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Effect of Hydropriming and Osmopriming on the Germination and Seedling Vigor of the East Indian Sandalwood (*Santalum album* L.)

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Abstract: The natural populations of East Indian sandalwood (*Santalum album* L.) are very confined and are facing a drastic decline over the past three decades due to overexploitation. *Santalum album* L. seeds, in general, have poor and staggered germination, which is the major constraint in raising seedlings in nursery and establishing plantations. In the present investigation, we studied the impact of hydro- and osmopriming on the germination attributes and seedling performance of *Santalum album* L. The Polyethylene glycol (PEG-6000) solutions at four concentrations of 5, 10, 15, and 20% and four durations of 2, 4, 6, and 8 days and hydropriming for the same durations were applied. Results indicated that the osmopriming of seeds at PEG solutions at 5% concentrations for 2 days recorded the highest germination (79%), which is 42% higher than the control group. Longer priming times (6–8 days) had no effect or negatively affected the germination and growth. Moreover, hydropriming had no significant impact on the germination percentage of sandalwood seeds. The positive growth after osmopriming was connected with higher β-amylase content, higher carbohydrate and fat content, and lower electrical conductance of the seeds. Osmopriming can be recommended as a suitable and low-cost technology in enhancing the seed germination and seedling growth of *Santalum album* L. to produce quality planting material. Further testing of osmoprimed seedlings under abiotic stress conditions may help to explore its possible acclimation potential for stress resistance.

Keywords: β amylase; carbohydrates; *Santalum album* L.; growth; seed germination; seed priming; vigor index

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1. Introduction

Seed priming has emerged as one of the cheapest low-risk methods and proven seed enhancement techniques adopted in crops for overcoming the germination constraints, poor seedling growth, and improper seedling establishment [1]. Priming has, thus, caught attention as a promising physiological technique, which involves control seed hydration and drying for the induction of a particular physiological state by use of different media [2], e.g., hydropriming (with water), halopriming (with salt solutions), osmopriming (with low-water-potential solutions), hormonal priming (with plant hormone solutions), nutripriming (with nutrients), and biopriming (with valuable microbes) [3]. The primed seeds normally exhibit a reduced emergence time, increased germination rate, greater germination uniformity, higher germination percentage due to different enzyme activations, metabolic activities, biochemical changes associated with cell repair, protein synthesis, and improvement of the antioxidant defense system, as compared to unprimed seeds [4,5].

Therefore, seed priming has been an emerging tool for plant stress physiology and crop stress management through these simple seed invigoration techniques [6]. The application of seed priming technologies is, moreover, time efficient and relatively inexpensive, compared to the potential benefits. Moreover, seed priming can be easily conducted by the nursery employees in standard storage conditions.

In hydropriming, the priming agent is water, and the priming duration is determined by controlling the seed imbibition [7], and the hydrated seeds are dried back to the original moisture level after a particular period of hydration under shade conditions [8]. Osmopriming is the soaking of seeds in aerated, different low-water-potential chemical solutions, e.g., polyethylene glycol (PEG), KNO_3, KCl, $MgSO_4$, $CaCl_2$, NaCl, mannitol, etc., [9]. It is a widely used commercial technique in seeds [10], used to study the transition of seeds from a dry and physiologically quiescent to a hydrated and physiologically active state [11]. The priming of seeds in osmoticums (osmopriming) and in water (hydropriming) has been reported to be a simple technique for attaining enhanced seed germination, seedling establishment, and biomass yield [12]. Priming with PEG has largely been explored as a good technique for alleviating abiotic stresses, e.g., drought and salinity stress in crop plants [13].

The seed priming approach in forest tree species for quality seedling material production is still limited. Seed priming studies of forest tree species are mostly confined to various coniferous species of pines [14,15], firs [16], alder, and birches [17], which include mainly hydropriming, osmopriming, and biopriming. *Santalum album* L. is a globally renounced tropical tree species for its fragrant heartwood, which yields the highly valuable aromatic essential oil [18–20]. The natural population of *Santalum album* L. is dwindling at an alarming rate, as an 80% reduction in its population in its natural habitat has been reported over the past three decades, leading to the categorization of the species in "Vulnerable" category of the IUCN list [21]. Raising quality seedlings in the nursery and planting is the key to achieving success in large-scale plantations [22,23]. A poor germination rate, staggered and prolonged germination period of the seeds, and a very slow rate of establishment of seedlings in the field are the major constraints in raising large-scale plantations of sandalwood [24]. This is due to the morpho-physiological dormancy possessed by the *Santalum album* L. seeds confirmed by [25]. The hard seed coat is impervious to water and gases, which causes a major reason for the poor germination of *Santalum album*. In this present study, we tried hydropriming and osmopriming with Polyethylene glycol-6000 (PEG-6000) solution to improve the germination and seedling performance of *Santalum album* L. The biochemical changes associated with priming were also studied to correlate the changes caused due to priming in the seeds. Our hypotheses were that: (i) We will observe significant differences of germination, seed characteristics, and growth parameters between control and primed treatments; (ii) Shorter priming duration will show better results (higher germination and higher growth) than longer priming duration; (iii) Osmopriming is more effective than hydropriming treatments.

2. Materials and Methods

2.1. Plant Material

The present study was carried out at the Department of Silviculture and Agroforestry, College of Forestry, Kerala Agricultural University, India. The *Santalum album* L. seeds were collected during February/March of 2022 from the Nachivayal Reserve Forest (10°25′0531″ N, 77°15′8950″ E) of Marayoor Sandal Division by the Pallanadu Vana Samrakshana Samithi, Idukki district. The seeds were globose to spherical in shape with an average weight of 0.1 g and a diameter of 0.75 cm. The collected seeds were cleaned and thoroughly mixed to improve homogeneity.

2.2. Seed Priming Treatments

The seeds were divided into lots, and the surface was sterilized in 1% mercuric chloride solution for 5 min and thoroughly washed. For hydropriming, the seeds were soaked in

distilled water for the duration 2, 4, 6, and 8 days in the sterilized glass bottles. The osmopriming of the seeds was performed using Polyethylene glycol (PEG-6000). Four different concentrations of osmotic solutions of PEG-6000 were made at 5, 10, 15, and 20% in distilled water, and the solution was applied on the seeds for the duration of 2, 4, 6, and 8 days. The osmotic potentials of the solutions were measured using a water potential meter (Psypro Wescor Corporation, Logan, UT, USA). Table 1 shows the osmotic potentials that correspond to the PEG-6000 solutions. Glass bottles were then covered with aluminum foil. Seeds without priming treatment were considered as a control. Upon completion of the priming treatments, the seeds were thoroughly washed with distilled water and placed on Whatman filter papers, allowing dehydration under the shade at 25 °C till the seeds retrieved their original moisture level, which was that of the pre-priming stage. The primed *Santalum album* L. seeds were pretreated with a 0.05% (*w*/*v*) gibberellic acid (GA_3) solution overnight.

Table 1. The osmotic potential (OP) of solutions of PEG-6000 concentrations.

PEG-6000 concentrations	0% (Hydro priming)	5%	10%	15%	20%
OP (MPa)	−0.06	−0.10	−0.20	−0.37	−0.059

2.3. Experimental Setup

Seeds were then placed to the germination trays filled with sand and were watered daily until germination was completed. The germination experiment was performed in CRD with 21 treatments and 3 equal replications with 50 seeds constituting one replication. Table 2 depicts the priming treatments adopted in the study. Daily germination counts were recorded for a period of 75 days by the time germination was completed. The percentage of germination, peak value (PV) of germination, and germination value (GV) were calculated as suggested by [26].

Table 2. The priming treatments adopted for the study.

Treatment Code	Method	Treatment Substrate Used	Concentration	Duration in (Days)
T_1	Hydropriming	Distilled water	-	2
T_2			-	4
T_3			-	6
T_4			-	8
T_5	Osmopriming	Polyethylene glycol (PEG-6000)	5%	2
T_6				4
T_7				6
T_8				8
T_9			10%	2
T_{10}				4
T_{11}				6
T_{12}				8
T_{13}			15%	2
T_{14}				4
T_{15}				6
T_{16}				8

Table 2. *Cont.*

Treatment Code	Method	Treatment Substrate Used	Concentration	Duration in (Days)
T_{17}				2
T_{18}			20%	4
T_{19}				6
T_{20}				8
T_{21}	(Control)	-	-	-

2.4. Physiological and Biolochemical Methods

The electrical conductivity of the seed leachates was determined using a direct reading conductivity meter (CDC 40101), and was expressed as dS cm^{-1}. To measure the electrical conductivity, the leachate was filtered to a conical flask immediately after priming treatments were completed. The total carbohydrate content of the seeds was estimated by the Anthrone reagent method [27], and the protein content by Lowry's method [28] by preparing standard curves and taking absorbance in spectrophotometer (Hach DR 6000, Germany). Crude fat content estimation was performed with Soxhlet extraction [29]. Amylase activities (α and β amylase) estimation was carried out by the method suggested by [30], taking maltose as the standard. The seedlings of 4 to 5 leaf stage were transplanted to the polythene bags filled with soil: sand: farm yard manure, in a ratio of 3:1:1. Growth attributes, such as shoot height, collar girth, leaf number, leaf area, root length, and dry biomass production, were recorded on Day 180 after transplanting (DAT). To estimate the total chlorophyll content of the seedlings, the leaf samples of 0.1 g in 7 mL of DMSO (dimethyl sulphoxide) was kept overnight, and quantification was performed as per [31] from the three selected mature leaves from the second whorl [32]. Twelve seedlings from each treatment were randomly selected to record the growth and biomass production of seedlings. Only 11 treatments were available for observing growth and biomass production, as the germination percentage and survival of seedlings were poor in osmopriming at higher concentration and durations. We calculated growth analysis indices such as specific leaf area, root: shoot biomass ratio [33], vigor index and seedling quality index. Vigor index was calculated using the following formula [34]:

$$VI = GP \times (SH + RL)$$

where GP = germination percentage, SH = shoot height, and RL = root length.

The seedling quality index was calculated as [35]

$$\text{Seedling quality index} = \frac{\text{Total seedling weight (g)}}{\frac{\text{Height(cm)}}{\text{Diameter (mm)}} + \frac{\text{Shoot dry weight (g)}}{\text{Root dryweight (g)}}}$$

2.5. Statistical Analysis

One-way analysis of variance was carried out for all the parameter in GRAPE 1.0.0 web application based on R software (https://www.kaugrapes.com, accessed on 11 January 2023), and the treatments were compared with LSD (least significant difference) at a 5% probability level for the post hoc test. The correlation analysis was conducted with cormorant package (available at http://github.com/r-link/corrmorant, accessed on 17 January 2023) in R 4.2.1 software (R Core Team, Vienna, Austria).

3. Results

3.1. Changes in Electrical Conductivity and Biochemical Composition of Primed Seeds

The treatment level averages of seed characteristics with LSD post hoc results are visualized in Table 3. Most of the osmopriming treatments lowered the electrical conductivity, except for T_7, T_8, T_9, and T_{13}, of the seed leachates, and hydropriming increased the electrical conductivity (EC), indicating that hydropriming for longer durations is not suitable

in improving the seed quality in *Santalum album* L. (Table 3). The EC of seeds on priming ranged from a lower value of 0.48 dS cm^{-1} (T_{18}) from osmopriming to 1.98 dS cm^{-1} (T_4) from hydropriming. The EC of seeds in osmopriming decreased with the concentration of PEG-6000 and increased with priming duration ($p = 0.05$).

Table 3. Changes in the electrical conductivity and biochemical composition of *Santalum album* L. seeds subjected to hydro- and osmopriming.

Treatment Code	Electrical Conductivity (dS cm^{-1})	Protein (mg g^{-1})	Carbohydrate (mg g^{-1})	Crude Fat (%)	α Amylase (μ mol min^{-1} mg^{-1})	β Amylase (μ mol min^{-1} mg^{-1})
T_1	1.12 ^{bc}	0.05 ^{a}	0.38 ^{a}	44.51 ^{c}	4.67 ^{hij}	1.28 ^{m}
T_2	1.55 ^{d}	0.06 ^{a}	0.36 ^{a}	38.93 ^{a}	4.76 ^{ghij}	1.30 ^{lm}
T_3	1.63 ^{d}	0.06 ^{a}	0.32 ^{a}	38.05 ^{a}	4.92 ^{efgh}	1.33 ^{kl}
T_4	1.98 ^{e}	0.07 ^{b}	0.28 ^{a}	41.17 ^{b}	5.13 ^{def}	1.36 ^{k}
T_5	1.04 ^{bc}	0.09 ^{c}	1.94 ^{c}	39.86 ^{a}	4.86 ^{ghi}	2.28 ^{ef}
T_6	1.04 ^{bc}	0.08 ^{b}	1.72 ^{c}	51.80 ^{g}	5.16 ^{de}	2.28 ^{ef}
T_7	1.24 ^{c}	0.08 ^{b}	1.54 ^{b}	51.14 ^{g}	5.29 ^{cd}	2.29 ^{def}
T_8	1.34 ^{cd}	0.09 ^{c}	1.28 ^{b}	49.73 ^{f}	5.48 ^{bc}	2.30 ^{de}
T_9	1.71 ^{de}	0.08 ^{b}	1.92 ^{c}	43.79 ^{b}	4.54 ^{j}	2.18 ^{i}
T_{10}	0.76 ^{ab}	0.07 ^{b}	1.85 ^{c}	49.43 ^{f}	4.79 ^{ghij}	2.19 ^{hi}
T_{11}	1.02 ^{bc}	0.07 ^{b}	1.83 ^{c}	46.12 ^{d}	4.90 ^{fghi}	2.26 ^{fg}
T_{12}	1.10 ^{bc}	0.08 ^{b}	1.43 ^{b}	43.75 ^{b}	4.92 ^{efg}	2.30 ^{de}
T_{13}	1.31 ^{cd}	0.09 ^{c}	1.85 ^{c}	47.09 ^{e}	4.96 ^{efg}	2.48 ^{b}
T_{14}	0.64 ^{a}	0.08 ^{b}	1.82 ^{c}	44.48 ^{bc}	5.58 ^{b}	2.52 ^{b}
T_{15}	0.65 ^{a}	0.07 ^{b}	1.12 ^{b}	46.17 ^{d}	5.44 ^{bc}	2.61 ^{a}
T_{16}	0.95 ^{b}	0.06 ^{a}	1.34 ^{b}	38.36 ^{a}	5.90 ^{a}	2.61 ^{a}
T_{17}	1.17 ^{bc}	0.09 ^{c}	1.62 ^{c}	46.98 ^{d}	4.66 ^{ij}	2.26 ^{fg}
T_{18}	0.48 ^{a}	0.07 ^{b}	1.56 ^{b}	55.14 ^{h}	4.59 ^{j}	2.23 ^{gh}
T_{19}	0.85 ^{ab}	0.07 ^{b}	1.33 ^{b}	47.29 ^{e}	4.88 ^{fghi}	2.33 ^{cd}
T_{20}	1.09 ^{bc}	0.06 ^{a}	1.39 ^{c}	46.02 ^{c}	5.44 ^{bc}	2.37 ^{c}
T_{21}	1.13 ^{bc}	0.06 ^{a}	1.05 ^{b}	54.03 ^{h}	4.99 ^{efg}	1.68 ^{j}
SEM	0.00	0.03	2.78	0.34	0.09	0.02

Significance level at $p \leq 0.05$. SEM: standard error of mean. Values within the same column with similar superscript are homogenous.

The stored proteins in the seeds ranged from 0.05 to 0.09 mg g^{-1} after priming. It can be noticed that hydropriming increased the protein content of seeds with duration. The carbohydrate content was highest in T_5 (1.94 mg g^{-1}) and lowest in T_4 (0.28 mg g^{-1}). Hydropriming caused lowering and osmopriming caused an increase in the amount of carbohydrates in the seeds compared to the control (T_{21}). During both hydro- and osmopriming, a sharp decrease in carbohydrate contents with increasing priming duration was observed. The crude fat content of the seeds after priming ranged from 38% (T_3) to 55% (T_{18}). The crude fat content of the seeds decreased with priming treatments compared to the control, with the exception of T_{18}. No specific trend was observed in the case of α amylase activity in the primed seeds, as compared to control, whereas the β amylase activity was lowered in the case of hydroprimed seeds and increased in osmoprimed seeds.

3.2. Seed Germination

Marked differences were observed in the germination percentage of the *Santalum album* L. seeds, influenced due to priming treatments ($p = 0.01$). The osmopriming of seeds in PEG-6000 solutions at 5% concentrations for 2 days (T_5) has resulted in the

highest germination (79%), followed by T_{13} (10% concentration for 2 days) and T_{17} (15% concentration for 2 days) having 78% germination (Figure 1). The lowest germination (3%) was noticed from osmopriming at 15% for 6 days (T_{15}). T_6, T_9, T_{10}, T_{14}, and T_{18}, all osmopriming treatments, outperformed the control, with germination percentages of more than 70%. The lowest imbibition period (12 days) and germination period (44 days) were in T_5, followed by T_6 (imbibition period of 12 days and germination period of 45 days) (Table 4). The osmoprimed T_5 treatment has the highest mean daily germination, peak value, and germination value, and the lowest was in T_{15}. All the hydropriming treatments significantly decreased the germination percentage, as compared to the control. With increase in hydropriming duration, the germination percentage decreased. However, in T_1, the MDG, PV, and GV were higher than control, indicating faster and uniform germination. The MDG and PV of the osmoprimed seeds were almost double those of the control for two days. The osmopriming of the seeds that were kept for less time were only reported to increase the germination percentage above the control (2 and 4 days).

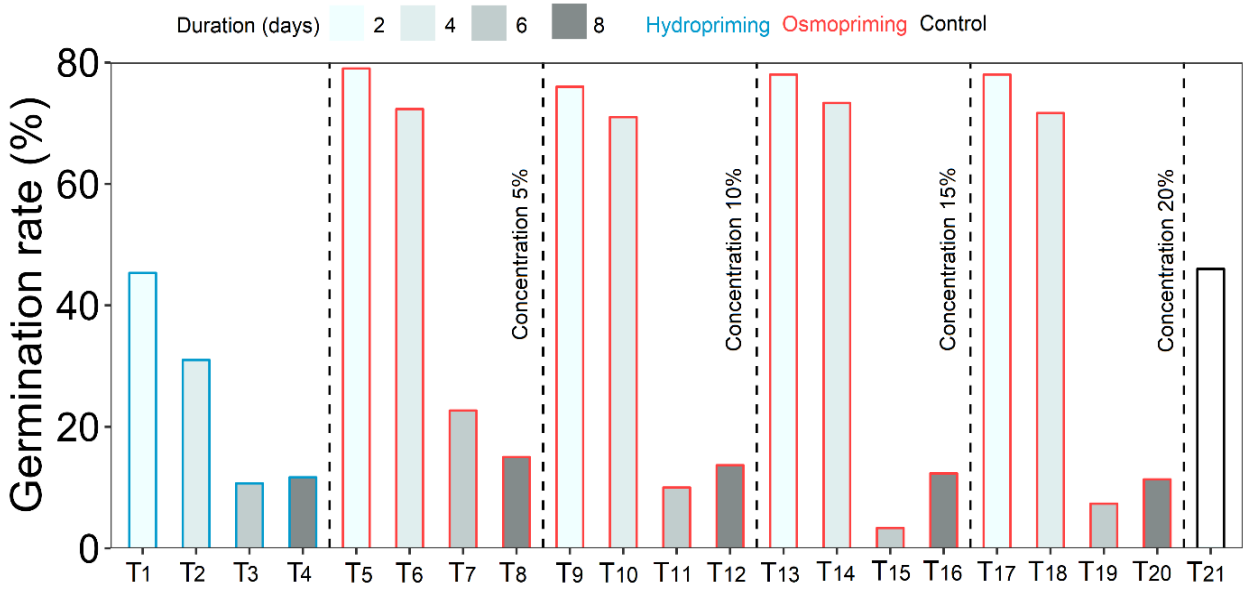

Figure 1. Effect of different hydropriming and osmopriming treatments on the germination percentage of the *Santalum album* L. seeds.

Table 4. Effect of hydropriming and osmopriming on germination attributes of *Santalum album* L. seeds.

Treatment Code	Imbibition Period (Days)	Germination Period (Days)	Mean Daily Germination	Peak Value	Germination Value	Speed of Germination
T_1	18	48	1.52	1.57	0.85	2.35
T_2	19	57	0.51	0.57	0.30	1.49
T_3	23	37	0.27	0.28	0.08	0.53
T_4	14	59	0.20	0.20	0.04	0.39
T_5	12	44	1.76	2.05	3.64	5.75
T_6	12	45	1.65	1.83	3.06	5.08
T_7	29	60	0.34	0.38	0.14	0.86
T_8	41	62	0.23	0.23	0.06	0.44
T_9	12	53	1.39	1.97	2.79	5.75

Table 4. *Cont.*

Treatment Code	Imbibition Period (Days)	Germination Period (Days)	Mean Daily Germination	Peak Value	Germination Value	Speed of Germination
T_{10}	14	49	1.43	1.77	2.55	4.66
T_{11}	38	63	0.15	0.15	0.03	0.31
T_{12}	44	62	0.18	0.18	0.03	0.33
T_{13}	12	60	1.29	1.71	2.37	4.97
T_{14}	14	46	1.58	1.84	2.95	4.62
T_{15}	39	52	0.06	0.04	0.00	0.12
T_{16}	43	61	0.20	0.20	0.04	0.37
T_{17}	13	49	1.58	1.89	3.02	5.42
T_{18}	16	54	1.31	1.64	2.18	4.29
T_{19}	21	47	0.15	0.15	0.02	0.31
T_{20}	48	62	0.18	0.17	0.03	0.31
T_{21}	14	56	0.82	0.86	0.73	2.38

3.3. Seedling Growth and Biomass Production

Analysis of variance revealed significant differences in the seedling height, collar girth, leaf number, leaf area, and root length of the *Santalum album* L. seedlings due to priming at 1% significance level. Results of biometric parameters of seedlings after 180 DAT indicated that the seedling height was the highest in T_5 (21.85 cm), followed by T_1 (21.80 cm), and the lowest value was recorded in T_4 (11.47 cm). The highest root length was recorded in T_7 (7.45 cm), and the lowest (2.42 cm) was in T_3. On the other hand, the collar girth was highest in T_9 (7.54 mm), followed by T_7 (7.45 mm) and T_5 (7.13 mm), and the lowest was in T_3 (4.07 mm). T_1 (21.50) was reported to be the highest in average leaf number, followed by T_5 (18.25), and the lowest was recorded in T_6 (11.50). However, the highest leaf area was recorded in T_7 (10.18 cm^2), and the lowest was in T_4 (5.74 cm^2) (Table 5).

Table 5. Effect of hydropriming and osmopriming on the biometric parameters of the seedlings at 180 DAT. Variability interval after the treatment means is a standard error; lower case superscript represents grouping by LSD post hoc test.

Treatment Code	Shoot Height (cm)	Collar Girth (mm)	Leaf Number	Leaf Area (cm^2)	Root Length (cm)
T_1	21.80 ± 0.27^{e}	4.16 ± 0.11^{a}	21.50 ± 0.63^{d}	9.47 ± 0.67^{a}	3.10 ± 0.36^{a}
T_2	17.07 ± 0.37^{c}	4.84 ± 0.13^{b}	19.50 ± 1.53^{bc}	6.53 ± 0.62^{b}	3.02 ± 0.11^{a}
T_3	12.87 ± 0.36^{b}	4.07 ± 0.09^{a}	11.75 ± 0.84^{a}	5.74 ± 0.22^{b}	2.42 ± 0.12^{a}
T_4	11.47 ± 0.23^{a}	4.10 ± 0.03^{a}	13.00 ± 0.69^{ab}	5.47 ± 0.23^{b}	2.67 ± 0.06^{a}
T_5	21.85 ± 0.34^{e}	7.13 ± 0.04^{f}	18.25 ± 0.61^{ab}	6.76 ± 0.37^{c}	6.30 ± 0.08^{bc}
T_6	18.82 ± 0.23^{d}	5.91 ± 0.31^{cd}	11.50 ± 0.63^{a}	7.54 ± 0.48^{c}	5.45 ± 0.11^{b}
T_7	21.75 ± 0.31^{e}	7.45 ± 0.08^{fg}	17.50 ± 1.53^{bc}	10.18 ± 0.78^{a}	7.45 ± 0.13^{cd}
T_8	18.22 ± 0.17^{d}	5.42 ± 0.12^{c}	12.00 ± 0.69^{a}	6.42 ± 0.50^{c}	5.45 ± 0.07^{b}
T_9	20.05 ± 0.12^{b}	7.54 ± 0.14^{g}	16.75 ± 1.11^{ab}	7.85 ± 0.38^{bc}	6.70 ± 0.78^{cd}
T_{10}	19.10 ± 0.17^{d}	6.70 ± 0.16^{f}	17.00 ± 1.45^{ab}	9.31 ± 0.52^{ab}	5.57 ± 0.12^{bc}
T_{21}	19.45 ± 0.42^{e}	6.35 ± 0.15^{f}	14.00 ± 1.39^{ab}	9.40 ± 0.57^{ab}	6.45 ± 0.14^{cd}
SEM	0.48	0.14	1.39	0.92	1.11

Results related to biomass production parameters, i.e., root, shoot, and the total biomass and chlorophyll content of the seedlings vary significantly in different treatments (p = 0.01) (Table 6). The highest total biomass was recorded in T_5 (0.62 g), followed by T_{21} (0.59 g), i.e., the control group, and the lowest was in T_4 (0.24 g). Subsequently, the highest shoot biomass was also recorded in T_5 (0.21 g), but the highest root dry weights were recorded in T_1, T_9, and T_{21} (0.14 g). The chlorophyll content was the highest (27.87 mg g^{-1}) in control seedlings, and the lowest values were recorded in T_{10} (15.43 mg g^{-1}).

Table 6. Effect of hydropriming and osmopriming on the biomass production and chlorophyll content of seedlings at 180 DAT. Variability interval after the treatment means is a standard error; lower case superscript represents grouping by LSD post hoc test.

Treatment Code	Shoot Dry Weight (g)	Root Dry Weight (g)	Total Dry Weight (g)	Chlorophyll (mg g^{-1})
T_1	0.18 ± 0.03 ^{c}	0.14 ± 0.03 ^{c}	0.52 ± 0.02 ^{b}	17.93 ± 0.16 ^{d}
T_2	0.08 ± 0.03 ^{b}	0.07 ± 0.05 ^{b}	0.28 ± 0.02 ^{b}	22.26 ± 0.18 ^{b}
T_3	0.10 ± 0.00 ^{b}	0.06 ± 0.05 ^{b}	0.26 ± 0.00 ^{b}	19.23 ± 0.21 ^{c}
T_4	0.09 ± 0.02 ^{b}	0.03 ± 0.04 ^{a}	0.24 ± 0.01 ^{b}	18.53 ± 0.29 ^{cd}
T_5	0.21 ± 0.02 ^{c}	0.11 ± 0.03 ^{a}	0.62 ± 0.02 ^{a}	25.94 ± 0.50 ^{b}
T_6	0.15 ± 0.02 ^{a}	0.12 ± 0.03 ^{a}	0.46 ± 0.12 ^{a}	19.29 ± 0.75 ^{d}
T_7	0.19 ± 0.03 ^{a}	0.13 ± 0.05 ^{a}	0.55 ± 0.16 ^{a}	23.96 ± 0.22 ^{c}
T_8	0.17 ± 0.02 ^{a}	0.08 ± 0.04 ^{a}	0.46 ± 0.14 ^{a}	16.86 ± 0.47 ^{e}
T_9	0.21 ± 0.02 ^{a}	0.14 ± 0.05 ^{a}	0.55 ± 0.17 ^{a}	19.23± 0.21 ^{d}
T_{10}	0.17 ± 0.01 ^{bc}	0.10 ± 0.04 ^{a}	0.40 ± 0.19 ^{a}	15.43 ± 0.22 ^{f}
T_{21}	0.20 ± 0.02 ^{c}	0.14 ± 0.04 ^{c}	0.59 ± 0.01 ^{c}	27.87 ± 0.42 ^{a}
SEM	0.012	0.005	0.02	4.28

Analysis of variance revealed significant differences in specific leaf area, vigor index, and quality index of the seedlings (p = 0.01). The highest specific leaf area was recorded in the T_{10} (60.05 cm^2 g^{-1}), followed by T_3 (47.17 cm^2 g^{-1}), the lowest was in T_5 at (21.33 cm^2 g^{-1}). There is no significant variation in the root: shoot biomass ratio noticed. The seedling vigor index that combines both germination and seedling attributes indicates that T_5 (1603.23) recorded the highest vigor index, and the lowest was in T_3 (102.17). T_9 recorded the highest Dickson's quality index (0.0451), followed by the control (0.431). T_4 (0.0167) recorded the lowest value related to quality index (Table 7).

Table 7. Effect of hydro- and osmopriming on the growth analysis indices of *Santalum album* L. seedlings. Variability interval after the treatment means is a standard error; lower case superscript represents grouping by LSD post hoc test.

Treatment Code	Specific Leaf Area (cm^2 g^{-1})	Root: Shoot Ratio	Vigor Index	Quality Index
T_1	44.60 ± 3.03 ^{c}	0.93 ± 0.14	952.54 ± 21.28 ^{c}	0.0268 ^{b}
T_2	43.38 ± 6.03 ^{c}	0.81 ± 0.10	472.03 ± 27.02 ^{b}	0.0213 ^{ab}
T_3	47.17 ± 3.56 ^{c}	0.77 ± 0.28	102.17 ± 3.73 ^{a}	0.0204 ^{ab}
T_4	37.69 ± 3.88 ^{abc}	0.36 ± 0.12	133.10 ± 6.20 ^{a}	0.0167 ^{c}
T_5	21.33 ± 1.04 ^{a}	0.57 ± 0.02	1603.23 ±26.16 ^{e}	0.0429 ^{a}
T_6	34.03 ± 1.92 ^{abc}	0.86 ± 0.07	1182.23 ±21.69 ^{d}	0.0334 ^{b}
T_7	42.30 ± 3.79 ^{c}	0.80 ± 0.12	1306.23 ± 50.75 ^{def}	0.0411 ^{a}
T_8	26.69 ± 2.51 ^{a}	0.57 ± 0.06	1256.95 ± 11.81 ^{de}	0.0302 ^{b}

Table 7. *Cont.*

Treatment Code	Specific Leaf Area (cm^2 g^{-1})	Root: Shoot Ratio	Vigor Index	Quality Index
T_9	35.02 ± 2.59 ^{abc}	0.78 ± 0.11	1317.23 ±14.62 ^{df}	0.0451 ^{a}
T_{10}	60.05 ± 4.86 ^{c}	0.66 ± 0.07	1387.43 ± 31.49 ^{def}	0.0317 ^{b}
T_{21}	33.12 ± 3.19 ^{a}	0.75 ± 0.03	839.29 ± 46.84 ^{d}	0.0431 ^{a}
SEM	0.012	0.005	0.02	4.28

A hierarchical cluster analysis was conducted with the average linkage method and Euclidian as the distance measure, so as to find the best performing seedlings. At the 10% level, five homogenous clusters were identified (Figure 2). The first cluster contained T_6, T_8, T_{10}, and T_{21}; the second T_5, T_7, and T_9; the third cluster, T_1; the fourth, T_3 and T_4, and the fifth, T_2. The comparison between growth and biomass production indicated that the second cluster was superior to others in these attributes. Clustering at 5% distance indicated that T_5 was the best treatment and the other two were identical.

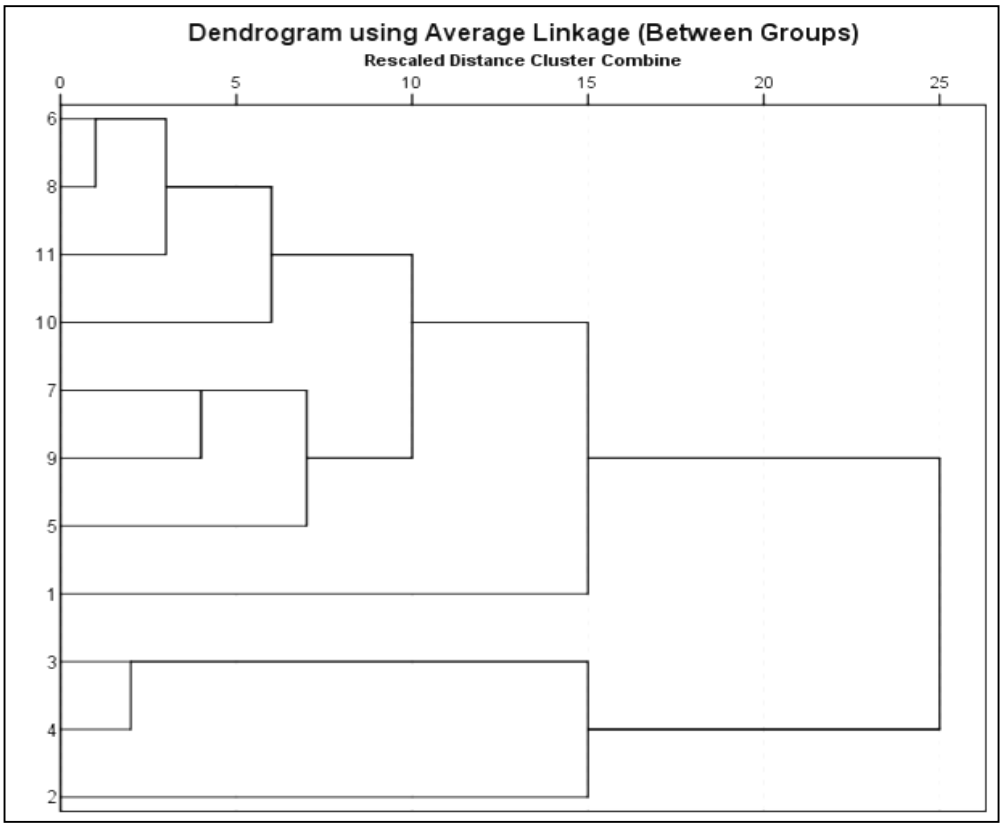

Figure 2. Dendrogram based on hierarchical cluster analysis with Euclidian distance measure with the seedling growth attributes of *Santalum album* L.

Correlation analysis (Figure 3) revealed a strong relationship between the characteristics of seeds and the growth performance of the seedlings. The EC of seeds showed significant negative correlation with all traits except chlorophyll content, SLA, and root:shoot ratio. On the other hand, the seed carbohydrate content showed positive significant correlation with collar girth, root length, and overall biomass accumulation of the seedlings. Similarly, seed fat content correlated positively with height growth, collar girth, leaf area, root length, and biomass accumulation. The α showed no significant correlation with any trait, but β amylase showed consistent positive correlation across growth traits, vigor index,

and quality index. Interestingly, the seed protein content of seeds correlated negatively with the specific leaf area and root: shoot ratio of the seedlings.

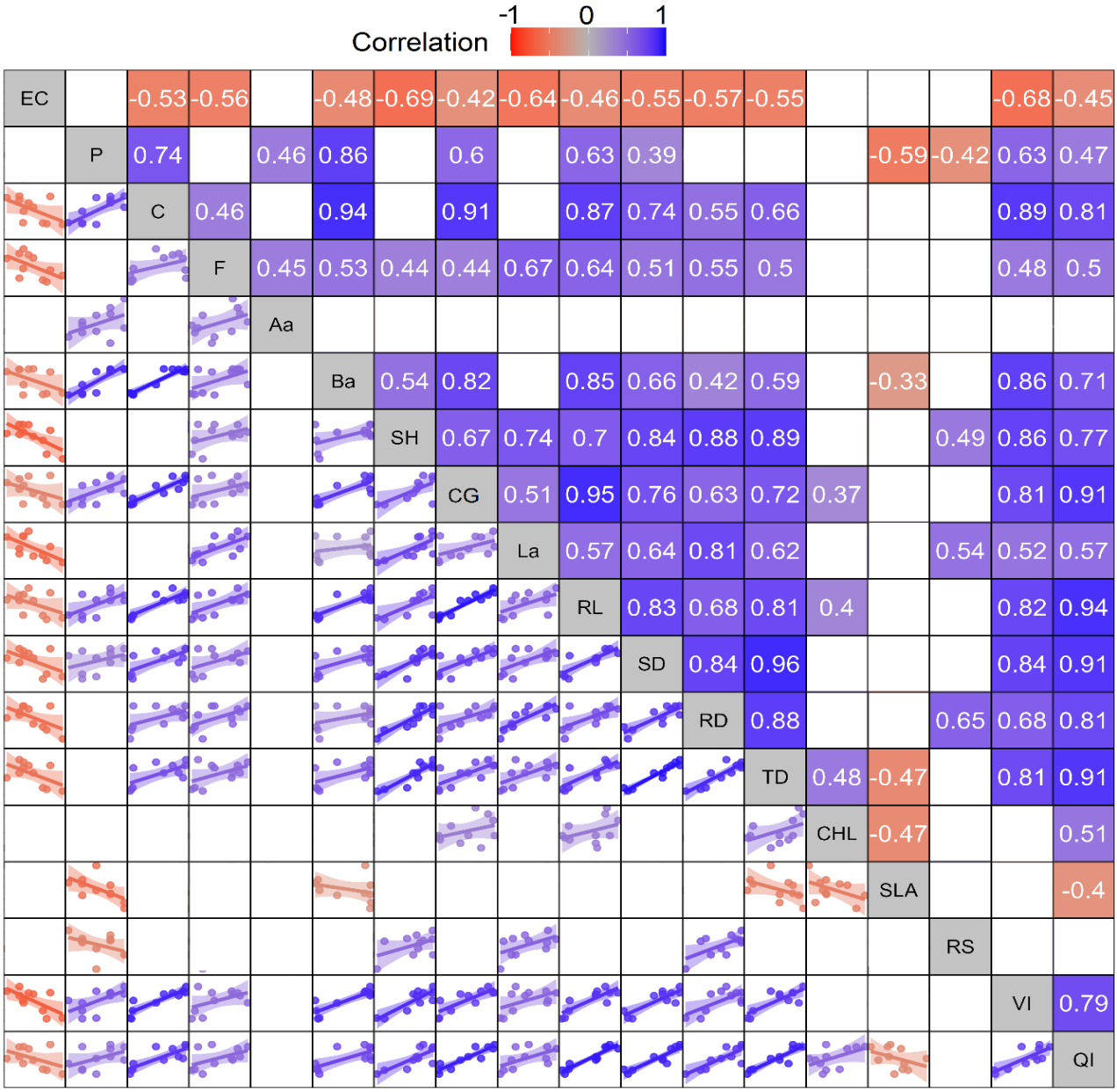

Figure 3. Correlation analysis between treatment level characteristics of seeds and seedlings with visualization of the linear relationship between the traits. EC—electrical conductance of seeds; P—protein content of seeds; C—carbohydrate content of the seeds; F—fat content of the seeds; A—α amylase content of the seeds; Ba—β amylase content of the seeds; SH—shoot height; CG—collar girth; La—leaf area; RL—root length; SD—shoot dry weight; RD—root dry weight; TD—total dry weight; CHL—chlorophyll content; SLA—specific leaf area; RS—root–shoot ratio; VI—vigor index; QI—quality index.

4. Discussion

Our results demonstrated that the priming of *Santalum album* L. seeds could improve the seed germination characteristics, growth, and vigor of the seedlings. The highest germination (79%) was recorded when the seeds were osmoprimed with 5, 15, and 20% concentrations of PEG-6000 for 2 days. Only the lower durations (2 and 4 days) of osmopriming could improve the germination above the control. This might be due to the fact

that osmopriming with PEG as substrate is the most optimal at higher water potentials and short priming times experienced in maize [36] and wheat [37]. During the application of more negative water potentials, which corresponds to a longer priming duration, as in our study (6 and 8 days) duration, oxidative processes may occur, and as a result, the disintegration of components detrimental to sprouting occurs, which might result in lower germination in least performing treatments. Chen and Arora (2013) [11] pointed out that the osmopriming induced hydrogen peroxide during germination, which could be a signaling molecule for germination improvement. Out of the 21 treatments, the lowest imbibition (12 days) and germination (44 days) periods were recorded in osmopriming at 5% of PEG for 2 and 4 days. Previous studies on the seed germination of *Santalum album* L. have revealed that the completion of germination of sandalwood seeds could take 4 to 22 weeks [38,39].

With the osmopriming approach in *Santalum album* L. seeds, the total germination period was reduced from 8 weeks (control) to approximately 6 weeks. Kumar and Rajalekshmi (2021) [40] also reported the superiority of osmopriming with PEG in terms of germination in winged bean. The results also show that hydropriming does not improve the germination percentage of *Santalum album* L. seeds above the control, and the germination percentage decreased with the increase in soaking duration, which was in conformity with [41], which studied Fir tree species. A decrease in the germination of hydroprimed seeds may be attributed to free radical accumulation, which stimulates lipid peroxidation during hydration, as reported in maize by [42].

Based on the correlation analysis, we can see that reduced electrical conductance increased the carbohydrate and fat content of the seed, and higher β amylase have a positive impact on the growth, vigor, and quality of the seedlings (Figure 3). The lower electrical conductivity of seed leachates indicates improved cellular membrane integrity during priming, which decreases leakage from the cells [43], and thus, helps in increasing seed viability and vigor [44]. It is notable in our study that the EC of seeds exposed to osmopriming was mostly lower than the control and corresponding hydropriming treatments. Additionally, with the increase in priming duration, the electrical conductivity increased. This might have led to the better germination of the seeds subjected to osmopriming, which was in accordance with the finding of [15] in *Pinus thunbergii*. The highest EC during hydropriming compared to the control corresponds to poor germination during hydropriming, which is supported by the findings of [45] in bitter gourd. The seed reserve material content is normally linked with germinations and speed of germination in *Pinus pinaster* [46]. The carbohydrate content in the seeds is believed to facilitate the greater germination value of that studied seeds, which are found in tree species with recalcitrant seed storage behavior [47]. In the present study, *Santalum album* L. seeds recorded large variations in their biochemical constituents with varying seed priming treatments. Considering the carbohydrate content, hydropriming induced a considerable decrease in the carbohydrate content of the seed (73.33% reduction) when compared to the control. Only specific osmopriming treatments could increase the carbohydrate content of *Santalum album* L. seeds, which performs a better germination. The metabolism of the seed protein is an important step during germination. In our study, we also observed an increase in the protein content of the seeds with osmopriming. All the osmopriming treatments were superior to the control, with an increase of almost 60% in protein content; on the other hand, hydropriming caused a reduction. There could be higher carbohydrate and protein content in the PEG-mediated primed seeds due to the larger molecular size of PEG-6000, which retards the entry of PEG molecules into the cells, thus preventing the deterioration of enzyme-related activities with the cells of seed. A decrease in the fat content of the seeds subjected to osmopriming was noticed, which might be due to the conversion of lipids into sugars, which is common in seeds with greater oil content [48]. Enzyme activity is associated with solubilizing stored food material and making it available to the germinating seeds. Although we did not obtain a specific trend in α—amylase activity due to treatment effects, the β—amylase activity was found to be increased in all the osmoprimed seeds compared to the control, as its major role is to

produce maltose that favors the germination process [49], whereas in hydroprimed seeds it was reduced significantly.

The clustering of the seedling parameters suggested that T_5 (PEG priming at 5% for 2 days) is the best treatment for the enhancement of seedling growth, which also corresponds to the highest speed of germination. The next best treatments were from osmopriming, i.e., T_7 and T_9. Hence, the treatments T_5 and T_9 can be recommended for quality planting stock production in *Santalum album* L. Osmopriming consists of the prior exposure of seeds in the low-water-potential solutions, which generates a series of pre-germination metabolic activities and prepares the seeds for radicle protrusion [50]. Polyethylene glycol (PEG) is a common osmopriming agent. Osmopriming has been reported to enhance germination and growth attributes in the forest trees, e.g., in *Pinus bungeana* [51] and *Pinus nigra* [52]. The improvement in germination and vigor might be due to the reserve mobilization of food material, the repair and build-up of nucleic acids, the enhanced synthesis of RNA, and proteins, and some enzymes during osmotic priming [50]. From the present study it can be concluded that osmopriming with polyethylene glycol has a positive effect on the germination and seedling growth of *Santalum album* L. seeds. The overpriming due to higher concentrations and durations of PEG-6000 was detrimental to seedling growth. This is supported by Elkoca et al. (2007) [53]; they concluded that overpriming may cause an oxygen deficiency and the build-up of inhibitors.

5. Conclusions

The analysis of the results leads to the following conclusions. The osmopriming of *Santalum album* L. seeds with polyethylene glycol (PEG-6000) significantly ($p < 0.05$) influenced seed germination and the subsequent seedling growth of *Santalum album* L., thus helping in the overcoming of the constraints related to the germination of this valuable tree species. Osmopriming seeds at 5, 15, and 20% concentrations for 2 days recorded the highest germination ($\geq$78%). Considering seedling growth as well, the osmopriming at 5% for 2 days was the best treatment in enhancing the germination and seedling performance of *Santalum album* L. A longer priming period (6–8 days) showed a significantly ($p < 0.05$) lower germination and growth rate than the 2-day period of seed priming. The positive impact of osmopriming on the growth of the seedlings was connected with lower electric conductance, higher β amylase concentration, and higher carbohydrate and fat concentration of seeds. The hydropriming showed no significant ($p < 0.05$) positive effect on the germination or growth of the *Santalum album* seedlings.

Hence, osmopriming can be recommended as a viable technology in East Indian sandalwood to produce quality plant stock in nurseries. The PEG-mediated osmopriming can be further tested for its responses to abiotic stress tolerance (drought, salinity, and temperature) in *Santalum album* L.

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References

1. Van Hulten, M.; Pelser, M.; Van Loon, C.; Pieterse, C.M.; Ton, J. Costs and benefits of priming for defense in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 5602–5607. [CrossRef] [PubMed]
2. Basra, S.M.A.; Farooq, M.; Tabassam, R.; Ahmad, N. Physiological and biochemical aspects of pre-sowing seed treatments in fine rice (*Oryza sativa*). *Seed Sci. Technol.* **2005**, *33*, 623–628. [CrossRef]
3. Jisha, K.C.; Vijayakumari, K.; Puthur, J.T. Seed priming for abiotic stress tolerance: An overview. *Acta Physiol. Plant.* **2013**, *35*, 1381–1396. [CrossRef]
4. McDonald, M.B. Seed priming. In *Seed Technology and Its Biological Basis*; Black, M., Bewley, J.D., Eds.; Sheffield Academic Press: Sheffield, UK, 2000; pp. 287–325.
5. Beckers, G.J.; Conrath, U. Priming for stress resistance: From the lab to the field. *Curr. Opin. Plant Biol.* **2007**, *10*, 425–431. [CrossRef]
6. Ashraf, M.; Foolad, M.R. Pre-sowing seed treatment—A shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. *Adv. Agron.* **2005**, *88*, 223–271. [CrossRef]
7. Kaur, S.; Gupta, A.K.; Kaur, N. Effect of osmo-and hydropriming of chickpea seeds on seedling growth and carbohydrate metabolism under water deficit stress. *Plant Growth Regul.* **2002**, *37*, 17–22. [CrossRef]
8. Thomas, U.C.; Varughese, K.; Thomas, A.; Sadanandan, S. Seed priming—For increased vigour, viability and productivity of upland rice. *Leisa India* **2000**, *4*, 14.
9. Farooq, M.; Basra, S.M.A.; Hafeez, K. Seed invigoration by osmohardening in coarse and fine rice. *Seed Sci. Technol.* **2006**, *34*, 181–187. [CrossRef]
10. Halmer, P. Methods to improve seed performance in the field. In *Handbook of Seed Physiology*; The Haworth Reference Press: New York, NY, USA; London, UK; Oxford, UK, 2004; pp. 125–165.
11. Chen, K.; Arora, R. Priming memory invokes seed stress tolerance. *Environ. Exp. Bot.* **2013**, *94*, 33–45. [CrossRef]
12. Moradi, A.; Younesi, O. Effects of osmo-and hydro-priming on seed parameters of grain sorghum (*Sorghum bicolor*). *Aust. J. Basic Appl. Sci.* **2009**, *3*, 1696–1700.
13. Lei, C.; Bagavathiannan, M.; Wang, H.; Sharpe, S.M.; Meng, W.; Yu, J. Osmopriming with polyethylene glycol (PEG) for abiotic stress tolerance in germinating crop seeds: A review. *Agronomy* **2021**, *11*, 2194. [CrossRef]
14. Wu, L.; Hallgren, S.W.; Ferris, D.M.; Conway, K.E. Effects of moist chilling and solid matrix priming on germination of loblolly pine (*Pinus taeda*) seeds. *New For.* **2001**, *21*, 1–16. [CrossRef]
15. Kim, D.H.; Han, S.H.; Lee, J.C. Germination and biochemical changes in accelerated aged and osmoprimed *Pinus thunbergii* seeds. *J. Korean Soc. For. Sci.* **2010**, *99*, 244–250.
16. Ma, Y.; Feurtado, J.A.; Kermode, A.R. Effect of solid matrix priming during moist chilling on dormancy breakage and germination of seeds of four fir species. *New For.* **2003**, *25*, 67–81. [CrossRef]
17. Atrip, N.D.; O'Reilly, C. The response of prechilled alder and birch seeds to drying, freezing, and storage. *Can. J. For. Res.* **2006**, *36*, 749–760. [CrossRef]
18. Jones, C.G.; Ghisalberti, E.; Plummer, J.A.; Barbour, E. Quantitative co-occurrence of sesquiterpenes; a tool for elucidating their biosynthesis in Indian sandalwood, Santalum album. *Phytochemistry* **2006**, *67*, 2463–2468. [CrossRef]
19. Kumar, A.A.; Srinivasa, Y.B.; Joshi, G.; Seetharam, A. Variability in and relation between tree growth, heartwood and oil content in sandalwood (*Santalum album*). *Curr. Sci.* **2011**, *100*, 827–830.
20. Baldovini, N.; Delasalle, C.; Joulain, D. Phytochemistry of the heartwood from fragrant Santalum species: A review. *Flavour Fragr. J.* **2011**, *26*, 7–26. [CrossRef]
21. Arunkumar, A.N.; Dhyani, A.; Joshi, G. Santalum album. In *The IUCN Red List of Threatened Species*; International Union for Conservation of Nature (IUCN): Gland, Switzerland, 2019. [CrossRef]
22. Cleary, B.D.; Greaves, R.D.; Hermann, R.K. *Regenerating Oregon's Forests: A Guide for the Regeneration Forester*; Oregon State University Extension Service: Corvallis, OR, USA, 1978.
23. Grossnickle, S.C. *Ecophysiology of Northern Spruce Species: The Performance of Planted Seedlings*; NRC Research Press: Ottawa, ON, Canada, 2000.
24. Nagaveni, H.C.; Srimathi, R.A. Studies on germination of sandal (*Santalum album* Linn.). Pre-treatment of sandal seeds. *Indian For.* **1981**, *107*, 348–354.
25. Baskin, C.C.; Baskin, J.M. Germination ecophysiology of herbaceous plant species in a temperate region. *Am. J. Bot.* **1988**, *75*, 286–305. [CrossRef]
26. Czabator, F.J. Germination value: An index combining speed and completeness of pine germination. *For. Sci.* **1962**, *8*, 386–396.
27. Yemm, E.W.; Willis, A. The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J.* **1954**, *57*, 508. [CrossRef] [PubMed]
28. Lowry, O.H.; Rosebrough, N.J.; Farr, A.; Randall, R.J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265–275. [CrossRef] [PubMed]
29. Kennedy, W.K.; Unrau, J. A rapid method for determining the oil content of safflower and sunflower seeds. *Agron. J.* **1949**, *41*, 93–95. [CrossRef]
30. Sadasivam, S.; Manickam, A. *Biochemical Methods*, 2nd ed.; New Age International Publishers (P) Ltd.: New Delhi, India, 1996.
31. Kaloyereas, S.A. A new method of determining drought resistance. *Plant Physiol.* **1958**, *33*, 232. [CrossRef]

32. Takebe, M.; Yoneyama, T. Measurement of leaf color scores and its implication to nitrogen nutrition of rice plants. *Jpn. Agric. Res. Q.* **1989**, *23*, 86–93.
33. Hunt, R. *Basic Growth Analysis: Plant Growth Analysis for Beginners*; Unwin Hyman: London, UK, 1990.
34. Abdul–Baki, A.A.; Anderson, J.D. Vigor determination in soybean by multiple criteria. *Crop Sci.* **1973**, *13*, 630–633. [CrossRef]
35. Dickson, A.; Leaf, A.; Hosner, J.F. Quality appraisal of White Spruce and White Pine seedling stock in nurseries. *For. Chron.* **1960**, *36*, 10–13. [CrossRef]
36. Foti, R.; Abureni, K.; Tigere, A.; Gotosa, J.; Gere, J. The efficacy of different seed priming osmotica on the establishment of maize (*Zea mays*) caryopses. *J. Arid Environ.* **2008**, *72*, 1127–1130. [CrossRef]
37. Salehzade, H.; Shishvan, M.I.; Ghiyasi, M.; Forouzin, F.; Siyahjani, A.A. Effect of seed priming on germination and seedling growth of wheat (*Triticum aestivum*). *Res. J. Biol. Sci.* **2009**, *4*, 629–631.
38. Srimathi, R.A.; Kulkarni, H.D.; Venkatesan, K.R. *Recent Advances in Research and Management of Sandal (Santalum album) in India*; Associated Publishing Co.: New Delhi, India, 1995; 416p.
39. Sutheesh, K.; Jijeesh, C.M.; Divya, T.P. Evaluation of organic and inorganic pre-treatment for better seed germination and seedling vigour in *Santalum album*. *Plant Arch.* **2016**, *16*, 143–150.
40. Kumar, V.K.; Rajalekshmi, R. Effect of hydro, halo and osmopriming on seed germination and seedling performance of *Psophocarpus tetragonolobus* (L.) DC. (winged bean). *J. Crop Sci. Biotechnol.* **2021**, *24*, 411–428. [CrossRef]
41. Rodríguez, R.Z.; Luis, G.H.M.; Bernardo, M.A.; Edgar, O.R.; Lara, C.E.; Troyo, D.; Matson, M.V.C. Effect of hydropriming and biopriming on seed germination and growth of two Mexican Fir tree species in danger of extinction. *Forests* **2015**, *6*, 3109–3122. [CrossRef]
42. Leprince, O.; Atherton, N.M.; Deltour, R.; Hendry, G.A.F. The involvement of respiration in free radical processes during loss of desiccation tolerance in germinating *Zea mays* (An electron paramagnetic resonance study). *Plant Physiol.* **1994**, *104*, 1333–1339. [CrossRef]
43. Copeland, O.; Mc Donald, M.B. *Principles of Seed Science and Technology*, 4th ed.; Springer: New York, NY, USA, 2001; 333p.
44. Sukesh, S.H.; Chandrashekhar, K.R. Biochemical changes during storage of seeds of *Hopea ponga* (Dennst.) Mabberly: An academic species of Western Ghats. *Res. J. Seed Sci.* **2011**, *4*, 106–116. [CrossRef]
45. Adhikari, B.; Dhital, P.R.; Ranabhat, S.; Poudel, H. Effect of seed hydro-priming durations on germination and seedling growth of bitter gourd (*Momordica charantia*). *PLoS ONE* **2021**, *16*, e0255258. [CrossRef]
46. Wahid, N.; Bounoua, L. The relationship between seed weight, germination and biochemical reserves of maritime pine (*Pinus pinaster* Ait.) in Morocco. *New For.* **2013**, *44*, 385–397. [CrossRef]
47. Lin, T.P.; Huang, N.H. The relationship between carbohydrate composition of some tree seeds and their longevity. *J. Exp. Bot.* **1994**, *45*, 1289–1294. [CrossRef]
48. Borek, S.; Ratajczak, W.; Ratajczak, L. Regulation of storage lipid metabolism in developing and germinating lupin (*Lupinus* spp.) seeds. *Acta Physiol. Plant.* **2015**, *37*, 119. [CrossRef]
49. Muscolo, A.; Sidari, M.; Anastasi, U.; Santonoceto, C.; Maggio, A. Effect of PEG-induced drought stress on seed germination of four lentil genotypes. *J. Plant Interact.* **2014**, *9*, 354–363. [CrossRef]
50. Sadeghi, H.; Khazaei, F.; Yari; Sheidaei, S. Effect of seed osmopriming on seed germination behavior and vigor of soybean (*Glycine max*). *ARPN J. Agric. Biol. Sci.* **2011**, *6*, 39–43.
51. Guo, S.J.; Wang, Y.C.; Wang, W.S. Effects of priming treatments on germination and biochemical characteristics of *Pinus bungeana* seeds. *For. Stud. China* **2012**, *14*, 200–204. [CrossRef]
52. Carvalho, A.; Gaivao, I.; Lima-Brito, J. Seed osmopriming with PEG solutions in seeds of three infraspecific taxa of *Pinus nigra*: Impacts on germination, mitosis and nuclear DNA. *For. Ecol. Manag.* **2020**, *456*, 117739. [CrossRef]
53. Elkoca, E.; Haliloglu, K.; Esitken, A.; Ercisli, S. Hydro-and osmopriming improve chickpea germination. *Acta Agric. Scand. Sect. B-Soil Plant Sci.* **2007**, *57*, 193–200. [CrossRef]