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Biochemical Changes and Germination Response of Indian Sandalwood (*Santalum album* L.) Seeds Subjected to Biopriming Techniques and Variable Post-priming Storage

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Understanding of the storage behaviour of endangered plant species such as Indian Sandalwood, *Santalum album* L. is important for successful conservation. We tested biopriming method with five biopriming agents (e.g. Effective microorganisms; Plant growth promoting rhizobacteria (PGPR-I

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and PGPR-II); *Pseudomonas fluorescens* and *Piriformospora indica*) with four uniform durations of treatments viz. 2, 4, 6 and 8 days. Hydropriming was also performed with similar condition for comparison of seed performance. The study examined the biochemical changes that occur in Sandalwood seeds during different post-priming storage periods (0, 30, 60, 90 days) to assess the efficacy of biopriming techniques on seed germination and health. Biopriming with *Pseudomonas fluorescens* for eight days recorded the highest germination percentage (70.67%) and the highest germination rate index (0.84). Longer durations of PGPR-I and *Piriformospora indica* treatment (6-8 days) lowered the imbibition period and mean germination time while increasing the germination rate. After a post-priming storage period of 30 days, only EM and *Pseudomonas fluorescens* treated seeds germinated while all other treatments failed to germinate. Absence of germination in any biopriming group at 60 and 90 days storage period indicated that the sandal seeds deteriorated over extended storage period. The reduction in seed viability of sandal seeds was consequent to the changes in the biochemical characteristics and action of enzymes (α and β amylases) involved in seed food reserve depletion and loss in membrane integrity.

Keywords: Seed storage; sandalwood; amylase activity; germination; membrane integrity; biopriming; hydropriming.

1. INTRODUCTION

Seed priming is a reliable method for improving seed quality. It is a controlled dehydration technique that allows partial seed hydration, enabling pre-germinative metabolic activity while preventing actual germination [1]. The benefits of seed priming include increased germination percentage and speed, preparation of seeds to germinate under a broader range of environmental conditions, and enhanced seedling vigour and growth, all in a cost-effective way [2,3]. These positive effects are attributed to the induction of the biochemical mechanisms of cell repair, activation of the antioxidant defence system, and the induction of enzymes that catalyze the decomposition and mobilization of storage compounds [4]. Biopriming is a popular approach among seed priming treatments which includes inoculation of seed with beneficial microorganisms (biological aspect) and seed hydration (physiological aspect) to induce changes in plant characteristics and facilitate uniform seed germination and growth associated with microorganism inoculation [5].

Santalum album Linn. (Indian sandalwood) is an evergreen, aggressive obligate hemi-parasitic tree renowned for its unique fragrance of its wood oil. The increased global demand for sandalwood has led to a decline in its population due to illegal harvesting and over-exploitation in the wild, pushing the species to the verge of extinction [6,7]. *Santalum album* is commonly propagated by seeds [8]. Seed traits of sandalwood are significantly affected by seed source, provenances, plus trees and clones. They are also greatly dependent on the storage method (Manonmani and Vanangamudi, 2001). However, the impact of post-priming storage on seed performance in sandalwood has not been extensively studied. Therefore, it is essential to identify the factors affecting seed viability during storage. The growing demand for large-scale planting stock necessitates the need for developing seed-handling protocols for the long-term viability of seeds. Seed deterioration during storage period is an irreversible and degenerative change in seed quality, attributed to various biophysical and biochemical changes in seed components, such as the loss of enzymatic activities, loss of membrane integrity, accumulation of toxic substances and genetic alterations [9,10].

Seed priming is a commercially successful practice, but the reduced longevity of primed seeds as compared to non-primed seeds during storage may limit its broader adoption [11,12]. The viability of several seeds deteriorates during extended storage periods [13], though the rate of deterioration varies significantly among different species [14]. The loss of viability in primed seeds during storage is a major barrier to the widespread adoption of the seed priming technique. To adopt the best storage conditions, it is essential to understand the biochemical processes that lead to the loss of seed viability, as this knowledge can help delay seed deterioration [15,9]. The perusal of literature indicated that the studies on seed storage behaviour after application of priming on sandalwood seeds is lacking. Understanding the post-priming storage behaviour in terms of seed viability would be valuable for the adoption of seed priming techniques for sandalwood.

2. MATERIALS AND METHODS

2.1 Plant Material and Biopriming Treatments

The experimentation was carried out at the Department of Silviculture and Agroforestry, College of Forestry, Kerala Agricultural University, 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 h and thoroughly mixed to improve homogeneity. For the germination test, three replications of 50 seeds from each treatment were tested.

The sandal seeds were subjected to biopriming with five biotic agents viz. (Effective microorganisms (EM); PGPR-I; PGPR-II; *Pseudomonas fluorescens* and *Piriforomospora indica*) and four uniform durations of treatments in days viz. 2, 4, 6 and 8 days with one group kept as control, i.e. without any biopriming treatment. Hydropriming with similar condition with distilled water was also performed. The Effective microorganisms (EM) stock solution was procured from Maple Biotech India Ltd., the only authorized producer of EM in India based on EM Research Organization Inc. (EMRO), Japan [16]. One ml of EM stock solution was diluted to one litre with distilled water. Total number of seed sample were taken and volume was measured using measuring cylinder. The seeds and the solution were made 1:2 volume for priming. 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 Agriculture, Vellanikkara, Kerala Agricultural University, Thrissur. The suspension culture of these three bioinoculants contains 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} produce 100% concentration for 50 sandal seeds. Hence, the ratio of suspension culture to the number of seeds to be primed were taken in the ratio 2:5 on volume basis. The culture of *Piriformospora indica*, an endophytic fungus was supplied by Department of Microbiology, College of Agriculture, Vellayani, Kerala Agricultural University. The fungus was cultured in Potato Dextrose Agar (PDA) medium and extracted following the protocol of Anith et al. [17]. For biopriming with *Piriformospora indica*, 5g of culture of this fungus was treated with 50 sandal seeds. Prior to biopriming treatments, seeds were surface sterilized in 1% mercuric chloride solution for 5 minutes and thoroughly washed before subjecting to biopriming. The glass bottles with seeds fully immersed in the priming solution were covered with aluminium foil and maintained at room temperature (29–30° C) for the specified durations. Sandal seeds were mixed with suspension culture in the sterilized glass bottles of 200 ml capacity and covered with aluminium foil. During treatment period, distilled water was added to make up the volume of priming solution sufficient to suspend seeds.

2.2 Post-priming Storage Period

The primed seeds were taken out of the respective biopriming agents and were thoroughly washed with distilled water thrice. Then it is allowed to dry on Whatman filter paper in shade at 25° C till the seeds achieved the moisture level prior to priming. The re-dried seeds were transferred to paper bags, kept in glass containers and stored at ambient condition in glass jars for 30, 60, 90 days period to study the effect of post-priming storage on seeds. The primed sandal seeds were pre-treated with a 0.05% (w/v) gibberellic acid (GA_3) solution overnight prior to sowing. The seeds were sown during May 2022 in germination trays filled with sand kept in the shade house at tree nursery of College of Forestry. Regular watering was done until the germination was completed.

2.3 Germination Observations

Daily germination counts were recorded for a period of 60 days by the time germination was completed. From these primary observations, germination percentage (G%), Mean time of germination (MTG), Germination rate index (GRI) was calculated. The germination percentage was calculated using the formulae [18];

$$\text{Germination percentage (G\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100 \quad (1)$$

The MTG and GRI were determined using the formulae suggested by [19] and [20] respectively;

Mean time of germination (MTG) = $\frac{\Sigma D_n}{\Sigma n}$; 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) =$\Sigma \frac{n_i}{t_i}$; 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)

2.4 Biochemical Analysis of Primed Seeds

In order to determine the electrical conductivity, the leachates of seeds immediately after priming were subjected to EC measurement in a conductivity meter (CDC 40101). The EC was directly obtained from the conductivity meter and was expressed in dS cm^{-1}. The total carbohydrate content of the seeds was estimated by the Anthrone reagent method [21], and the protein content by Lowry's method [22] by preparing standard curves and taking absorbance in a spectrophotometer (Hach DR 6000, Germany). Crude fat content estimation was performed with Soxhlet extraction [23]. Amylase activities (α and β amylase) estimation was determined using the 5- dinitosalicylic (DNS) method [24].

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 18th May, 2024). One-way analysis of variance was carried out for all the parameter 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 the Bartlett test and arc-sine transformed for analysis wherever appropriate.

3. RESULTS AND DISCUSSION

3.1 Seed Germination

The data on the influence of biopriming treatments on germination attributes is presented in Table 1 for seeds undergone no storage. The imbibition period ranged from a lowest 13 days in (*Pseudomonas fluorescens*, 4 days treatment) to a highest 41 days in (Effective microorganisms, 6 days treatment). However, imbibition period was 24 days for control seeds. Among the biopriming treatments, germination period of seeds ranged from 29 days (PGPR-II, 8 days treatment) to 58 days (EM, 4 days treatment and *Pseudomonas fluorescens*, 4 days treatment) among the biopriming treatments while the corresponding range for hydroprimed seeds was 40 to 55 days. Marked differences were observed in the germination percentage of the sandal seeds due to biopriming treatments as compared to the corresponding hydropriming treatments and control seeds. The highest germination percentage was obtained for the seeds subjected to biopriming with *Pseudomonas fluorescens* for 8 days (70.67%), and the lowest was recorded from Effective microorganisms (EM) and PGPR-I treated with 8 days (6.67%) each. From the hydropriming treatments, the 2 days treated seeds resulted the highest germination percentage (36%). The control seeds obtained a germination percentage of 28.67%. The mean time of germination (MTG) value in days ranged from 27 days in (PGPR-II, 2 days) to 58 days in (hydropriming, 8 days). The control seeds obtained a MTG value of 57 days. Germination rate index (GRI) value ranged from 0.11 to 0.84. The highest value of GRI was obtained in seeds treated with *Pseudomonas fluorescens* for 8 days and lowest in Effective microorganisms for 8 days. The control seeds reported a lower germination index value of 0.28. The superiority of *Pseudomonas fluorescens* as comparison to other biotic agents regarding improvement in germination attributes may be due to better homeostasis of plant hormones that regulates the various aspects of plant growth including seed germination [25], [26], that helps in increasing speed and uniformity of germination. The improvement in germination attributes with PGPR-I in the present study may be attributed to rhizosphere colonization and production of phytohormones that triggers the seed germination process [27].

Table 2 represents the germination attributes of seeds kept for 30 days storage. There was no germination obtained from seeds bioprimed with PGPR-I, PGPR-II, *Piriformospora indica*; corresponding hydroprimed seeds and control seeds. Imbibition period of seeds ranged from 17 days in (*Pseudomonas fluorescens*, 4 days treatment) to 40 days in (EM, 6 days treatment).

Table 1. Effect of biopriming techniques on germination attributes of sandal seeds (without any storage period)

Biopriming treatments		Imbibition period (in days)	Germination period (in days)	Germination percentage (%)	Mean time of germination (MTG) (in days)	Germination rate index (GRI)
Effective microorganisms	2 days	36	58	31.33^{fgh} (0.593)	46	0.25
	4 days	38	56	27.33^{hi} (0.549)	53	0.28
	6 days	41	54	10.67^{lm} (0.331)	55	0.11
	8 days	29	52	6.67^{m} (0.258)	56	0.07
PGPR-I	2 days	14	45	34.00^{fgh} (0.621)	51	0.44
	4 days	22	48	52.00^{cd} (0.805)	47	0.6
	6 days	25	52	47.33^{d} (0.758)	46	0.49
	8 days	15	50	58.67^{bc} (0.872)	42	0.62
PGPR-II	2 days	24	39	17.33^{jk} (0.429)	27	0.23
	4 days	22	35	13.33^{kl} (0.373)	29	0.25
	6 days	23	41	10.67^{lm} (0.332)	35	0.14
	8 days	19	29	6.67^{m} (0.260)	39	0.13
Pseudomonas fluorescens	2 days	14	55	64.67^{ab} (0.935)	55	0.63
	4 days	13	50	60.00^{b} (0.888)	48	0.72
	6 days	17	58	50.67^{d} (0.792)	52	0.51
	8 days	17	43	70.67^{a} (0.998)	44	0.84
Piriformospora indica	2 days	32	50	20.67^{ij} (0.471)	56	0.22
	4 days	29	54	29.33^{gh} (0.571)	47	0.25
	6 days	24	47	38.67^{ef} (0.670)	53	0.42
	8 days	28	57	44.67^{de} (0.731)	50	0.42
Hydropriming	2 days	21	55	36.00^{fg} (0.643)	40	0.36
	4 days	23	59	27.33^{hi} (0.549)	50	0.27
	6 days	25	40	16.67^{jk} (0.419)	52	0.25
	8 days	19	52	10.67^{lm} (0.331)	58	0.11
Control (non- primed)		24	59	28.67^{gh} (0.564)	57	0.28

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

Table 2. Effect of biopriming techniques on germination attributes of sandal seeds (without any storage period) cont. of Table 1

Biopriming treatments		Imbibition period (in days)	Germination period (in days)	Germination percentage (%)	Mean time of germination (MTG)	Germination rate index (GRI)
Effective microorganisms	2 days	28	50	36.67^{a} (0.650)	49	0.36
	4 days	31	52	26.00^{b} (0.533)	51	0.19
	6 days	40	57	10.00^{d} (0.320)	50	0.08
	8 days	-	-	-	-	-
Pseudomonas fluorescens	2 days	23	56	26.00^{b} (0.533)	43	0.28
	4 days	17	48	22.67^{b} (0.494)	46	0.29
	6 days	26	51	12.00^{cd} (0.350)	49	0.15
	8 days	30	43	18.67^{bc} (0.445)	51	0.25

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

Germination period of seeds ranged from 43 days in (*Pseudomonas fluorescens*, 8 days treatment) to 57 days in (EM, 6 days treatment). The highest germination percentage was obtained for the seeds subjected to biopriming with EM for 2 days (36.67%), and the lowest in EM for 6 days (10%). The mean time of germination (MTG) value in days ranged from 43 to 51 days. The lowest value of MTG for this storage period was obtained in seeds treated with *Pseudomonas fluorescens* for 2 days (43) and highest in Effective microorganisms for 4 days and *Pseudomonas fluorescens*, 8 days (51). Germination rate index (GRI) value ranged from 0.08 to 0.36 with the highest value of GRI was obtained in seeds treated with EM for 2 days and lowest in *Pseudomonas fluorescens* for 4 days. For the seeds bioprimed with *Pseudomonas fluorescens*, it can be inferred that, the lack of germination of the seeds subjected to one-month storage after priming can be due to the reversion of benefits obtained by priming during storage. This was in compliance with the studies in sweet corn [28] and bitter gourd seeds [29], which indicated that the reduced storability of the primed seeds may be due to the decreased activity of antioxidant enzymes resulting an increase in the lipid peroxidation activity and production of reactive oxygen species.

3.2 Changes in Seed Biochemical Composition Due to Biopriming

3.2.1 At no storage period

Table 3 represents the seed biochemical composition of at 'no storage' period. Most of the biopriming treatments increased the seed carbohydrate content except EM, 6 days; EM, 8 days and PGPR-II treatments. Almost a two-fold increase in carbohydrate content was observed in *Piriformospora indica*, 8 days (1.58 mg g^{-1}) as comparison to control seeds (0.63 mg g^{-1}). The seed reserve material content is normally correlated with germination percentages or speed of germination [30,31]. The carbohydrate content in the seeds facilitates the greater germination value in seeds of tree species with recalcitrant seed storage behaviour [32]. *Pseudomonas fluorescens* influenced the highest increase in carbohydrate content followed by PGPR-I. The carbohydrate content was positively correlated with the germination rate of *Glycine max* L. seeds [33]; the result of the present study is in conformity with this. The increase in carbohydrate content in the seeds increased with increasing duration of treatments (in days) in the biopriming treatments e.g. PGPR-I, *Pseudomonas fluorescens* and *Piriformospora indica***.** Whereas, it was found in reverse trend in PGPR-II and EM. The effect of priming is associated with a decrease in the level of lipid peroxidation and restoration of antioxidant defence systems [34]. Fatty acid content was negatively correlated with the germination percentage of *Gossypium spp.* seeds [35], the results of the present study conforms with these findings. Considerable variation was observed in crude fat content of seeds as influenced by biopriming treatments. The crude fat content was highest noticed in PGPR-I, 2 days (56.30%) and lowest in EM, 2 days (43.33%). Electrical conductivity (EC) that explains the degradation of cell membrane system seeds, decreased significantly due to biopriming treatments as comparison to control seeds (1.07 dS cm^{-1}). EC value ranged from 0.09 dS cm^{-1} in (*Piriformospora indica*, 8 days) to 0.67 dS cm^{-1} in (EM, 8 days) among the biopriming treatments. 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. Our result aligns with the finding of [36] that biopriming resulted in a low electrical conductivity value compared to hydropriming and control. The stored proteins in the seeds ranged from 0.04 mg g^{-1} (PGPR-II, 8 days) to 0.08 (*Piriformospora indica*, 8 days) mg g^{-1} after biopriming. The stored proteins increase during the seed priming process [37,30]. 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. Which were also better performed regarding germination percentage also. In all the biopriming treatments the α and β amylases activity increased as comparison to control seeds. That implies the influence of biopriming treatments for significantly increasing the amylases activity in seeds. α amylase activity ranged from 4.54 m mol^{-1} mg^{-1} in (PGPR-II, 8 days) to 5.69 m mol^{-1} mg^{-1} in (*Pseudomonas fluorescens*, 8 days), whereas β amylase activity ranged from 2.26 m mol^{-1} mg^{-1} in (PGPR-II, 6 days) to 2.61 m mol^{-1} mg^{-1} in (PGPR-I, 6 days and PGPR-I, 8 days) among the biopriming treatments. The increase in the activity of amylases indicated that the depletion of seed food reserves (starch) amounts causes faster germination and better seedling health.

3.2.2 At 30 days storage period

The changes in the biochemical attributes of the seeds after 30 days post-priming storage period is presented in Table 4. The utilization of seed reserves is an important characteristic of seed quality. To adopt the best storage conditions, the specific information which throw light on the biochemical processes leading to the loss of seed viability is essential to delay seed deterioration. The results revealed that seeds treated with *Pseudomonas fluorescens* exhibited highest carbohydrate content, where all the duration of treatments were at par for this biopriming agent. The carbohydrate content ranged from 0.42 mg g^{-1} to 0.49 mg g^{-1} in hydropriming treatment group which was lower than all the biopriming treatment groups. Among the biopriming treatments, highest crude fat content (50.50%) was obtained in seeds bioprimed with *Piriformospora indica* for 2 days and the lowest value (39.02%) was obtained from EM, 8 days treated seeds. The crude fat content was found to be decreasing with increasing in duration of treatment after the 30 days storage period for all the biopriming agents except *Pseudomonas fluorescens*, where the value for all the duration was found to be statistically at par with each other. The electrical conductivity of the bioprimed seeds ranged from 0.095 dS cm^{-1} in (*Piriformospora indica*, 8 days treatment) to 0.606 dS cm^{-1} in (EM, 8 days treatment). The hydropriming treatments influenced increasing the electrical conductivity substantially as compared to other biopriming agents and the value ranged from 1.316 dS cm^{-1} to 1.622 dS cm^{-1}. To adopt the best storage conditions, the specific information which throw light on the biochemical processes leading to the loss of seed viability is essential to delay seed deterioration [38]. The protein content of the seeds was found to be statistically non-significant among the biopriming treatments. The α amylases activity of the seeds ranged from 3.14 m mol^{-1} mg^{-1} in (PGPR-I, 8 days treatment) to 4.87 m mol^{-1} mg^{-1} in (*Pseudomonas fluorescens,* 2 days treatment). The β amylases activity of the seeds ranged from 1.88 m mol^{-1} mg^{-1} (*Piriformospora indica*, 8 days treatment) to 2.38 m mol^{-1} mg^{-1} (*Pseudomonas fluorescens*, 8 days treatment). The decline of seed viability and germination during seed ageing can be due to biochemical changes as reported in *Dendrocalamus sikkimensis* [39].

3.2.3 At 60 days and 90 days storage period

The occurrence of no germination in any biopriming group at 60 and 90 days storage period reveals that the sandal seeds deteriorated at extended storage period. The changes in the biochemical compositions of the seeds presented vide Tables 5 and 6 suggests the possible reason for seed deterioration. Similar results were found for primed sweet corn seeds by Chiu et al. (2002) and tomato seeds [39]. Primed sweet corn seed exhibited poor germination and seedling growth performance after 3 months of storage at 25°C than non-primed seed. Delayed and lower germination was recorded in primed tomato seeds, when stored at 30°C for 6 months as compared with the control respectively. In general, seed deterioration and aging are considered as a force to reckon the depletion in food reserve, increased fat acidity, increased enzyme activity, and membrane permeability. Our result regarding the decrease in carbohydrate content and crude fat content at these storage periods solidify the seed deterioration process with an increase in the electrical conductance of the seed leachates. The highest value of EC was recorded from hydroprimed seeds at the end of 60 days and 90 days storage period, that reveals that seeds get deteriorated. This observation is in accordance with the results of [40] of stored soyabean, where they reported a loss of viability and high EC content at 60 days storage period. Inactivation of proteins during storage period is another factor of loss in viability of the sandal seeds after 60 and 90 days storage period that is in conformity with the results of [41]. They concluded that seed deterioration reduces the metabolic ability of the cell to repair the damages caused during aging. During storage, deteriorative changes related to aging occur in seeds, this is mainly due to modulation of various enzyme activities present in the seeds. The starch content in seeds decrease as a result of ageing due to the hydrolysis action of alpha and beta amylase resulting in an increase in the activity of amylases [42]. The reduction in seed viability of sandal seeds in storage can be due to changes in the biochemical characteristics and function of enzymes involved in seed reserve food depletion as noticed from several investigations [43,44].

Table 3. Influence of biopriming treatments on biochemical changes associated with sandal seeds subjected to no storage period

Biopriming treatments		Carbohydrate (mg g^{-1})	Crude fat (%)	Electrical conductivity (dS cm^{-1})	Protein (mg g^{-1})	α amylases (m mol^{-1} mg^{-1})	β amylases (m mol^{-1} mg^{-1})
Effective microorganisms	2 days	0.71^{ij}	43.33^{k}	0.520^{ghi}	0.067^{bc}	5.29^{de}	2.34^{efgh}
	4 days	0.70^{ijk}	44.42^{jk}	0.530^{ghi}	0.057^{cde}	5.16^{de}	2.28^{ghi}
	6 days	0.63^{klm}	45.72^{j}	0.651^{ef}	0.063^{bcd}	4.86^{f}	2.29^{fghi}
	8 days	0.64^{klm}	47.64^{i}	0.676^{e}	0.053^{def}	4.55^{ghi}	2.30^{fghi}
PGPR -I	2 days	0.91^{ef}	56.30^{a}	0.587^{efg}	0.043^{fgh}	4.66^{fg}	2.48^{bcd}
	4 days	0.96^{e}	54.81^{b}	0.542^{fgh}	0.050^{efg}	4.59^{ghi}	2.52^{ab}
	6 days	0.99^{d}	52.75^{cde}	0.501^{ghi}	0.063^{bcd}	4.88^{f}	2.61^{a}
	8 days	1.07^{d}	50.48^{f}	0.421^{i}	0.067^{bc}	5.44^{abc}	2.61^{a}
PGPR -II	2 days	0.68^{ijk}	50.30^{fg}	0.465^{hi}	0.057^{cde}	4.92^{f}	2.55^{ab}
	4 days	0.66^{klm}	52.22^{e}	0.493^{ghi}	0.053^{def}	4.90^{f}	2.32^{efghi}
	6 days	0.61^{mn}	52.66^{de}	0.560^{fgh}	0.050^{efg}	4.79^{fg}	2.26^{hi}
	8 days	0.61^{lm}	53.70^{bcd}	0.553^{fgh}	0.040^{gh}	4.54^{hi}	2.30^{fghi}
Pseudomonas fluorescens	2 days	1.39^{c}	54.10^{bc}	0.223^{j}	0.063^{bcd}	5.27^{e}	2.39^{def}
	4 days	1.42^{c}	52.58^{de}	0.190^{jk}	0.067^{bc}	5.50^{bcd}	2.49^{bcd}
	6 days	1.47^{b}	51.87^{e}	0.207^{jk}	0.070^{ab}	5.62^{ab}	2.49^{bc}
	8 days	1.58^{a}	49.78^{fgh}	0.177^{jk}	0.073^{ab}	5.69^{a}	2.53^{ab}
Piriformospora indica	2 days	0.75^{hi}	50.38^{fg}	0.207^{jk}	0.067^{bc}	5.30^{cde}	2.34^{efgh}
	4 days	0.82^{gh}	49.37^{fgh}	0.205^{jk}	0.073^{ab}	5.52^{bcde}	2.36^{efgh}
	6 days	0.81^{fg}	48.94^{ghi}	0.121^{jk}	0.073^{ab}	5.37^{cde}	2.38^{efg}
	8 days	0.85^{fg}	48.77^{hi}	0.095^{k}	0.080^{a}	5.35^{cde}	2.42^{cde}
Hydropriming	2 days	0.54^{o}	43.94^{k}	1.316^{c}	0.043^{fgh}	4.67^{gh}	2.28^{ghi}
	4 days	0.54^{no}	44.31^{k}	1.405^{c}	0.047^{efgh}	4.54^{ghi}	2.23^{ij}
	6 days	0.54^{o}	47.65^{i}	1.622^{b}	0.043^{fgh}	4.35^{ij}	2.14^{jk}
	8 days	0.52^{o}	47.79^{i}	1.728^{a}	0.037^{h}	4.13^{j}	2.07^{k}
Control		0.63^{jkl}	54.13^{bc}	1.074^{d}	0.056^{cde}	4.29^{ij}	1.78^{l}
SEM		0.041**	0.464**	0.036**	0.004**	0.082*	0.032*

*$P \leq 0.05$, **$P \leq 0.01$

Table 4. Influence of biopriming treatments on biochemical changes associated with sandal seeds subjected to 30 days storage period

Biopriming treatments		Carbohydrate (mg g^{-1})	Crude fat (%)	Electrical conductivity (dS cm^{-1})	Protein (mg g^{-1})	α amylases (m mol^{-1} mg^{-1})	β amylases (m mol^{-1} mg^{-1})
Effective	2 days	0.58^{bcde}	42.11^{ij}	0.538^{def}	0.047	4.12^{cde}	2.36^{ab}
microorganisms	4 days	0.57^{def}	40.55^{kl}	0.535^{def}	0.040	4.09^{cde}	2.32^{abc}
	6 days	0.58^{cdef}	39.71^{lm}	0.568^{def}	0.043	4.07^{cde}	2.16^{efgh}
	8 days	0.54^{efgh}	39.02^{m}	0.606^{d}	0.047	3.91^{def}	2.08^{hi}
PGPR -I	2 days	0.63^{b}	49.56^{ab}	0.587^{de}	0.047	3.82^{efg}	2.26^{bcde}
	4 days	0.61^{bcd}	49.17^{abcd}	0.542^{def}	0.047	3.48^{ghi}	2.23^{cdefg}
	6 days	0.61^{bcd}	49.22^{abc}	0.501^{defg}	0.037	3.40^{hij}	2.24^{cdef}
	8 days	0.56^{defg}	47.82^{def}	0.421^{g}	0.033	3.14^{j}	2.11^{ghi}
PGPR -II	2 days	0.53^{efghi}	48.01^{cdef}	0.465^{fg}	0.043	3.67^{fghi}	2.30^{abcd}
	4 days	0.52^{ghi}	46.70^{fg}	0.493^{efg}	0.053	3.58^{ghi}	2.19^{defgh}
	6 days	0.523^{fghi}	46.13^{gh}	0.560^{def}	0.047	4.05^{hij}	2.07^{hi}
	8 days	0.49^{hi}	45.00^{h}	0.553^{def}	0.047	4.03^{hij}	2.08^{hi}
Pseudomonas	2 days	1.28^{a}	48.99^{bcd}	0.223^{h}	0.043	4.87^{a}	2.38^{a}
fluorescens	4 days	1.26^{a}	49.24^{abc}	0.190^{hi}	0.040	4.41^{ab}	2.22^{cdefg}
	6 days	1.24^{a}	48.62^{bcde}	0.207^{h}	0.043	4.27^{bcd}	2.19^{defgh}
	8 days	1.23^{a}	48.56^{bcde}	0.177^{hi}	0.040	4.29^{bcd}	2.03^{i}
Piriformospora	2 days	0.63^{bc}	50.50^{a}	0.207^{h}	0.057	4.34^{bc}	2.13^{fghi}
indica	4 days	0.61^{bcd}	48.83^{bcd}	0.205^{h}	0.057	4.23^{bcd}	2.07^{hi}
	6 days	0.60^{bcd}	47.34^{efg}	0.121^{hi}	0.053	4.18^{bcd}	1.92^{j}
	8 days	0.57^{def}	46.93^{fg}	0.095^{i}	0.047	3.93^{def}	1.88^{jk}
Hydropriming	2 days	0.49^{i}	43.04^{i}	1.316^{c}	0.153	3.74^{efg}	1.80^{k}
	4 days	0.49^{hi}	41.47^{jk}	1.405^{c}	0.037	3.58^{fgh}	1.67^{l}
	6 days	0.42^{j}	40.81^{kl}	1.622^{b}	0.030	3.36^{ij}	1.50^{m}
	8 days	0.44^{j}	40.04^{lm}	1.728^{a}	0.033	3.22^{j}	1.48^{n}
SEM		0.016**	0.426**	0.033*	0.025	0.025**	0.036**

*$P \leq 0.05$, ** $P \leq 0.01$

Table 5. Influence of biopriming treatments on biochemical changes associated with sandal seeds subjected to 60 days storage period

Biopriming treatments		Carbohydrate (mg g^{-1})	Crude fat (%)	Electrical conductivity (dS cm^{-1})	Protein (mg g^{-1})	α amylases (m mol^{-1} mg^{-1})	β amylases (m mol^{-1} mg^{-1})
Effective microorganisms	2 days	0.50^{cdef}	37.89^{lm}	0.667^{bcd}	0.033^{bcde}	3.44^{abcde}	1.294^{ab}
	4 days	0.48^{cdefg}	37.77^{lm}	0.671^{bcd}	0.030^{cdef}	3.34^{abcdef}	1.221^{defg}
	6 days	0.45^{defg}	37.63^{m}	0.690^{bc}	0.030^{cdef}	3.17^{cdefg}	1.175^{ghi}
	8 days	0.45^{defg}	36.77^{m}	0.728^{b}	0.023^{ef}	3.02^{g}	1.058^{k}
PGPR -I	2 days	0.46^{defg}	46.73^{ab}	0.425^{hi}	0.030^{cdef}	3.46^{abcd}	1.294^{ab}
	4 days	0.45^{defg}	45.02^{cdef}	0.490^{fgh}	0.027^{def}	3.28^{bcdefg}	1.272^{abcd}
	6 days	0.43^{fgh}	44.57^{defg}	0.479^{fgh}	0.027^{def}	3.17^{cdefg}	1.194^{fgh}
	8 days	0.41^{ghi}	42.84^{hij}	0.536^{efg}	0.027^{def}	3.13^{efg}	1.093^{jk}
PGPR -II	2 days	0.48^{cdefg}	44.28^{efgh}	0.487^{fgh}	0.047^{a}	3.34^{abcdef}	1.255^{abcde}
	4 days	0.47^{cdefg}	43.68^{fghi}	0.550^{efg}	0.040^{abc}	3.19^{bcdefg}	1.237^{bcdef}
	6 days	0.43^{fgh}	42.49^{ij}	0.576^{def}	0.047^{a}	3.150^{defg}	1.204^{efgh}
	8 days	0.46^{defg}	41.72^{j}	0.620^{cde}	0.047^{a}	3.083^{fg}	1.157^{hi}
Pseudomonas fluorescens	2 days	0.89^{b}	47.42^{a}	0.364^{i}	0.040^{abc}	3.633^{a}	1.283^{abc}
	4 days	1.02^{a}	47.44^{a}	0.329^{ij}	0.047^{a}	3.487^{abc}	1.237^{bcdef}
	6 days	0.88^{b}	45.82^{bcd}	0.257^{ij}	0.040^{abc}	3.397^{abcdef}	1.170^{ghi}
	8 days	0.83^{b}	45.70^{bcde}	0.343^{ij}	0.037^{abcd}	3.223^{bcdefg}	1.127^{ij}
Piriformospora indica	2 days	0.53^{c}	46.18^{abc}	0.464^{gh}	0.043^{ab}	3.497^{ab}	1.241^{bcdef}
	4 days	0.52^{cd}	45.09^{cdef}	0.487^{fgh}	0.037^{abcd}	$3.330^{abcdefg}$	1.229^{cdefg}
	6 days	0.52^{cd}	46.15^{abc}	0.525^{efgh}	0.030^{cdef}	3.203^{bcdefg}	1.204^{efgh}
	8 days	0.51^{cde}	43.33^{ghi}	0.537^{efg}	0.033^{bcde}	3.163^{defg}	1.091^{jk}
Hydropriming	2 days	0.44^{efg}	39.62^{k}	1.856^{a}	0.023^{ef}	2.657^{h}	1.300a
	4 days	0.42^{gh}	39.25^{kl}	1.915^{a}	0.023^{ef}	2.617^{h}	1.162^{hi}
	6 days	0.37^{hi}	39.26^{kl}	1.854^{a}	0.023^{ef}	2.570^{h}	1.127^{ij}
	8 days	0.35^{i}	39.20^{kl}	1.921^{a}	0.020^{f}	2.387^{h}	1.072^{jk}
SEM		0.022**	0.479**	0.031**	0.003**	0.094**	0.018**

** $P \leq 0.01$

Table 6. Influence of biopriming treatments on biochemical changes associated with sandal seeds subjected to 90 days storage period

Biopriming treatment		Carbohydrate (mg g^{-1})	Crude fat (%)	Electrical conductivity (dS cm^{-1})	Protein (mg g^{-1})	α amylases (m mol^{-1} mg^{-1})	β amylases (m mol^{-1} mg^{-1})
Effective microorganisms	2 days	0.39^{fg}	36.83^{f}	0.733^{cd}	0.027^{abc}	2.83^{cde}	1.08^{bcde}
	4 days	0.39^{fg}	35.86^{fg}	0.746^{bcd}	0.027^{abc}	2.46^{ghi}	1.04^{e}
	6 days	0.37^{fgh}	34.79^{g}	0.755^{bc}	0.023^{bc}	2.29^{ijk}	1.07^{cde}
	8 days	0.36^{ghij}	34.88^{g}	0.759^{bc}	0.020^{c}	2.08^{k}	1.04^{e}
PGPR -I	2 days	0.36^{ghi}	41.50^{b}	0.704^{cdef}	0.030^{abc}	2.92^{cd}	1.10^{bcde}
	4 days	0.34^{hijk}	40.61^{bc}	0.707^{cdef}	0.023^{bc}	2.66^{efg}	1.09^{bcde}
	6 days	0.33^{ijk}	39.81^{cde}	0.691^{cdef}	0.023^{bc}	2.71^{def}	1.07^{cde}
	8 days	0.34^{hij}	39.68^{cde}	0.730^{cd}	0.023^{bc}	3.28^{a}	1.06^{de}
PGPR -II	2 days	0.40^{f}	41.29^{b}	0.694^{cdef}	0.037^{a}	2.64^{efgh}	1.13^{bc}
	4 days	0.39^{fg}	40.61^{bc}	0.715^{cde}	0.033^{ab}	2.56^{fgh}	1.09^{bcde}
	6 days	0.39^{fg}	39.91^{cde}	0.717^{cde}	0.033^{ab}	2.66^{efg}	1.09^{bcde}
	8 days	0.39^{fg}	40.30^{bcd}	0.686^{cdef}	0.027^{abc}	2.74^{cdef}	1.08^{bcde}
Pseudomonas fluorescens	2 days	0.77^{a}	43.14^{a}	0.856^{b}	0.027^{abc}	3.17^{ab}	1.20^{a}
	4 days	0.72^{b}	44.06^{a}	0.590^{efgh}	0.020^{c}	2.98^{bc}	1.14^{b}
	6 days	0.69^{bc}	44.24^{a}	0.493^{hi}	0.020^{c}	2.77^{cdef}	1.09^{bcde}
	8 days	0.68^{c}	44.09^{a}	0.457^{i}	0.023^{bc}	2.83^{cde}	1.07^{cde}
Piriformospora indica	2 days	0.48^{d}	43.08^{a}	0.584^{fgh}	0.033^{ab}	2.89^{cde}	1.09^{bcde}
	4 days	0.47^{d}	43.11^{a}	0.552^{ghi}	0.027^{abc}	2.94^{cd}	1.06^{cde}
	6 days	0.440^{e}	42.99^{a}	0.584^{fgh}	0.027^{abc}	2.63^{efgh}	1.07^{cde}
	8 days	0.38^{fg}	43.24^{a}	0.618^{defg}	0.020^{c}	2.41^{hij}	1.06^{de}
Hydropriming	2 days	0.33^{ijk}	39.27^{de}	1.889^{a}	0.023^{bc}	2.14^{k}	1.12^{bcd}
	4 days	0.30^{kl}	39.13^{de}	1.882^{a}	0.023^{bc}	2.18^{jk}	1.04^{e}
	6 days	0.28^{l}	38.71^{e}	1.854^{a}	0.023^{bc}	2.06^{k}	1.07^{cde}
	8 days	0.27^{l}	38.85^{e}	1.885^{a}	0.020^{c}	1.83^{l}	1.04^{e}
SEM		0.11**	0.395**	0.038**	0.003**	0.078**	0.021**

** $P \leq 0.01$

4. CONCLUSION

The results from the present study throw an important light on the impact of biopriming techniques on the seed health. The impact of biopriming treatments on improving the germination attributes were noticed at no storage period. *Pseudomonas fluorescence* followed by PGPR-I and *Piriformospora indica* resulted in overcoming the slow, staggered and low germination percentage problem of sandal seeds. Viability losses of primed seeds during storage is a major limiting factor to wide adoption of seed priming technique, that has been observed in the present study. At the 30 days storage period, bioprimed seeds belonged to EM and *Pseudomonas fluorescens* only germinated. The germination improvement noticed in EM mediated biopriming at the 30 days storage period can be further investigated to harness the best effect of EM on storability, where other biopriming agents failed to germinate with an extended storage period. Seed deterioration at 60 and 90 days storage period has been corresponding to the loss of enzymatic activities, the loss of membrane integrity and depletion in food reserve which consequent with dropped performance of germination and complete viability loss at extended storage period.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Moeinzadeh A, Sharif-Zadeh F, Ahmadzadeh M, Tajabadi FH. Biopriming of Sunflower (*'Helianthus annuus'* L.) Seed with *'Pseudomonas fluorescens*' for Improvement of Seed Invigoration and Seedling Growth. Aust J Crop Sci. 2020;4(7):564–570.
2. Raj AB, Raj SK. Seed priming: An approach towards agricultural sustainability. J Nat Appl Sci. 2019;11(1):227-234.
3. Thakur M, Tiwari S, Kataria S, Anand A. Recent advances in seed priming strategies for enhancing planting value of vegetable seeds. Sci Hortic. 2022;305:111355.
4. Di Girolamo G, Barbanti L. Treatment conditions and biochemical processes influencing seed priming effectiveness. Ital J Agron. 2012;7(2):e25-e25.
5. Bisen K, Keswani C, Mishra S, Saxena A, Rakshit A, Singh HB. Unrealized potential of seed biopriming for versatile agriculture. Nutrient use efficiency: from basics to advances. 2015;193-206.
6. Venkatesan KR, Srimathi RA, Kulkarni HD. Survey of sandal populations. Recent advances in research and management of sandal. 1995;3–52.
7. Meera C, Rao N, Ganeshaiah KN, Uma Shaanker R, Swaminath MH. Conservation of sandal genetic resources in India and threats to sandal resources in Karnataka. Myforest. 2000;36(2):125–132.
8. Manonmani V, Vanangamudi K. Effect of seed source and size on seed germination and seedling vigour of sandal (*Santalum album*). J Trop For Sci. 2002;14(1),150–155.
9. Ebone LA, Caverzan A, Chavarria G. Physiologic alterations in orthodox seeds due to deterioration processes. Plant Physiol Biochem. 2019;145,34-42.
10. Kurek K, Plitta-Michalak B, Ratajczak, E. Reactive oxygen species as potential drivers of the seed aging process. Plants. 2019;8(6):174.
11. Schwember AR, Bradford KJ. Drying rates following priming affect temperature sensitivity of germination and longevity of lettuce seeds. HortSci. 2005;40(3):778-781.
12. Hussain S, Zheng M, Khan F, Khaliq A, Fahad S, Peng S, Huang J, Cui K, Nie, L. Benefits of rice seed priming are offset permanently by prolonged storage and the storage conditions. Sci Rep. 2015;5(1): 8101.
13. Nagel M, Börner A. The longevity of crop seeds stored under ambient conditions. Seed Sci Res. 2010;20(1):1-12.

14. De Vitis M, Hay FR, Dickie JB, Trivedi C, Choi J, Fiegener, R. Seed storage: maintaining seed viability and vigor for restoration use. Restor Ecol. 2020;28:S249-S255.
15. Khan, FA, Maqbool R, Narayan S, Bhat SA, Narayan R, Khan FU. Reversal of age-induced seed deterioration through priming in vegetable crops–a review. Adv Plants Agric Res. 2016;4(6):1-9.
16. Higa, T. Effective Microorganisms: A new dimension for nature farming. In Proceedings of the Second International Conference on Kyusei Nature Farming. US Department of Agriculture, Washington, DC, USA. 1994;20-22.
17. Anith KN, Faseela KM, Archana PA, Prathapan KD. Compatibility of *Piriformospora indica* and *Trichoderma harzianum* as dual inoculants in black pepper (*Piper nigrum* L.). Symbiosis. 2011; 55,11-17.
18. Krichen K, Mariem HB, Chaieb M. Ecophysiological requirements on seed germination of a Mediterranean perennial grass (*Stipa tenacissima* L.) under controlled temperatures and water stress. S Afr J Bot. 2014;94:210–217
19. Oliveira CEDS, Steiner F, Zuffo AM, Zoz T, Alves CZ, Aguiar VCBD. Seed priming improves the germination and growth rate of melon seedlings under saline stress. Ciencia Rural. 2019;49: e20180588.
20. Yemm EW, Willis A. The estimation of carbohydrates in plant extracts by anthrone. Biochem J. 1954;57(3):508–514.
21. Lowry OH. Measurement with the folin phenol reagent. J Biol Chem. 1951;93:265–275.
22. Kennedy WK, Unrau J. A rapid method for determining the oil content of safflower and sunflower seeds. Agron J. 1949;41:93–95.
23. Wang J, Zhang G, Chen J, Shen Q, Wu F. Genotypic and environmental variation in barley beta-amylase activity and its relation to protein content. Food Chem. 2003;83(2):163-165.
24. Meliani A, Bensoltane A, Benidire L, Oufdou K. Plant growth-promotion and IAA secretion with *Pseudomonas fluorescens* and *Pseudomonas putida.* J Bot Sci. 2017;6(2):16–24.
25. Ortiz-Castro R, Campos-García J, López-Bucio J. *Pseudomonas putida* and *Pseudomonas fluorescens* influence Arabidopsis root system architecture through an auxin response mediated by bioactive cyclodipeptides. J. Plant Growth Regul. 2020;39(1):254–265.
26. Grobelak A, Napora A, Kacprzak MJEE. Using plant growth-promoting rhizobacteria (PGPR) to improve plant growth. Ecol. Eng. 2015;84:22-28.
27. Chiu KY, Chen CL, Sung JM. Effect of priming temperature on storability of primed sh-2 sweet corn seed. Crop Sci. 2002;42(6):1996-2003.
28. Yeh YM, Chiu KY, Chen CL, Sung JM. Partial vacuum extends the longevity of primed bitter gourd seeds by enhancing their anti-oxidative activities during storage. Sci. Hortic. 2005;104(1),101-112.
29. Sharma S, Gambhir S, Munshi SK. Changes in lipid and carbohydrate composition of germinating soybean seeds under different storage conditions. Asian J Plant Sci. 2007;6(3),502–507.
30. Soriano D, Huante P, Gamboa-deBuen A, Orozco-Segovia A. Effects of burial and storage on germination and seed reserves of 18 tree species in a tropical deciduous forest in Mexico. Oecologia. 2014;174(1): 33–44.
31. Lin TP, Huang NH. The relationship between carbohydrate composition of some tree seeds and their longevity. J Exp Bot. 1994;45(278):1289–1294.
32. Hussain S, Iqbal N, Rahman T, Liu T, Brestic M, Safdar ME, Asghar MA, Farooq MU, Shafiq I, Ali A, Shoaib M. Shade effect on carbohydrates dynamics and stem strength of soybean genotypes. Environ. Exp. Bot. 2019;162:374–382.
33. Bailly C, Benamar A, Corbineau F, Côme D, Antioxidant systems in sunflower (*Helianthus annuus* L.) seeds as affected by priming. Seed Sci Res. 2000;10(1):35–42.
34. Hoffpauir CL, Poe SE, Wiles LU, Hicks M. Germination and free fatty acids in seed stock lots of cottonseed. J Am Oil Chem Soc. 1950;27(9):347–348.
35. Forti C, Shankar A, Singh A, Balestrazzi A, Prasad V, Macovei A. Hydropriming and biopriming improve *Medicago truncatula* seed germination and upregulate DNA repair and antioxidant genes. Genes. 2020;11(3):242.
36. Job D, Capron I, Job C, Dacher F, Corbineau F, Côme D. Identification of germination-specific protein markers and their use in seed priming technology. In: Seed biology: advances and applications.

In: Proceedings of the Sixth International Workshop on Seeds, Merida, Mexico, 2000;449-459, Wallingford UK: CABI Publishing.
37. Perry DA. Problems of the development and application of vigour tests to vegetable seeds. In: Symposium on Seed Problems in Horticulture. 1977;83:141–146.
38. Lakshmi CJ, Jijeesh CM and Seethalakshmi KK. Impact of accelerated aging process on seed quality and biochemical changes of *Dendrocalamus sikkimensis* Gamble. Acta Physiol. Plant. 2021;43(2):34.
39. Argerich CA, Bradford KJ, Tarquis AM. The effects of priming and ageing on resistance to deterioration of tomato seeds. J Exp Bot.1989;40(5):593-598.
40. Akter N, Haque MM, Islam MR, Alam KM. Seed Quality of Stored Soybean (*Glycine max* L.) as Influenced by Storage Containers and Storage Periods. The Agric. 2014;12(1):85-95.
41. Dahuja ANIL, Yadav S. Biochemical basis of seed deterioration-an overview. Seed Res. 2015; 43(1):1-8.
42. Zhou Y, Hoover R, Liu Q. Relationship between α-amylase degradation and the structure and physicochemical properties of legume starches. Carbohydr Polym. 2004;57(3): 299-317.
43. Telewski FW, Zeevaart JA. The 120-yr period for Dr. Beal's seed viability experiment. Am. J. Bot. 2002;89(8):1285-1288.
44. Rajjou L, Debeaujon I. Seed longevity: survival and maintenance of high germination ability of dry seeds. C. r. Biol. 2008;331(10):796-805.

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