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Effect of Pre-Sowing Treatments on Seed Germination and Seedling Qualities of Sandalwood (*Santalum album* L.)

M. Shankar and A. S. Devakumar
Department of Forestry and Environmental Science, College of Agriculture, UAS, GKVK, Bengaluru - 560 065
E-mail: shankarkakching@gmail.com

Abstract

In the study, seed germination and seedling qualities of *Santalum album* (Sandalwood) was conducted in order to assess the germination of different seed sources and pre-sowing treatments. In this experiment seeds of sandalwood were collected from three different locations and subjected to two different concentrations of gibberelliic acid (GA_3) *i.e.,* 100 ppm and 500 ppm for 24 hours. Germination related parameters such as germination percentage, mean daily germination, peak value, germination value varied significantly compared to control. Quality seedling characters such as seedling height and seedling vigour index also shown the highest values in seed treated with 500 ppm GA_3 for 24 hours. Among the three seed sources, the highest seed germination of 67 per cent was recorded in the seeds which were treated with 500 ppm GA_3 for 24 hours, collected from Marayoor, Idukki District, Kerala and the lowest of 6 per cent was recorded in control seed collected from UAS, GKVK, Bengaluru. This experiment indicates that the seeds from Marayoor sandal division, Idukki district, Kerala is the best source for seed germination and seedling qualities and GA_3 500 ppm for 24 hours is the best pre-sowing treatment to obtain maximum planting material.

Keywords: Santalum album, Gibberellic acid, Germination rate, Mean daily germination, Seedling vigour index

Sandalwood is the fragrant heartwood of some species of genus *Santalum*. The widely distributed and economically important *Santalum* genus belonging to the family Santalaceae which is being completely or partially parasitic. Sandal is an evergreen large shrubs or small trees growing upto 20 m in height. In India, *S. album* is mainly distributed in the Deccan Plateau. The total extent of its distribution is around 9600 km^2 of which 8200 km^2 is in the states of Karnataka and Tamil Nadu (Srinivasan *et al*., 1992). Ecologically sandal has adapted to various agro-climatic and soil conditions for *in situ* regeneration with an exception of waterlogged areas and very cold places. It is grown predominantly in laterite soil having rainfall ranging from 1200 mm to 1600 mm with maximum temperature of 45°C and minimum temperature 7°C. Sandalwood trees are famous and very costly because of its fragrant heartwood and oil. The sandal area is declining drastically due to over-exploitation, poor seed germination, poor regeneration and failure of artificial regeneration. Extensive extraction of heartwood has severely decimated the natural stands of the trees in forests and has rendered many populations fragmented (Rao *et. al*., 2007) In order to cater the global demand, afforestation programmes need to be hastened, for which that quality planting stock production is of prime importance. The major problem that can be addressed during planting stock production in nurseries is the low seed germination. Sandal seeds are reported to have post drop dormancy of 50-60 days due to impermeable outer covering. Germination of sandal is found to be sporadic and takes 4-12 weeks to complete germination. Various pre-sowing treatments are tried in sandal to obtain quick and maximum germination. Scarification and soaking in gibberellic acid were found useful. Thus, in the present study pre-soaking of GA_3 was tried to see its effect on germination of seeds from different locations.

Material and Methods

Seed collection

The seeds were collected from three different locations *viz*., University of Agricultural Science, GKVK,

TABLE 1

Geographical information of seed source locations of *Santalum album*

Location	Latitude and longitude	Altitude	Annual rainfall	Temperature Maximum and minimum	Soil type
UAS, GKVK Bengaluru	13.0767°N N, 77.5776° E	924 m	528-1374 mm	7.8 °C- 38.9 °C	Red lateritic andfine loamy to Clayey soils.
IWST, Bengaluru	13.0112° N, 77.5702° E	900 m	528-1374 mm	7.8 °C- 38.9 °C	Red lateritic andfine loamy to clayey soils.
Marayoor, Sandal Division, Kerala	10° 16 29° N, 77° 9 361° E	500 m-2300 m	2500 mm-4250mm	36 °C-20 °C	Forest loam with a high organic matter content

Bengaluru, Karnataka, IWST, Bengaluru, Karnataka and Marayoor Sandal Division, Idukki District, Kerala during the month of October-November 2016. After the collection, fruits were soaked in sterile water for thirty minutes then depulped and shade dried for five days.

Sieved river sand was filled in a plastic trays. Separate trays were used for germinating seeds from different location.

Pretreatment and experimental design: Seeds were soaked in water (Control) and 100 ppm and 500 ppm of GA_3 for 24 hours collected from three different location *viz.,* L1 (UAS, GKVK Bengaluru), L2 (IWST, Bengaluru) and L3 (Marayoor, Idukki District, Kerala) and sown separately in a randomized manner in three replications and 100 seeds per replicate. Factorial Complete Random Design (FCRD) with three replications was used for the experiment. The treatment combination consisted of two factors, *viz.,* seed source/location and pre-sowing treatment of seeds. There were three pre-sowing methods in this experiment. In total, there were nine treatment combinations denoted as: T1: S1L1, S2L1, S3L1; T2: S1L2, S2L2, S3L2; T3: S1L3, S2L3, S3L3 (Table 2). The germination tray beds were maintained regularly with watering and treated with fungicide to avoid fungal attack.

Germination parameters: Different types of germination parameters *viz*., percentage of germination, mean daily germination, peak value of germination, germination value, root length, shoot length, seedling height and seedling vigour index were used to assess the germination characters.

TABLE 2

Treatment details

Location	Treatments
L1 (UAS, GKVK, Bengaluru)	S1 (Control)
	S2 (GA_3 @ 100 ppm for 24 hours)
	S3 (GA_3 @ 500 ppm for 24 hours)
L2 (IWST, Bengaluru)	S1 (Control)
	S2 (GA_3 @ 100 ppm for 24 hours)
	S3 (GA_3 @ 500 ppm for 24 hours)
L3 (Marayoor, Idukki district, Kerala)	S1 (Control)
	S2 (GA_3 @ 100 ppm for 24 hours)
	S3 (GA_3 @ 500 ppm for 24 hours)

i) Germination percentage: Germination test was carried out by adopting sand media germination method as per the procedures of ISTA (Anonymous, 1996). The number of normal seedlings was counted on the 60th day (final count) of germination from all the replications. The average of three replications was expressed as germination percentage.

$$\text{Germination (\%)} = \frac{\text{Number of seed/s germinated}}{\text{Total no. of seed sown}} \times 100$$

ii) Mean daily germination: Mean daily germination is estimated by adopting formula as prescribed by ISTA (Anon., 1996).

$$\text{Mean daily germination (\%)} = \frac{\text{Cumulative germination per cent}}{\text{Total number of days}}$$

iii) Peak value of germination: Peak value of germination is maximum mean daily germination reached at any stage of germination period which is calculated by following formula :

$$\text{Peak value (PV) of germination} = \frac{\text{Total germination per cent}}{\text{Number of days required to reach the peak germination}}$$

iv) Germination value: It is a composite value that combines both germination speed and total germination provides an objective means of evaluating the result of germination test. Total germination is calculated as the cumulative percentage of full seed germination at the end of the test. Germination value (GV) was then calculated from the formula given by Czabator (1962).

Germination value (GV) = Mean germination value X peak value

v) Seedling height: The height of the seedlings was measured from collar region to the apex in ten randomly selected normal seedlings at the end of germination period and the mean was recorded as shoot length in centimeters.

vi) Seedling vigour index: It is calculated by adopting the method suggested by Abdul Baki and Anderson (1973) and expressed as number.

Seedling vigour index = Germination per cent x Seedling height.

Data analysis

One way analysis of variance was carried out in SPSS 17 for windows to find the difference seedling attributes due to seed pretreatments. Duncan's Multiple Range Test was conducted to compare the treatment means.

Results and Discussion

Effects of variati]on of seed sources on seed germination

Germination started somewhat earlier from the seed originating from the L3 than L2 and L1 (Table 3). The germination stopped later in L2 than L1. In all treatments, germination completed by 60 days after sowing of seeds in tray with sand media. The highest germination success was found at 67 per cent in L3 and the lowest was 6 per cent in L1 (Table 3).

Effects of pre-sowing treatments on seed germination

Germination is advanced for the seeds soaked in 500 ppm GA_3 for 24 hours (S3) followed by seed soaked in 100 ppm GA_3 for 24 hours (S2) than control (S1) in all three different seed sources (Table 3). The seed germination percentage were highly significant different in 500 ppm GA_3 treatment (S3) in allocation L1, L2 and L3. The highest seed germination percentage was found at 67 per cent in 500 ppm GA_3 for 24 hours (S3) in L3 followed by 56 per cent at 500 ppm GA_3 for 24 hours (S3) in L2. The lowest seed

Table 3

Effect of various pre-sowing treatments and seed sources on germination parameters and seedling qualities in *Santalum album* L.

Seed sources	Treatments	Germination percentage	Shoot length (cm)	Root length (cm)	Seedling length (cm)
L1	S1	6	6.30	5.60	11.70
	S2	10	9.73	5.13	14.87
	S3	12	8.77	5.82	14.60
L2	S1	23	9.70	5.77	15.47
	S2	34	11.17	6.17	17.33
	S3	56	12.13	6.85	19.40
L3	S1	30	10.98	6.53	17.53
	S2	47	11.27	6.80	18.07
	S3	67	12.93	7.97	21.07
	SEm±	1.25	0.52	0.23	0.71
	CD @ 5%	3.75	1.56	0.68	2.08

germination percentage of 6 percent (S1) was recorded in control (S1) followed by 10 per cent at seed soaking with 100 ppm GA_3 (S2) both in L1 location. There was non-significant difference at treatment S2 and S3 in L1 location (Table 3).

The data on mean daily germination, peak value and germination value were influenced by different pre-sowing treatments with different locations. The highest mean daily germination (1.111), peak value (1.429) and germination value (1.586) were recorded in seeds soaked with 500 ppm GA_3 for 24 hours (S3) in location L3, followed by mean daily germination (0.777), peak value (1.102) and germination value (0.856) in seed soaked with 100 ppm GA_3 for 24 hours (S2) in location L2. The lowest mean daily germination (0.100), peak value (0.158) and germination value (0.016) were recorded in Control (S1) at location L1 (Fig.1; Fig.2 and Fig.3).

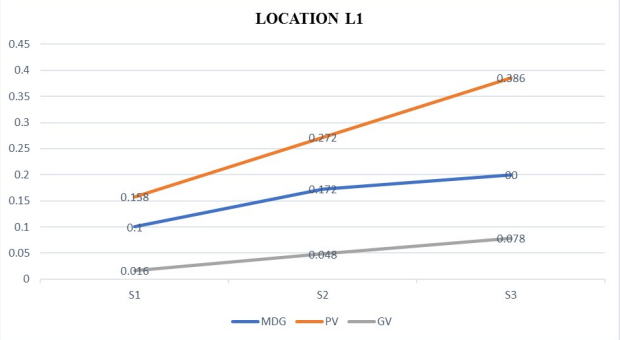

Fig. 1: The Mean daily germination (MDG), Peak value (PV) and Germination value (GV) of Santalum album seeds for location L1.

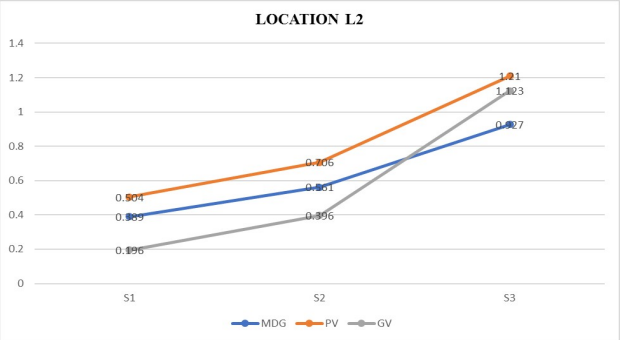

Fig. 2: The Mean daily germination (MDG), Peak value (PV) and Germination value (GV) of Santalum album seeds for location L2.

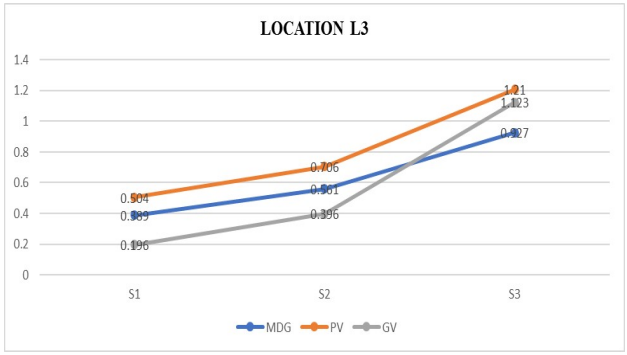

Fig. 3: The Mean daily germination (MDG), Peak value (PV) and Germination value (GV) of Santalum album seeds for location L3.

Pre-sowing treatments and different seed sources exhibited significant variation in shoot length, root length and seedling height of *Santalum album* seedlings. Higher shoot length (12.93 cm), root length (7.97 cm) and seedling height (21.07 cm) were found in seeds soaked with 500 ppm GA_3 for 24 hours (S3) in location L3, followed by shoot length (12.13 cm), root length (6.85 cm) and seedling height (19.40 cm) in seeds soaked with 100 ppm GA_3 for 24 hours (S3) in location L2. The lowest shoot length (6.30 cm) and seedling height (11.70 cm) were found in control (S1) in location L1 but the lowest root length (5.13 cm) was found in seeds soaked with 100 ppm GA_3 for 24 hours (S2) in Location L1. There were no significant difference in shoot length between the treatments of S2 and S3 in location L1, S2 and S3 in location L2 (Table 3).

There were significant differences observed due to different pre-sowing treatments and different seed sources in seedling vigour index value. Seeds treated with 500 ppm GA_3 for 24 hours (S3) in location L3 recorded the highest seedling vigour index value (1406) followed by seeds soaked with 500 ppm GA_3 for 24 hours in location L2 (1081). The lowest seedling vigour index value (71) was recorded in control (S1) at location L1. Statistically treatments S1, S2 and S3 in location L1 were on par with each other (Fig.4).

Seed dormancy is the major riddle in the planting stock production of tree species. Various pre-sowing treatments are tried on seeds all over the world to get quick and uniform germination. There are many reports on seed dormancy and pre-sowing treatments in

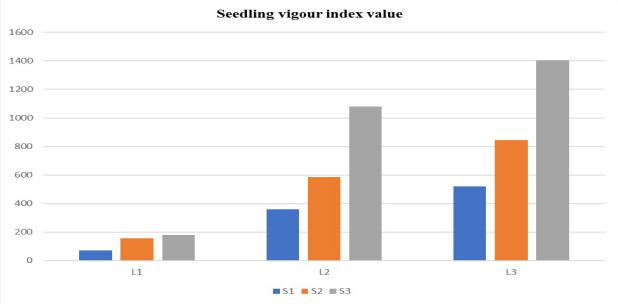

Fig.4: Effect of various pre-sowing treatments and different seed sources on Seedling vigour index values of Santalum album seedlings

sandal. In sandal, the stony endocarp, although not to be called seed coat, is referred to as seed coat literally, though it is a false seed coat causes dormancy.

In respect of geographical factors in locations the seed sources exhibited variability. As the seeds were freshly harvested and experimented within a month the viability factors did not effect on germination parameters in our experiment. Similar results have been reported by Brand *et al*. (2006). The variability of seed germination depends on microclimate, local environmental conditions and plant population in the growing area. It also depends on the age of mother tree and its habitat which may influence in the production of fertile seeds. The time taken for the initiation of germination is largely influenced by pretreatment of seeds. The time taken for the completion of germination is also variable in different cases as observed by Khera *et.al.* (2008). The other germination parameters showed remarkable difference, suggesting the locational potentiality supported by agro-climatic and edaphic factors.

In sandal, freshly collected seeds show dormancy for many months. It is likely that the enforced dormancy of seed is due to the presence of chemical substances in the seed coat which are impervious to water and gases (Das and Tah, 2013). In the present investigation, the treatment with pretreatment with GA_3 emerged as the best one as proven by earlier workers irrespective of the seed source location. Seed germination is promoted by gibberellin (GA) in many plant species. Abscisic Acid (ABA) is an example of a hormone (endogenous), which inhibits seed germination, while gibberellic acid (GA_3) is known to promote seed germination (Rehman and Park, 2010). Several GA signalling factors are known to induce the expression of genes encoding enzymes that mobilise food reserves, including starches, proteins and lipids, stored in the endosperm during seed germination (Peng and Harberd, 2008). Sutheesh *et.al.* (2016) also reported that the *Santalum album* seeds soaked in GA_3 in 500 mg $^{-1}$ GA_3 for 24 hrs. increased germination speed, germination percentage and seedling growth in comparison to the control treatments.

The present study indicates that the seeds from Marayoor Sandal Division, Idukki District, Kerala performed best in terms of seed germination and seedling qualities when subjected to the pre-sowing treatment of 500 ppm GA_3 for 24 hours. The location of IWST, Bengaluru also performed well. The pre-sowing treatment was also found to influence the seedling growth. The treatment with 500 ppm GA_3 for 24 hours proved to be the best pretreatment as compared to 100 ppm in *Santalum album* seeds. Thus seeds from Marayoor, Idukki, District, Kerala source can be used to fulfill the need of seed germination programme to raise effective quality seedling stock for sandal tree improvement and afforestration programme.

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