight.

Effects of interactions between the seed source and pre-sowing seed treatments; seed source and growing environment and pre-sowing seed treatment and growing environment were found to be non significant for the all quality parameters viz. root:shoot ratio, sturdiness quotient and quality index.

CONCLUSION

The following broad conclusions can be drawn from the present studies.

1. It is concluded that the pre-sowing seed treatments had the significant effect on all the germination, growth and quality parameters. Treatment of seeds with GA_3 0.5 % for 24 hours has shown the best performance for seed germination parameters, but treating the seeds with H_2SO_4 (10% for 5 min) gave better results for growth and quality parameters.
2. Seeds treated with cold water for 48 hours performed poorly among all pre-sowing seed treatments and had almost lowest values for germination, seedling growth and quality parameters.
3. Seed sources had no significant effect on all the germination, growth and quality parameters except number of leaves and root shoot ratio.
4. Growing environments also had no significant effect on all germination, growth and quality parameters.
5. All the interactions between the seed source and pre-sowing seed treatments; seed source and growing environment and pre-sowing seed treatments and growing environment were found to be statistically non significant for the all germination, growth and quality parameters.

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