International Journal of Economic Plants

https://ojs.pphouse.org/index.php/IJEP

IJEP November 2024, 11(4): 489-498

Crossref

Article IJEP5726

Full Research

Doi: HTTPS://DOI.ORG/10.23910/2/2024.5726

Biopriming Techniques for Improving Germination and Seedling Growth in Indian Sandalwood (*Santalum album* L.)

Harish Debta[1*], T. K. Kunhamu[1], A. V. Santhoshkumar[2], V. Jamaludheen[1], N. K. Binu[2] and K. C. Jisha[3]

[1]Dept. of Silviculture and Agroforestry, [2]Dept. of Forest Biology and Tree Improvement, College of Forestry, Kerala Agricultural University, Thrissur, Kerala (680 656), India

[3]Dept. of Botany, MES Asmabi College, Vemballur, Thrissur, Kerela (680 671), India

Corresponding Author

Harish Debta

e-mail: harishdebta007@gmail.com

Article History

Received on 21st August, 2024

Received in revised form on 02nd November, 2024

Accepted in final form on 14th November, 2024

Abstract

The present study was conducted during April, 2022 to September, 2023 with an aim to screen out the most effective biopriming treatment for improving the regeneration related constraints and seedling growth of Indian sandalwood (*Santalum album* L.), an IUCN- Vulnerable category tree species, that are under severe threat due to illegal harvesting and over exploitation in the wild. We tried five biopriming agents (e.g. Effective microorganisms; Plant growth promoting rhizobacteria (PGPR-I and PGPR-II); *Pseudomonas fluorescens* and *Piriformospora indica*) with four uniform durations of treatments i.e. 2, 4, 6 and 8 days. The results revealed that biopriming with *Pseudomonas fluorescens* for eight days recorded the highest germination percentage (70.67%) and the highest germination rate index (0.84). *Pseudomonas fluorescens* mediated biopriming followed by longer durations of PGPR-I and *Piriformospora indica* treatment (6-8 days) effectively lowered the imbibition period, mean germination time and increased germination rate. The seedling vigour index improved substantially due to the application of *Pseudomonas fluorescens* and PGPR-I. Hence, we recommend biopriming with *Pseudomonas fluorescens*, longer duration of PGPR-I and *Piriformospora indica* as a potential technique for overcoming the germination constraints in sandalwood and producing seedlings with high vigour.

Keywords: Vigour index, germination rate, amylase activity, electrical conductance, PGPR

1. Introduction

Biopriming is an emerging technique of seed priming which integrates the physiological (seed hydration) and biological (seed inoculation with the beneficial organisms) mechanisms (Moeinzadeh et al., 2010). Controlled hydration to achieve a critical moisture content is fundamental to all priming techniques that induce a set of biochemical changes such as enzyme activation, metabolism of germination inhibitors, repair of cell damage, and imbibition to promote germination (Heydecker et al., 1973; Farooq et al., 2006). Biopriming with beneficial microbes improves crop protection by enhancing seed quality, seedling vigour, and the plant's ability to withstand suboptimal growth conditions (Sharifi, 2012; Rakshit et al., 2015). Several studies have demonstrated that seed priming improves germination rates and growth uniformity, thereby reducing the emergence time of many horticultural and agricultural crops (Thomas et al., 2000; Kaya et al., 2006; Ghobadi et al., 2012). The use of microbial inoculants using plant growth-promoting rhizobacteria (PGPR) application through biopriming involves soaking the seeds in liquid bacterial culture suspension for a particular period, which initiates physiological processes inside the seed and enhances the bacterial abundance in the spermosphere while preventing plumule and radicle emergence until the seed is sown (Wright et al., 2003; Pravisya et al., 2019)

The previous line of work on seed priming for raising quality seedlings in forest tree species mostly centered around temperate conifers forest species and often undermining the low-cost and environment-friendly techniques such as biopriming (Ma et al., 2003, Kim et al., 2010; Park et al., 2013; Ramos-Montano et al., 2020). Vegetative propagation of many forest species are difficult, requiring specialised approaches and resources compared to agricultural and horticultural crops. In this context, seed priming assumes a potential strategy to be explored as a low-cost, environmentally friendly, and pragmatic approach for tree farmers to grow

high-vigour and stress-tolerant seedlings (Rodriguez et al., 2015; Becerra-Vazquez et al., 2020).

Santalum album L. (Indian Sandalwood), belongs to the family Santalaceae, which includes approximately 36 genera and more than 400 species of semi-parasitic shrubs and trees. The distribution of the species generaextends from 30°N to 40°S, from Indonesia in the east to Juan Fernandez Islands (Chile) in the west and from the Hawaiian Archipelago in the north to New Zealand inthe south (Harbaugh et al., 2010; Arun Kumar et al., 2012). The Indian Sandalwood consists of maximum oil (6%) and α- and β- santalol (90%) as compared to other *Santalum* spp. in trade (Shankaranarayana and Theagarajan, 2000). The wood is second only to ivory for intricate carvings. It is indigenous to Southeast Asia and south India regions, exclusively growing well in the drier part of Western Ghats. Due to habitat degradation and reduction of population sizesandalwood was registered as vulnerable in the IUCN Red List in 1994. The dry deciduous forests of the Deccan region are reported as the 'genetic hotspots' of sandalwood resources in the subcontinent (Rai, 1990). In the Indian subcontinent, sandalwood resources are fast depleting due to indiscriminate harvesting in natural habitats and the lack of established plantations, resulting in erosion of the gene pool and reduction in genetic diversity in natural populations (Venkatesan et al., 1995; Meera et al., 2000).

The dormancy of the seeds is a major constraint in the regeneration of sandalwood. Baskin and Baskin (1988) observed that the seeds possess physiological or morphophysiological dormancy the leading causes of seed dormancy are impermeable seed coats; mechanically resistant seed coats, rudimentary embryos; physiologically immature embryos and morphologically mature but physiologically dormant embryos. In the present study, we tried seed biopriming techniques with five different biotic agents to improve the germination constraints, followed by raising quality seedling of the highly esteemed aromatic oil-producing tree species *Santalum album*. The present study highlights the effect of biopriming techniques on germination response with biochemical changes that occur in the seeds due to biopriming treatments and seedling growth.

2. Materials and Methods

2.1. Plant material, experimental conditions and treatments

The experiment was conducted at the Department of Silviculture and Agroforestry, College of Forestry, Kerala Agricultural University, Kerala (680656), India. Seeds of *Santalum album* were obtained from the Marayoor Sandal Division, under the Kerala Forest Department, during February/March 2022. The seeds were collected by the Pallanadu Vana Samrakshana Samithi, Idukki district, on behalf of the State Forest Department from the designated seed production areas (SPA) of the Nachivayal Reserve Forest (10°25'00531''N, 77°15'8950'' E) of Marayoor Sandal Division. Collected seeds were cleaned, dried in the shade for 48 hours and thoroughly mixed to improve homogeneity.

The sandal seeds were subjected to biopriming with five biotic agents viz.(Effective microorganisms (EM), PGPR-I, PGPR-II, *Pseudomonas fluorescens* and *Piriformospora indica*) and four uniformdurations of treatments in days viz. (2, 4, 6 and 8 days). Additionally, a control, without any biopriming treatment, was maintained. The study was conducted in a completely randomised design with three replicates for each treatment, and each treatment unit consisted of 50 seeds. The details of seed biopriming treatments are listed in Table 1. The Effective microorganisms (EM) stock solution was procured from Maple Biotech India Ltd., an authorised producer of EM Research Organization Inc. (EMRO), Japan (Higa, 1994). One ml of EM stock solution was diluted to one litre with distilled water and used for biopriming. The talc-based culture of *Pseudomonas fluorescens*, plant growth promoting rhizobacteria (PGPR); PGPR-I and PGPR-II of KAU were obtained from Department of Microbiology, College of

Table 1: Details of seed biopriming treatments adopted in the study

No. allotted	Biopriming substrate	Duration of treatment (in days)	Treatment code
1	Effective microorganisms	2	EM T_2
2		4	EM T_4
3		6	EM T_6
4		8	EM T_8
5	PGPR –I	2	PG1 T_2
6		4	PG1 T_4
7		6	PG1 T_6
8		8	PG1 T_8
9	PGPR-II	2	PG2 T_2
10		4	PG2 T_4
11		6	PG2 T_6
12		8	PG2 T_8
13	*Pseudomonas fluorescens*	2	PF T_2
14		4	PF T_4
15		6	PF T_6
16		8	PF T_8
17	*Piriformospora indica*	2	PI T_2
18		4	PI T_4
19		6	PI T_6
20		8	PI T_8
21	Control (non-primed)	-	C

Horticulture, Vellanikkara, Kerala Agricultural University. The suspension culture of these three bioinoculants contained minimum of 10^8 c.f.u. ml^{-1} of bioagents. For the study, 20 g of the suspension culture of these three agents @ 10^8 c.f.u. ml^{-1} was used to produce 100% concentration for 50 sandal seeds. The culture of *Piriformospora indica*, an endophytic fungus was obtained from Department of Microbiology, College of Agriculture, Vellayani, Kerala Agricultural University. For biopriming with *Piriformospora indica*, 5 g of culture of this fungus in Potato Dextrose Agar medium was treated with 50 sandal seeds (Anith et al., 2011). Prior to biopriming treatments, seeds were surface sterilised in 1% mercuric chloride solution for 5 minutes and thoroughly washed. The glass bottles with seeds fully immersed in the priming solution. Suspension culture were covered with aluminium foil and maintained at room temperature (29–30°C) for the specified durations. Distilled water was added to make up the volume of priming solution sufficient to immserse seeds.

Upon completion of the biopriming treatments, the seeds were thoroughly washed with distilled water and placed on filter papers, under the shade till the seeds attained the pre-priming stage moisture level. The primed sandal seeds were pre-treated with a 0.05% (w/v) gibberellic acid (GA_3) solution overnight. The seeds were sown in germination trays filled with sand and maintained in the shade house throughout the experiment. Regular watering was done until the germination was completed.

2.2. Germination observations

Daily germination counts were recorded for 60 days by the time germination was completed. From these primary observations, germination percentage (G%), Mean time of germination (MTG), and Germination rate index (GRI) were calculated. The germination percentage was calculated using the formulae (Scott et al., 1984);

Germination percentage (G%) = (Number of seeds germinated)/(Total number of seeds sown)×100.....(1)

The MTG and GRI were determined using the formulae suggested by Krichen et al., 2014 and Oliveira et al., 2019 respectively;

Mean time of germination (MTG)=$(\Sigma D_n)/(\Sigma n)$; where n is the number of seeds germinated on day D, and D is the number of days counted from the beginning of germination .....(2)

Germination rate index (GRI) =$\Sigma ni/ti$; where n_i is the number of seeds germinated at time t_i, t_i is the time (in days) at which n_i seeds have germinated .....(3)

2.3. Biochemical analysis of primed seeds

In order to determine the electrical conductivity, the leachates of seeds immediately after priming were subjected to Electrical Conductivity (EC) measurement in a conductivity meter (CDC 40101). The total carbohydrate content of the seeds was estimated by the Anthrone reagent method (Yemm and Willis, 1954), and the protein content by Lowry's method (Lowry et al., 1951). Crude fat content estimation was performed with Soxhlet extraction (Kennedy and Unrau, 1949). Amylase activities (α and β amylase) estimation was carried out by the method suggested by Sadasivam and Manickam (1996), taking maltose as the standard.

2.4. Physiological methods for seedling growth observations

The seedlings of 4 to 5 leaf stage were transplanted into the polythene bags filled with soil: sand: farm yard manure, in a ratio of 3:1:1. Growth attributes like seedling height, collar diameter, leaf number, leaf area, root length, seedling fresh and dry weight were recorded at 180th days after transplanting (DAT). Vigour index was calculated using the formula (Abul-Baki and Anderson, 1973):

VI=GP×(SL+RL) .....(4)

where GP=Germination percentage, SL=Shoot height and RL=Root length.

2.5. Statistical analysis

Statistical analyses were performed using GRAPE 1.0.0 web application based on R software (https://www.kaugrapes.com, accessed on 14th March 2024). One-way analysis of variance was carried out for all the parameters, and the treatments were compared with Duncan Multiple Range Test (DMRT) at 5% probability for the post-hoc test. Data were submitted to normality by the Levene's test and homoscedasticity by Bartlett test. Arc-sine transformation was done for statistical analysis, wherever appropriate.

3. Results and Discussion

3.1. Seed germination

The analysed data on the influence of biopriming treatments on germination attributes is depicted in Table 2. Marked differences were observed in the germination percentage of the sandal seeds due to biopriming treatments. The highest germination percentage was observed from treatment PF T_8 (70.67%) against the non-primed control seeds (28.67%). The lowest germination percentage was recorded from EM T_8 (6.67%). Germination percentage recorded from PGPR–I, PGPR–II, *Piriformospora indica* biopriming treatments were 58.67% (PG1 T_8); 17.33% (PG2 T_2) and 44.67% (PI T_8), respectively. The imbibition period ranged from 13 days (PF T_4) to 41 days (EM T_6). The imbibition period for control treatment was 24 days. The mean time of germination (MTG) value ranged from 27 days in (PG2 T_2 days) to 57 days in (control seeds). Germination rate index (GRI) value ranged from 0.11 to 0.84. The germination rate index (GRI) value, an indicator of speed of germinationwas highest in PF T_8 (0.84) which was also the best treatment regarding germination percentage. The lowest GRI value was noticed in EM T_8 (0.11). The control seeds reported a germination index value of 0.28.

3.2. Changes in seed biochemical composition due to biopriming

The results of the experiment on biochemical composition of

Table 2: Efficacy of biopriming techniques on the germination attributes of sandal seeds

Biopriming treatments	Imbibition period (in days)	Germination period (in days)	Germination percentage (%)	Mean time of germination (MTG) (in days)	Germination rate index (GRI)
EM T_2	36	58	31.33^{fg} (0.594)	46	0.25
EM T_4	38	56	27.33^{g} (0.549)	53	0.28
EM T_6	41	54	10.67^{jk} (0.331)	55	0.11
EM T_8	29	52	6.67^{k} (0.258)	56	0.07
PG1 T_2	14	45	34.00^{fg} (0.622)	51	0.44
PG1 T_4	22	48	52.00^{cd} (0.806)	47	0.6
PG1 T_6	25	52	47.33^{d} (0.759)	46	0.49
PG1 T_8	15	50	58.67^{bc} (0.873)	42	0.62
PG2 T_2	24	39	17.33^{hi} (0.429)	27	0.23
PG2 T_4	22	35	13.33^{ij} (0.373)	29	0.25
PG2 T_6	23	41	10.67^{jk} (0.332)	35	0.14
PG2 T_8	19	29	6.67^{k} (0.261)	39	0.13
PF T_2	14	55	64.67^{ab} (0.935)	55	0.63
PF T_4	13	50	60.00^{b} (0.888)	48	0.72
PF T_6	17	58	50.67^{d} (0.792)	52	0.51
PF T_8	17	43	70.67^{a} (0.999)	44	0.84
PI T_2	32	50	20.67h (0.472)	56	0.22
PI T_4	29	54	29.33^{g} (0.572)	47	0.25
PI T_6	24	47	38.66^{ef} (0.671)	53	0.42
PI T_8	28	57	44.67^{de} (0.732)	50	0.42
C	24	59	28.67^{g} (0.564)	57	0.28

Data in parenthesis represent arcsine transformed values; Means with different superscripts (alphabets) differ significantly (p<0.01)

seed are presented in Table 3. Unlike germination characters studies, the seeds differed significantly in response to the various biopriming treatments. Most biopriming treatments increased the seed carbohydrate content except EM T_6, EM T_8, PGPR-II treatments. A two-fold increase in carbohydrate content was observed in PF T_8 (1.58 mg g^{-1}) compared to control seeds (0.63 mg g^{-1}). *Pseudomonas fluorescens* influenced the highest increase in carbohydrate content, followed by PGPR-I. Considerable variation was observed in the crude fat content of seeds as influenced by biopriming treatments. The crude fat content was highest noticed in PG1 T_2 (56.30%) and lowest in EM T_2 (43.33%). Electrical conductivity (EC) consequent to degradation of the cell membrane in seeds, which decreased significantly due to biopriming treatments as compared control seeds (1.07 dS cm^{-1}). EC value ranged from 0.09 dS cm^{-1} in (PI T_8) to 0.67 dS cm^{-1} in (EM T_8) among the biopriming treatments. The stored proteins in the seeds ranged from 0.04 mg g^{-1} (PG2 T_8) to 0.08 (PI T_8) mg g^{-1} after biopriming. In all the biopriming treatments, the α and β amylase activity increased compared to control seeds. That implies the influence of biopriming treatments for significantly increasing the amylase activity in seeds. α amylase activity ranged from 4.54 m mol^{-1} mg^{-1} in (PG2 T_8) to 5.69 m mol^{-1} mg^{-1} in (PF T_8), whereas β amylase activity ranged from 2.26 m mol^{-1} mg^{-1} in (PG2 T_6) to 2.61 m mol^{-1} mg^{-1} in (PG1 T_6 and PG1 T_8) among the biopriming treatments.

3.1. Seedling growth and biomass production

Growth attributes of the sandal seedlings originated from the seeds subjected to biopriming treatments 180 days after transplanting (DAT) and exhibited significant variation across various bioprimingtreatments (Table 3). Results of biometric parameters of seedlings after 180 DAT indicated that the seedling height was the highest in PF T_4 (70.97 cm) and lowest in PG2 T_8 (47.83 cm), whereas the control seedlings recorded a value of 47.67 cm. All the *Pseudomonas fluorescens* were statistically at par with each other regarding seedling height. Meanwhile, the highest collar diameter was recorded in EM T_6 (3.67 mm) and EM T_8, EM T_4 and PG2 T_2 treatments, which

Table 3: Changes in the seed biochemical compositionof the sandal seeds subjected to biopriming treatments

Biopriming treatments (With treatment code)	Carbohydrate (mg g^{-1})	Crude fat (%)	Electrical conductivity (dS cm^{-1})	Protein (mg g^{-1})	α amylase (m mol^{-1} mg^{-1})	β amylase (m mol^{-1} mg^{-1})
EM T_2	0.71^{jk}	43.33^{i}	0.52^{de}	0.07^{bc}	5.29^{cde}	2.34^{efg}
EM T_4	0.70^{jkl}	44.42^{i}	0.53^{de}	0.06^{cde}	5.16^{e}	2.28^{fg}
EM T_6	0.63^{lm}	45.72^{h}	0.65^{bc}	0.06^{bcd}	4.86^{fg}	2.29^{fg}
EM T_8	0.64^{lm}	47.64^{g}	0.67^{b}	0.05^{def}	4.55^{i}	2.30^{fg}
PG1 T_2	0.91^{fg}	56.30^{a}	0.59^{bcd}	0.04^{fg}	$4.66g^{hi}$	2.48^{bcd}
PG1 T_4	0.96^{ef}	54.81^{b}	0.54^{cd}	0.05^{efg}	4.59^{hi}	2.52^{abc}
PG1 T_6	0.99^{e}	52.75^{cd}	0.50^{de}	0.06^{bcd}	4.88^{fg}	2.61^{a}
PG1 T_8	1.07^{d}	50.48^{e}	0.42^{e}	0.07^{bc}	5.44^{bcd}	2.61^{a}
PG2 T_2	0.68^{jklm}	50.30^{e}	0.46^{de}	0.06^{cde}	4.92^{f}	2.55^{ab}
PG2 T_4	0.66^{klm}	52.22^{d}	0.49^{de}	0.05^{def}	4.90^{f}	2.32^{efg}
PG2 T_6	0.62^{m}	52.66^{cd}	0.56^{cd}	0.05^{efg}	4.79^{fgh}	2.26^{g}
PG2 T_8	0.62^{m}	53.70^{bc}	0.55^{cd}	0.04^{g}	4.54^{i}	2.30^{fg}
PF T_2	1.39^{c}	54.10^{b}	0.22^{f}	0.06^{bcd}	5.27^{de}	2.39^{def}
PF T_4	1.42^{bc}	52.58^{cd}	0.19fg	0.07^{bc}	5.50^{abcd}	2.49^{bcd}
PF T_6	1.47^{b}	51.87^{d}	0.21^{fg}	0.07^{ab}	5.62^{ab}	2.49^{bcd}
PF T_8	1.58^{a}	49.78^{ef}	0.18^{fg}	0.07^{ab}	5.69^{a}	2.53^{abc}
PI T_2	0.75^{ij}	50.38^{e}	0.21^{fg}	0.07^{bc}	5.31^{cde}	2.34^{efg}
PI T_4	0.82^{h}	49.37^{ef}	0.21^{fg}	0.07^{ab}	5.52^{ab}	2.36^{efg}
PI T_6	0.81^{hi}	48.94^{f}	0.12^{fg}	0.07^{ab}	5.37^{cde}	2.38^{def}
PI T_8	0.85^{gh}	48.77^{fg}	0.09^{g}	0.08^{a}	5.35^{cde}	2.42^{cde}
C	0.63^{lm}	54.13^{b}	1.07^{a}	0.06^{cde}	4.29^{j}	1.78^{h}
SEm±	0.023	0.432	0.037	0.004	0.073	0.034

Means with different superscripts (alphabets) differ significantly (p<0.01); SEm±= Standard error of the mean

were statistically at par. Among the biopriming treatments, the lowest value was observed in PI T_8 (1.82 mm). PG1 T_8 reported the highest leaf number (32.67 numbers), while the lowest was for EM T_8 (16 numbers). On the other hand, the highest leaf area was observed in PG1 T_8 (156.62 cm^2) and the lowest in PG2 T_8 (98.33 cm^2). Highest increment in root length was noticed for PI T_6 (54.20 cm), followed by PI T_4 (50.87 cm). The lowest value of root length was recorded in PG2 T8 (26.13 cm). Number of lateral roots did not differ significantly among the biopriming treatments and control seedlings. The effect of biopriming was apparent in the biomass production of the sandal seedlings e.g. seedling fresh weight and seedling dry weight. The highest seedling fresh weight was obtained in PG1 T_8 (4.71 g) and lowest in EM T8 (3.01 g), whereas the control seedlings recorded a weight of 3.50 g. On the other hand, PG1 T_2 recorded the highest seedling dry weight (1.78 g) and EM T_6 (1.16 g), the lowest. Control seedlings valued at 1.47 g for seedling dry weight. The vigour index designed to assess seedlings' quality, that considered germination and biometric parameter are presented in Table 4 and Figure 1. *Pseudomonas fluorescens* treatments outperformed other biopriming treatments regarding the seedling vigour index. PF T_8 recorded the highest vigour index (8257.67), followed by PF T_2 (6797.06), PF T_4 (6759.47). PG2 T_8 was the lowest regarding the vigour index, with a value (494.40). Treatments PG2 T_2; PG2 T_4; EM T_6; PG2 T_6; EM T_8, and PG2 T_8 were substandard compared to the control seedlings in overall seedling vigour index.

Seed priming is a highly effective and resource efficient technique for enhancing seed quality, promoting optimal germination and seedling establishment (Blunk et al., 2019; Pedrini et al., 2020). Our results demonstrated that the biopriming of sandal seeds could improve the seed germination characteristics, growth and vigour of seedlings. Biopriming with *Pseudomonas fluorescens* culture could improve the germination and seedling growth attributes in sandal followed by PGPR–I. The performance of seed germination and seedling quality parameters were increased at higher duration of biopriming with *Piriformospora indica*. In nursery practice, sandal seed germination percentage is reported to be very low (15–35%) due to morpho-

Table 4 Effect of biopriming treatments on the growth and biomass accumulation of sandal seedlings at 180 days after transplanting

Biopriming treatment code	Seedling height (cm)	Collar diameter (mm)	Leaf number	Leaf area (cm^2)	Root length (cm)	Number of lateral roots	Seedling fresh weight (g)	Seedling dry weight (g)
EM T_2	60.20^{bcde}	2.28^{efghi}	20.33^{defg}	121.81^{bcde}	44.60^{cde}	21.67	3.85^{cdefg}	1.57^{abc}
EM T_4	54.87^{cdef}	3.52^{a}	22.00^{bcdefg}	142.09^{ab}	35.63^{hijk}	21.00	3.68^{cdefgh}	1.52^{abcd}
EM T_6	53.77^{def}	3.67^{a}	18.67^{fg}	140.39^{ab}	38.27^{fghi}	18.67	3.24^{fgh}	1.16^{e}
EM T_8	52.47^{def}	3.56^{a}	16.00^{g}	135.22^{abc}	34.03^{ijkl}	20.67	3.01^{h}	1.18^{de}
PG1 T_2	50.60^{ef}	2.31^{defghi}	20.67^{cdefg}	128.99^{abcd}	30.47^{klm}	17.67	4.63^{ab}	1.78^{a}
PG1 T_4	58.43^{bcde}	2.74^{cd}	32.67^{a}	126.98^{abcde}	37.20^{ghi}	17.33	4.40^{abc}	1.70^{ab}
PG1 T_6	52.63^{def}	2.57^{cdef}	19.67^{efg}	143.32^{ab}	36.17^{ghij}	19.33	4.15^{abcde}	1.40^{bcde}
PG1 T_8	56.97^{cdef}	2.67^{cde}	25.00^{abcdef}	156.62^{a}	39.63^{efghi}	21.67	4.71^{a}	1.67^{ab}
PG2 T_2	60.83^{bcde}	3.36^{a}	21.33^{bcdefg}	115.07^{bcde}	30.93^{jklm}	23.00	3.56^{defgh}	1.58^{abc}
PG2 T_4	59.43^{bcde}	2.97^{bc}	23.67^{bcdefg}	114.42^{bcde}	36.90^{ghi}	19.67	3.43^{efgh}	1.23^{cde}
PG2 T_6	53.00^{def}	2.55^{cdefg}	22.33^{bcdefg}	110.15^{cde}	29.70^{lm}	21.33	3.22^{fgh}	1.43^{abcde}
PG2 T_8	47.83^{f}	2.41^{defg}	26.00^{abcdef}	98.33^{e}	26.13^{m}	18.67	3.14^{gh}	1.19^{de}
PF T_2	61.47^{abcd}	2.23^{efghij}	27.33^{abcde}	129.63^{abcd}	44.07^{cde}	19.00	3.94^{bcdef}	1.64^{ab}
PF T_4	70.97^{a}	2.16^{fghij}	27.67^{abcde}	140.87^{ab}	41.50^{defg}	21.00	4.32^{abc}	1.73^{ab}
PF T_6	70.70^{a}	2.32^{defghi}	29.33^{ab}	143.04^{ab}	40.60^{efgh}	19.33	4.27^{abcd}	1.63^{ab}
PF T_8	68.23^{ab}	2.52^{defg}	27.00^{abcdef}	128.28^{abcd}	48.60^{bc}	20.33	4.30^{abcd}	1.55^{abc}
PI T_2	64.90^{abc}	2.03^{hij}	24.00^{bcdefg}	129.76^{abcd}	43.43^{cdef}	21.33	3.72^{cdefgh}	1.39^{bcde}
PI T_4	57.30^{cdef}	2.09^{ghij}	29.00^{abc}	139.28^{abc}	50.87^{ab}	22.00	4.11^{abcde}	1.68^{ab}
PI T_6	60.43^{bcde}	1.87^{ij}	28.33^{abcd}	132.19^{abc}	54.20^{a}	21.33	3.82^{cdefg}	1.52^{abcd}
PI T_8	57.23^{cdef}	1.82^{j}	27.33^{abcde}	117.43^{bcde}	47.10^{bcd}	24.33	3.74^{cdefgh}	1.44^{abcde}
C	47.67^{f}	1.97^{hij}	19.67^{efg}	102.32^{de}	$28.94l^{m}$	18.67	3.50^{efgh}	1.47^{abcde}
SEm±	3.053	0.138	2.461	8.788	1.778	1.853 (p= 0.585)	0.223	0.105

Means with different superscripts (alphabets) differ significantly (p<0.01); SEm±=Standard error of mean

physiological dormancy of the seed and the prolonged germination period is a major constraint in raising seedlings commercially (Manonmani and Vanangamudi, 2002; Nagaveni and Vijayalakshmi, 2007). With the adaptation of biopriming techniques, the germination percentage could be increased to 70.67% in seeds primed with *Pseudomonas fluorescens* for 8 days. With the adoption of biopriming, in the present study, the total germination period of sandal seeds was also reduced to 43 days from 59 days (control) with a 146% increase in germination percentage. These results conform with the findings of Rodriguez et al. (2015), they reported that biopriming of seeds of *Abies hickelii* with *Pseudomonas fluorescens* increased the germination percent by 91% over control. The biopriming treatments could also reduce the days to initiate germination to 13 days compared to the 24 days of control seeds. From the findings of the present study, it can be concluded that biopriming with *Pseudomonas fluorescens*, PGPR–I and *Piriformospora indica*could to overcome seed dormancy in sandal seeds, resulting in greater and uniform germination. The superiority of *Pseudomonas fluorescens* over other biotic agents in enhancing germination attributes may result from its ability to maintain better homeostasis of plant hormones,which regulate various aspects of plant growth, including seed germination (Meliani et al., 2017; Ortiz-Castro et al., 2020). Contrasting to PGPR–I, PGPR–II was not competent enough to improve the germination attributes, and the germination percentages (6.67% to 17.33%) observed from these treatments were even lower than those of the control seeds. The wide effect on the performance of PGPR–I and PGPR–II on seed germination attributes may be due to the different cultures of bacterial strains and is supported by the study of Nezarat and Gholami (2009), they researched screening out of different plant growth promoting rhizobacteria for improving seed germination

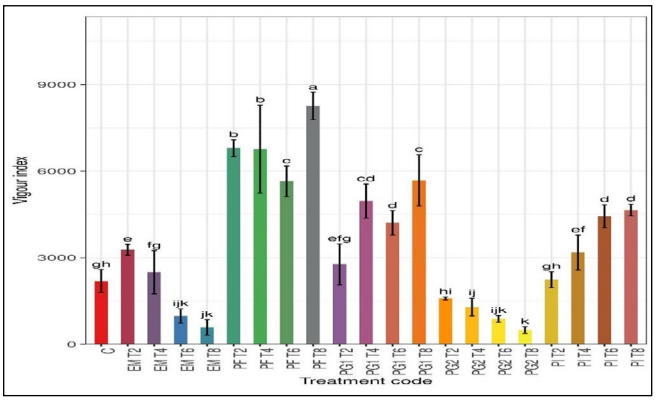

Figure 1: Seedling vigour index corresponds to biopriming treatments and control seedlings at 180 DAT

and seedling growth and yield of. The results obtained from biopriming with Effective microorganisms in sandal showed that the germination percentage was decreasing with in the duration of treatment in days. A higher duration of treatment with Effective microorganisms was found to be detrimental to seeds. Many biochemical and physiological factors are involved in seed priming, including the cellular/solute osmotic potential, plant growth regulators, and enzymatic activity, which are governed by priming. Efficacy of priming treatments is influenced by the priming substrate, duration of treatment and priming environment and species (Thomas et al., 2000; Ma et al., 2003; Kanto et al., 2015).

The seed reserve material content is normally correlated with germination percentages or speed of germination (Sharma et al., 2007; Soriano et al., 2014). Carbohydrates are a vital seed reserve that provides energy and nutrients to support seed germination until the seedling can establish photosynthesis and produce its carbohydrates. The carbohydrate content in the seeds facilitates the greater germination value in seeds of tree species with recalcitrant seed storage behaviour (Lin and Huang, 1994). The highest carbohydrate content was observed in the seeds bioprimed with *Pseudomonas fluorescens* followed by *Piriformospora indica*. The carbohydrate content was positively correlated with the germination rate of *Glycine max* L. seeds (Hussain et al., 2019); the present study is results conform with this. The highest increase in carbohydrate content recorded in *Pseudomonas fluorescens* treatments resulted in the highest germination percentage and rate. A decrease in the fat content of the seeds subjected to biopriming was noticed in the case of all biotic agents except PGPR–II, which was also related to the lowest performing regarding germination. The effect of priming is associated with a decrease in the level of lipid peroxidation and restoration of antioxidant defence systems (Bailly et al., 2000). Fatty acid content was negatively correlated with the germination percentage of *Gossypium spp.* seeds (Hoffpauir et al., 1950), the results of the present study conforms with these findings. This might be due to the conversion of lipids into sugars, which is common in seeds with greater oil content (Borek et al., 2015). An increase in leachate content when soaked in water is often a symptom of deteriorated seeds. Primary objective of priming is to reduce the seed leachate's electrical conductivity (EC) (Perry, 1977). Lower values of EC of leachates of seeds subjected to biopriming (*Piriformospora indica* and *Pseudomonas fluorescens*) in the present study indicate a reduction in seed leakage, leading to better membrane integrity of sandal seeds. The highest germination percentage was also recorded from the *Pseudomonas fluorescens* and PGPR-I treatments, implying biopriming with this biotic agent leads to a membrane repair mechanism, enhancing its stability and better germination. This aligns with the finding of Patel et al. (2017) that biopriming resulted in a low electrical conductivity value compared to hydropriming and control. The stored proteins increase during the seed priming process (Job et al., 2000; Soriano et al., 2014). From the present study, due to the influence of biopriming, the highest protein content was obtained from seeds bioprimed with *Piriformospora indica* followed by *Pseudomonas fluorescens* treatments. Biopriming treatments impart a 30% increase in the seed protein content. Biopriming has been reported to increase the seed protein content in safflower (Silva et al., 2013) and soybean (Sharifi, 2012). The increase in the activity of amylases indicated that the depletion of seed food reserves (starch) amounts causes faster germination and better seedling health. The highest level of α amylase activity was noticed in seeds bioprimed with *Pseudomonas fluorescens* at the longer duration of treatment (4 and 6 days), followed by *Piriformospora indica*. In the case of β amylase activity, PGPR-I was the highest performing, followed by *Pseudomonas fluorescens*. Similar results have been reported by Lee et al. (2000) in primed rice seed.

Seed priming enhances and promotes faster growth of young seedlings with enhanced quality (Sharma et al., 2014; Rehman et al., 2015). PF T_8 (Biopriming with *Pseudomonas fluorescens* for 8 days) is the best treatment regarding seedling vigour (Figure 1), which is also superior regarding germination percentage (Table 2). Seeds subjected to bioprimed with *Pseudomonas fluorescens* exhibited superior growth and biomass production in terms of seedling height, leaf number, leaf area, root length, seedling fresh weight and dry weight at 180 days after transplanting (DAT) followed by and PGPR-I and *Piriformospora indica* treatments. These results supporting the superiority of *Pseudomonas fluorescens* regarding seedling growth attributes were similar with the findings reported in chilli (Ananthi et al., 2014) and cabbage (Vij et al., 2022). Enhancements in germination and vigour may arise from processes such as the mobilisation of reserve food materials, the repair and synthesis of nucleic acids, increased RNA and protein synthesis, and enhanced enzyme activity during biopriming. Jijeesh and Sudhakara (2016) also obtained a high positive correlation between the vigour index and biochemical constituents of crude oil and soluble and total carbohydrates in *Tectona grandis*. Plant growth-promoting bacteria have been reported to increase

the production of some phytohormones such as gibberellic acid, auxin etc. that trigger plant root development, shoot growth and increase the biomass of crop plants (Patten and Glick, 2002; Spaepen et al., 2007). The present result showing better performance of PGPR–I bioprimed seeds for growth attributes may be ascribed to this reason. The better root length observed in *Piriformospora indica* group at 180 DAT may be due to endophytic root colonising properties of the fungus, which stimulates the production of plant growth-promoting hormones such as auxins and cytokinins, which regulate various physiological processes (Shrivastava and Varma, 2014). Better root architecture leads to efficient nutrient uptake and improvement in plant growth; this result regarding better performance of *Piriformospora indica* was in accordance with the findings reported by Cheng et al. (2022) in longan seedlings, Sirrenberg et al. (2007) in *Arabidopsis* plant.

4. Conclusion

Biopriming techniques significantly influenced the seed germination characteristics and seedling growth of *Santalum album* L. compared to control. Among the biopriming agents used in the present study, *Pseudomonas fluorescence* significantly influenced seed germination and seedling growth of sandal, resulting in enhanced germination and seedling performance, followed by PGPR-I and *Piriformospora indica*. The positive impact of biopriming techniques (with *Pseudomonas fluorescence,* PGPR-I and *Piriformospora indica*) on the growth of the seedlings was connected with lower electrical conductance, higher amylase activity, and higher carbohydrate and lower fat concentration of seeds. Biopriming with *Pseudomonas fluorescence,* PGPR-I and *Piriformospora indica* with longer duration of treatment i.e. 4-8 days and 6-8 days, respectively can be recommended as an effective and low-cost technology to enhance seed germination and seedling growth of sandals to produce quality plant stock in nurseries.

5. Acknowledgement

The financial support received from Kerala Agricultural University for conducting the research is duly acknowledged.

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