

Original Research Article

Effect of bio-regulators on seed germination of *Santalum album* L. under laboratory conditions.

Abstract

The present investigation entitled “Studies on natural regeneration of *Santalum album* L. in low hills of Himachal Pradesh ” was carried out during the year 2020-2022 in the Department of Silviculture and Agroforestry, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan (H.P). The interaction effect of seed collection time (S), locations (L) and pre-sowing seed treatments (T) revealed that the seeds collected during September-October from Dholra (Bilaspur) site; treated with GA₃ 400ppm (S₁L₂T₅) showed maximum germination percentage (75.00%), germination capacity (80.56%), germination energy (44.44%), mean daily germination (2.50) and peak value (7.27).

Introduction

Tree evergreen in nature, 10-15 m height and 1-2 m girth at full maturity when it reaches the age of 60-80 years (Ghosh *et al.*, 1985; Jain *et al.*, 1999). On ideal sites, the tree attains a height of 18 m and a girth of 2 to 4 m. Tree flourishes well above sea level up to 1200 m altitude with different soil types and varying climatic conditions. The bark is reddish-brown to dark brown, smooth in young trees, rough with deep vertical fissures in matures tree. Leaves are opposite, decussate, flowers unscented, straw yellow coloured turning to deep purplish-brown on maturation and occurs in axillary or terminal cymose panicles. Flowering generally occurs twice a year from March to May and September to December. The fruit is a single-seeded succulent drupe, purplish-black when mature.

Distribution of the sandalwood is between 30°N and 40°S from Indonesia in West to Juan Fernandez Island in the north to New Zealand in the South. The species is mainly found in India, Indonesia and Australia. In India, its distribution is mostly in the deciduous forests of the Deccan region of Peninsular India and is found mainly growing naturally in the states of Karnataka and

Tamil Nadu. In Himachal Pradesh, the sandalwood is found growing naturally in districts of Bilaspur, Kangra and Sirmour.

Sandalwood belongs to family Santalaceae, native to India. It is strongly associated with Indian religion and culture (Arun Kumar *et al.* 2016). It grows well in a wide range of soils and climatic conditions of tropical and subtropical areas of India. India is the leading producer of sandalwood oil for perfumery and pharmaceutical industries. The active ingredients present in sandalwood oil are α Santalol and β Santalol. The β Santalol is responsible for 90% of its characteristic odoriferous nature. The value of sandalwood oil is high where the β -santalol content is high in the oil According to IUCN 2000, considered as vulnerable species in nature.

Apart from its medicinal uses its wood is also used for making wood carving articles. Many items like rosary from seeds, soaps, perfumes, incense stick and powder etc. It has an important place in Hindu religious ceremonies. In India, the natural plantations of sandalwood is gradually diminishing due to changes in the land use pattern, habitat destruction, illegal felling and theft of plantations (Arun Kumar *et al.*,2012). Seed dormancy is the most limiting factor for seed germination and thus it causes prolongation in the seed germination. The seeds of sandalwood are impermeable to water and oxygen thus inhibits the germination of the seed. Various other factors also cause seed dormancy like impermeable seed coats, mechanically resistant seed coats, rudimentary and physiological immature embryo, morphologically mature and physiological immature embryos (Das and Tah, 2013). Hence, there is a need to enhance the seed germination by breaking the seed dormancy through various pre-sowing seed treatments. Hence, the present experiment has been taken up to study bio-regulators on seed germination of *Santalum album* L. under laboratory conditions to break the seed dormancy and ensure superior quality seedlings, and supply of quality planting materials to promote the cultivation of sandalwood to meet the demand from the industry.

Material and Methods

The present investigation in the department of Silviculture and Agroforestry, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni (Solan), Himachal Pradesh during 2019-2020 and 2020-2021.

Location of seed source: Three

L₁: Jawalaji (Kangra)

L₂: Dholra (Bilaspur)

L₃: Dhaulakuan (Sirmour)

Seed Collection time: Two

S₁: September - October

S₂: March - April

Plant bio-regulator: Gibberellin

Gibberellin concentrations used: Five including control

T₁: control

T₂: 100ppm

T₃: 200ppm

T₄: 300ppm

T₅: 400ppm

Number of treatment/combination (3x2x5) : 30

Replication : 3

Design : Complete Randomized Design (Factorial)

Sowing date : 6th February 2021 and 14th May 2021

Experimental Methodology:

The seeds were collected from three different sites *viz*; Jawalaji (Kangra), Dholra (Bilaspur) and (Dhaulakuan) Sirmour during the month of October 2020 and April 2021. The sites varying at an altitude ranging from 380-850m above mean sea level. The collected seeds were subjected to the drying under shade for 25 days and all the foreign matter from the seeds was removed.

The seeds of each category were treated with pre-sowing seed treatments mentioned as above. The seeds were soaked for 24 hours in respective Gibberellic acid (GA₃) solution. After pre-sowing seed treatments, all the seeds were treated with 1.00 per cent Dithane M-45 to prevent from fungal attack. Thereafter the seeds (12 numbers) were placed in the each petriplates and the petriplates were kept in the germinator. During the experimental period, the temperature

and the humidity was maintained at 25°C and 85-100 per cent respectively. The seeds were watered at regular intervals and were regularly examined for germination.

The experiments were conducted in the laboratory of the Department of Silviculture and Agroforestry, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan located at 30° 5' N latitude and 76° 11' E longitude.

Observations recorded:

Germination percent (%):

Germination percent (GP) was calculated as the total number of seeds germinated at the end of experiment out of total seeds sown in each treatment.

$$\frac{\text{Total number of seed germinated}}{\text{Total number of seed sown}} \times 100$$

Germination capacity (%):

Germination capacity (GC) of a seed lot was calculated as the cumulative number of seed that had germinated at the end of test period plus the number of un-germinated viable seed at the end of the test expressed in percentage (Paul, 1974).

Germination energy (%):

Germination energy (GE) was calculated on the basis of the percentage of the total number of seeds that had germinated when the germination reached its peak generally taken as the highest number of germination in 24 hours period (Czabator, 1962).

$$\frac{\text{Number of seed germinated upto the time of peak germination}}{\text{Total number of seed sown}} \times 100$$

Mean daily germination:

Mean daily germination (MDG) was calculated as the cumulative percentage of full seed germination at the end of the test divided by the number of days taken from sowing to the end of the test or total percentage germination divided by total days in the test gives the mean daily germination (Paul, 1974).

Peak value:

The maximum mean daily germination reached at any time during the period of test gives the peak value (Paul, 1974).

STATISTICAL ANALYSIS

The data generated from the present investigation were subjected to statistical analysis in accordance with the procedure outlined by Panse and Sukhatme (2000).

Result and discussion

The data presented in Table 1 revealed significant interaction effect of collection time (S), location (L), pre-sowing seed treatments (T). The maximum germination percentage (75.00%) was observed in seeds collected during September-October from Dholra (Bilaspur) site, treated with GA₃ 400ppm (S₁L₂T₅) while minimum (30.56%) in seeds collected during September-October from Dhaulakuan (Sirmour) site under control (S₁L₃T₁). The maximum germination capacity (80.56%) was observed when the seeds were collected during September-October from Dholra (Bilaspur) site, treated with GA₃ 400ppm (S₁L₂T₅) followed by S₁L₁T₅, S₁L₃T₅ and S₂L₁T₄ whereas minimum (36.11%) in seeds collected during March-April from Jawalaji (Kangra) and Dhaulakuan (Sirmour) site with control (S₂L₁T₁ and S₂L₃T₁) followed by S₂L₁T₁, S₁L₁T₁ and S₂L₂T₁. The interaction effect of seed collection time (S), location (L), pre-sowing seed treatments (T) were observed significant on germination capacity (Table 2).

Table 1: Interaction effect of seed collection time (S), locations (L) and pre-sowing seed treatments (T) on germination percent of *Santalum album* L. under laboratory condition

Treatments	S ₁ (September-October)			Mean	S ₂ (March-April)			Mean
	L ₁ (Jawalaji)	L ₂ (Dholra)	L ₃ (Dhaulakuan)		L ₁ (Jawalaji)	L ₂ (Dholra)	L ₃ (Dhaulakuan)	
T ₁ (control)	36.11 (36.92)	41.67 (40.19)	30.56 (33.54)	36.11 (36.88)	33.33 (35.23)	38.89 (38.56)	33.33 (35.25)	35.18 (36.35)
T ₂ (GA ₃ 100ppm)	52.78 (46.57)	58.33 (49.78)	38.89 (38.57)	50.00 (44.97)	41.67 (40.19)	36.11 (36.92)	36.11 (36.92)	37.96 (38.01)
T ₃ (GA ₃ 200ppm)	50.00 (44.98)	63.89 (53.05)	41.67 (40.19)	51.85 (46.07)	44.45 (41.79)	47.22 (43.39)	33.33 (35.25)	41.67 (40.14)
T ₄ (GA ₃ 300ppm)	66.67 (54.72)	66.67 (54.72)	63.89 (53.04)	65.74 (54.16)	72.22 (58.17)	58.33 (49.78)	61.11 (51.40)	63.89 (53.12)
T ₅ (GA ₃ 400ppm)	72.22 (58.18)	75.00 (59.98)	66.67 (54.72)	71.30 (57.62)	69.45 (56.43)	66.67 (54.71)	58.33 (49.78)	64.82 (53.64)
Mean	55.56 (48.27)	61.11 (51.54)	48.33 (44.01)	55.00 (47.94)	52.22 (46.37)	49.44 (44.67)	44.44 (41.72)	48.70 (44.25)
			S.E.m±		C.D _{0.05}			
Collection time (S)			0.19		0.53			
Location (L)			0.23		0.65			

Interaction S × L	0.33	0.92
Pre sowing treatments (T)	0.30	0.84
Interaction S × T	0.42	1.19
Interaction L × T	0.51	1.46
Interaction S × L × T	0.73	2.06

The values in parenthesis are transformed values (angular transformation)

Table 2: Interaction effect of seed collection time (S), locations (L) and pre-sowing seed treatments (T) on germination capacity of *Santalum album* L. under laboratory condition

Treatments	S ₁ (September-October)			Mean	S ₂ (March-April)			Mean
	L ₁ (Jawalaji)	L ₂ (Dholra)	L ₃ (Dhaulakuan)		L ₁ (Jawalaji)	L ₂ (Dholra)	L ₃ (Dhaulakuan)	
T₁ (control)	38.89 (38.56)	44.44 (41.79)	38.89 (38.56)	40.74 (39.64)	36.11 (36.92)	38.89 (38.56)	36.11 (36.92)	37.04 (37.47)
T₂ (GA₃ 100ppm)	58.34 (49.78)	66.67 (54.72)	47.22 (43.39)	57.41 (49.30)	47.22 (43.39)	44.44 (41.79)	38.89 (38.56)	43.52 (41.25)
T₃ (GA₃ 200ppm)	58.33 (49.78)	69.45 (56.44)	52.78 (46.57)	60.19 (50.93)	75.00 (60.01)	52.78 (46.57)	44.44 (41.79)	57.41 (49.46)
T₄ (GA₃ 300ppm)	75.00 (59.98)	69.44 (56.43)	63.89 (53.04)	69.44 (56.48)	77.78 (61.88)	61.11 (51.40)	69.44 (56.42)	69.44 (56.57)
T₅ (GA₃ 400ppm)	77.78 (61.85)	80.56 (63.84)	77.78 (61.85)	78.70 (62.51)	72.22 (58.19)	72.22 (58.19)	69.44 (56.42)	71.29 (57.60)
Mean	61.67 (51.99)	66.11 (54.65)	56.11 (48.68)	61.30 (51.77)	61.67 (52.08)	53.89 (47.30)	51.67 (46.02)	55.74 (48.47)
	S.E.m±				C.D_{0.05}			
Collection time (S)	0.26				0.72			
Location (L)	0.31				0.89			
Interaction S × L	0.44				1.25			
Pre sowing treatments (T)	0.40				1.14			
Interaction S × T	0.57				1.62			
Interaction L × T	0.70				1.98			
Interaction S × L × T	0.99				2.80			

The values in parenthesis are transformed values (angular transformation)

The results (Table 3) revealed maximum germination energy (44.44%) in seeds collected during September-October from Dholra (Bilaspur) site treated with GA₃ 400ppm (S₁L₂T₅) whereas, minimum germination (16.67%) was recorded in the seeds collected during March-April from Jawalaji (Kangra) and Dhaulakuan (Sirmour) site with hot water treatment (S₂L₁T₂ and S₂L₃T₂). The interaction effect of seed collection time (S), location (L) and pre-sowing seed treatments (T) were found significant on germination energy.

Table 3: Interaction effect of seed collection time (S), locations (L) and pre-sowing seed treatments (T) on germination energy of *Santalum album* L. under laboratory condition

Treatments	S ₁ (September-October)			Mean	S ₂ (March-April)			Mean
	L ₁ (Jawalaji)	L ₂ (Dholra)	L ₃ (Dhaulakuan)		L ₁ (Jawalaji)	L ₂ (Dholra)	L ₃ (Dhaulakuan)	
T ₁ (control)	19.44 (26.15)	25.00 (29.99)	22.22 (28.12)	22.22 (28.09)	19.44 (26.15)	19.45 (26.15)	19.45 (26.16)	19.45 (26.15)
T ₂ (GA ₃ 100ppm)	30.55 (33.54)	27.78 (31.79)	19.45 (25.15)	25.93 (30.50)	16.67 (24.09)	19.44 (26.15)	16.67 (24.09)	17.59 (24.78)
T ₃ (GA ₃ 200ppm)	30.56 (33.54)	30.56 (33.54)	25.00 (29.99)	28.71 (32.36)	19.44 (26.15)	22.22 (28.11)	22.22 (28.12)	21.30 (27.46)
T ₄ (GA ₃ 300ppm)	30.56 (33.54)	30.56 (33.54)	25.00 (29.99)	28.71 (32.36)	30.56 (33.54)	30.55 (33.54)	30.56 (33.54)	30.56 (33.54)
T ₅ (GA ₃ 400ppm)	41.67 (40.19)	44.44 (41.79)	36.11 (36.92)	40.74 (39.63)	30.55 (33.54)	30.56 (33.54)	27.78 (31.79)	29.63 (32.96)
Mean	30.56 (33.39)	31.67 (34.13)	25.56 (30.23)	29.26 (32.59)	23.33 (28.70)	24.44 (29.50)	23.33 (28.74)	23.70 (28.98)
			S.E.m±		C.D_{0.05}			
Collection time (S)			0.11		0.31			
Location (L)			0.13		0.38			
Interaction S × L			0.19		0.53			
Pre sowing treatments (T)			0.17		0.49			
Interaction S × T			0.24		0.69			
Interaction L × T			0.30		0.84			
Interaction S × L × T			0.42		1.19			

The values in parenthesis are transformed values (angular transformation)

The data (Table 4) inferred significant interaction effect of seed collection time (S), location (L) and pre-sowing seed treatments (T) on mean daily germination. The maximum mean daily germination (2.50) was recorded in seeds collected during September-October from Dholra (Bilaspur) site treated with GA₃ 400ppm (S₁L₂T₅) whereas, minimum (1.02) in seeds collected during September-October from Dhaulakuan (Sirmour) site with control (S₁L₃T₁).

Table 4: Interaction effect of seed collection time (S), locations (L) and pre-sowing seed treatments (T) on mean daily germination of *Santalum album* L. under laboratory condition

Treatments	S ₁ (September-October)			Mean	S ₂ (March-April)			Mean
	L ₁ (Jawalaji)	L ₂ (Dholra)	L ₃ (Dhaulakuan)		L ₁ (Jawalaji)	L ₂ (Dholra)	L ₃ (Dhaulakuan)	
T ₁ (control)	1.20	1.39	1.02	1.20	1.11	1.30	1.11	1.17
T ₂ (GA ₃ 100ppm)	1.76	1.94	1.30	1.67	1.39	1.20	1.20	1.26
T ₃ (GA ₃ 200ppm)	1.67	2.13	1.39	1.73	1.48	1.57	1.11	1.39
T ₄ (GA ₃ 300ppm)	2.22	2.22	2.13	2.19	2.41	1.94	2.04	2.13
T ₅ (GA ₃ 400ppm)	2.41	2.50	2.22	2.38	2.32	2.22	1.95	2.16
Mean	1.85	2.04	1.61	1.83	1.74	1.65	1.48	1.62
		S.E.m±			C.D _{0.05}			
Collection time (S)		0.01			0.02			
Location (L)		0.01			0.02			
Interaction S × L		0.01			0.03			
Pre sowing treatments (T)		0.01			0.03			
Interaction S × T		0.01			0.04			
Interaction L × T		0.02			0.05			
Interaction S × L × T		0.02			0.07			

The maximum peak value (7.27) was observed in seeds collected during September-October from Dholra (Bilaspur) site treated with GA₃ 400ppm (S₁L₂T₅) whereas, minimum (2.13) in seeds collected during March-April from Dhaulakuan (Sirmour) site with control (S₂L₃T₁). The results (Table 5) revealed significant interaction effect of seed collection time (S), location (L) and pre-sowing seed treatments (T) on peak value.

Table 5: Interaction effect of seed collection time (S), locations (L) and pre-sowing seed treatments (T) on peak value of *Santalum album* L. under laboratory condition

Treatments	S ₁ (September-October)			Mean	S ₂ (March-April)			Mean
	L ₁ (Jawalaji)	L ₂ (Dholra)	L ₃ (Dhaulakuan)		L ₁ (Jawalaji)	L ₂ (Dholra)	L ₃ (Dhaulakuan)	
T ₁ (control)	2.65	2.99	2.25	2.63	2.23	2.55	2.13	2.30
T ₂ (GA ₃ 100ppm)	4.42	4.51	3.00	3.98	2.99	2.47	2.59	2.68
T ₃ (GA ₃ 200ppm)	4.29	5.06	3.29	4.21	3.25	3.47	2.63	3.12
T ₄ (GA ₃ 300ppm)	5.89	5.58	5.64	5.70	5.71	4.27	4.71	4.90
T ₅ (GA ₃ 400ppm)	6.78	7.27	5.58	6.54	5.64	5.29	4.74	5.22
Mean	4.81	5.08	3.95	4.61	3.96	3.61	3.36	3.65
		S.E.m±			C.D _{0.05}			
Collection time (S)		0.04			0.12			
Location (L)		0.05			0.14			
Interaction S × L		0.07			0.20			
Pre sowing treatments (T)		0.07			0.19			
Interaction S × T		0.09			0.26			
Interaction L × T		0.11			0.32			

The maximum germination percentage (75.00%), germination capacity (80.56%), germination energy (44.44%), mean daily germination (2.50) and peak value (7.27) were observed in seeds collected during September-October from Dholra (Bilaspur) site, treated with GA₃ 400ppm (S₁L₂T₅). It was observed that the seeds collected during September-October from Dholra (Bilaspur) site, treated with GA₃ 400ppm showed significantly higher germinability. The present results are in agreement with the findings of Subasinghe *et al.*, (2014) who also reported 80.00 per cent germination when the sandalwood seeds treated with GA₃, which might be due to the effect of GA₃ in cytological activities. The cytological enzyme leads to the increase in the plasticity of the cell wall and the absorption of water. GA₃ releases the seeds from dormancy by promoting protein synthesis, elongation of coleoptiles and production of ethylene (Liu *et al.*, 2010). Jayawardena *et al.*, (2015) reported maximum germination (98.00%) in sandalwood seeds treated with GA₃ 500ppm, suggesting the GA₃ had a significant effect on breaking the seed dormancy and increase the germination of the seed to a great extent.

Similar study was conducted by Rawat *et al.*, (2006) aim to understand the role of GA₃ treatment for seed germination in different provenances of *Abies pindrow*, *Cupressus torulosa* and *Picea smithiana* in Gharwal Himalayas. In *Abies pindrow* 45.00 per cent, *Cupressus torulosa* 57.00 per cent and *Picea smithiana* 56.00 per cent germination percentage was achieved with GA₃ as compare to control. However, Das, (2014) collected the seeds of *Acacia catechu* and *Elaeocarpus floribundus* from matured and healthy trees from four different locations and treated with different pre-sowing seed treatments methods reported highest germination 91.26 per cent was found in hot water treatment (80°C for 10 minutes) treatment in *Acacia catechu* and the highest germination 89.81 per cent of *Elaeocarpus floribundus* was recorded in H₂SO₄ (80.00% for 20 minutes) treatment. Other than enhancing the germination many authors have reported the enhanced seedling growth due to pre-sowing seed treatments studied by Koirala *et al.*, (2000) in *Adenantha pavonia*, Khan *et al.*, (2001) in *Melia azedarach* and Hossain *et al.*, (2005) in *Terminalia belerica*.

References

- Arun Kumar AN, Geeta Joshi, Rekha Warriar R. Know your trees. 2016. Santalum album (Indian sandal wood). Envis Newsletter, Van Vigyan. 2(4):2-11.
- Czabator FJ. 1962. Germination value: An index combining speed and completeness of Pine seed germination. *Forest Sciences* 8: 386-396
- Das SD, Tah J. 2013. Effect of GA₃ on seed germination of sandal (*Santalum album* L.). *International Journal of Current Science.*; 8:79-84.
- Ghosh SK, Balasundaran M and Ali MIM. 1985. Studies on spike disease of Sandal. KFRI Research Report No. 37. Kerala Forest Research Institute, Peechi, Thrissur: 56pp.
- Jain SH, Angadi VG, Ravikumar G, Theagarajan KS and Shankaranarayana KH. 1999. Studies on cultivation and chemical utilization of sandal (*Santalum album* L.). *Fafai Journal* 3: 49-53.
- Khan BM, Koirala B and Hossian MK. 2001. Effect of different pre-sowing treatments on germination and seedling growth attributes in Ghora Neem (*Melia azedarach* L.). *Malaysian Forester* 64: 14-21
- Panse VG and Sukhatme PV. 2000. Statistical methods for Agricultural workers. ICAR, New Delhi: 157-165pp.
- Paul WD. 1974. Germination control of stratified sugar maple seeds. *Forest Chronicle* 50: 112-113
- Rawat BS, Sharma CM and Ghildiyal SK. 2006. Improvement of seed germination in three important conifer species by Gibberellic acid (GA₃). *Lyonia* 11: 23-30