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RESEARCH ARTICLE

Influence of seed priming to alleviate the seed dormancy and growth performance in wild moth bean (*Vigna aconitifolia* (Jacq.) *Marechal*)

K Venkatalakshmi[1]*, V Vijayalakshmi[1], K Praimathy[2], E S Sruthi Priya[2], R Subhitsha[2], K Parameshwari[1] & P Sivakumar[1]

[1]Department of Agronomy, Agricultural College and Research Institute, Kudumiyanmalai, Pudukottai 622 104, India

[2]M.S.Swaminathan Agricultural College and Research Institute, Eachangkottai, Thanjavur 613 006, India

*Email:venkatalakshmi@tnau.ac.in

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Abstract

To determine the most effective techniques for breaking moth bean seed dormancy and evaluating the impact of seed priming on growth performance and storage potential, experiments were conducted in 2022–23 at the Agricultural College and Research Institute, Eachangkottai, Thanjavur. The study examined various treatments for reducing seed dormancy and enhancing growth performance in wild moth beans. Freshly harvested wild moth bean seeds were subjected to seven different seed priming methods: soaking in water for 4 hours and 6 hours; soaking in gibberellic acid (GA_3) at 100 ppm for 30 min and 60 min; and soaking in potassium nitrate (0.02 M) for 30 min. Compared against untreated control. The results revealed that seeds soaked in GA_3 (100 ppm) showed significantly higher germination and superior seedling growth parameters than the other treatments. Regarding the soaking durations, seeds soaked in GA_3 (100 ppm) for 60 min recorded higher seed germination and seedling vigour. Seed quality measures were assessed at trimonthly intervals while dormancy broken seeds were kept in Thanjavur's ambient settings for 9 months. As per the results of the storage experiment, wild moth bean seeds were kept for 9 months without significantly reducing their germination rate. Further, a linear reduction was observed in seedling length. A significant increase was recorded in moisture, EC, dehydrogenase, alpha-amylase and superoxide dismutase. Current research concluded that wild moth bean seeds were treated with GA_3 (100 ppm for 60 min) as a seed priming procedure to break dormancy and preserve seed vigour for up to nine months of storage.

Keywords

antioxidant activity; enzymatic activity; gibberellic acid; seed dormancy; seed priming; seed quality

Introduction

Moth bean (*Vigna aconitifolia (Jacq.) Marechal)* is dominantly grown in tropical areas of Asia and is native to India. It is cultivated on 14.00 lakh hectares of land in India, yielding 1.753 million tons with 133 kg of productivity per ha (1). Moth beans fix nitrogen in the atmosphere and are the high-protein source of food, fodder, feed, green manuring and pasture. Being a drought-tolerant crop, it mitigates greenhouse emissions and has various medicinal properties. Hence, it serves as a multi-purpose crop.

Grain yield can be improved and the tolerance of moth beans to significant biotic and abiotic stresses can be enhanced by utilizing wild variants of the crop. Seeds of wild moth beans have been gathered from Kollimalli hills. Mature wild bean moth bean seeds exhibit low germination percentages. Additionally, their germination speed, growth and development are suboptimal. This may be due to dormancy. This experiment aimed to break the seed dormancy of wild moth beans to preserve germination and seedling vigour immediately after harvest and throughout the storage period. Both fresh and aged wild moth seeds may reduce their seed dormancy by applying various seed priming chemicals, including potassium nitrate and phytohormones like GA_3.

Materials and Methods

Wild moth bean seeds have been sourced from farmers in Kollihills, Tamil Nadu, who consistently cultivate moth beans. The experiments were conducted in the Seed Science and Technology Laboratory of the Agricultural College and Research Institute, Eachangkottai, Thanjavur.

Seed dormancy-breaking treatments

Seeds were imposed to various dormancy-breaking seed priming treatments such as soaking in water (4 hr), soaking in water (6 hours), soaking in Gibberallic Acid (GA_3) 100 ppm for 30 min; soaking in GA_3 (100 ppm for 60 min); soaking in KNO_3 (0.02 M for 30 min) and soaking in KNO_3 (0.02 M for 60 min). These treatments were compared against the untreated control and subsequently evaluated in seed germination studies.

Vigour Index I

The proposed method evaluated the seed lot's vigour index as per equation 1 (2).

Vigour index I =
Average Seedling length (cm) × germination (%) (Eqn.1)

Vigour Index II

Ten normal seedlings from each replication were randomly selected after the germination test. For 16 hr, seedlings have been dried at 60 °C in a hot air oven. Milligrams (mg) have been employed to determine seedlings' dry weight. Combining seedlings' dry matter content and germination rate yields vigour index-II in equation 2.

Vigour index-II =

Average dry weight (mg) × germination (%) (Eqn. 2)

Seed germination studies

A germination test was conducted on wild moth bean seed fraction by employing 100 seeds in four separate repetitions complying with between papers (BP) procedures at room temperature. The number of normal seedlings was counted at the end of eight days (final count) and the mean was given as a percentage (3). Dry weight (mg per 10 seedlings), seedling vigour index (SVI), seedling length (cm) and seed germination observations were reported. The best dormancy -breaking treatments were identified and forwarded to seed storage studies.

Moisture content

The hot air oven method was employed to determine moisture content. In a hot air oven, moth bean seeds (10 g) were maintained at 103 °C for 17 hr (4). Weight (W/W) basis was employed to calculate the moisture content percentage, as per Equation 3.

Moisture content (%) = $(M_2 - M_3 / M_2 - M_1) \times 100$ (Eqn. 3)

Here,

M_1 = weight of a container with a lid

M_2 = of the container with lid + seeds before drying

Weight of container with lid + seeds after drying

Seedling length

From each germination test replication, 10 normal seedlings were randomly selected, with their length measured in centimetres (cm). The average length was measured and recorded.

Dry matter content of seedlings

Ten normal seedlings from each replication were placed in a butter paper bag and then dried for 16 hours at 60°C in a hot air oven to determine the dry matter content of the seedlings. After removing and allowing dried seedlings to cool at room temperature, the final weight was measured in milligrams (mg per seedling).

Seed storage studies

For nine months, treated seedlings remained in cloth and super bags in the Thanjavur environment. The seeds were evaluated periodically at intervals of three months for seed quality and biochemical parameters.

Electrical conductivity (dSm^{-1})

In four replicates, 50 seeds from each treatment were soaked in distilled water (50 mL) for 9 hours. Water employed to soak seeds was identified as leachate. An electrical conductivity meter was employed to test seed leachate's electrical conductivity, which could then be expressed as dSm^{-1} (5).

α -amylase assay

α-amylase activity was measured employing the procedure outlined (6). A 0.2 M citrate buffer (pH 5.5) was employed to extract seeds that had been centrifuged at 10,000 g and then a supernatant was utilized for the enzyme assay. Subsequently, 0.2 mL of enzyme extract was diluted into 1 mL of distilled water. Further, the experiment began by adding starch substrate (1 mL) for 1 hr. Potato starch (150 mg) was added to 100 mL of solution that included $CaCl_2$ (200 µ mol) and KH_2PO_4 (600 mg) for generating starch substrate. After 1 min. For heating, the mixture was centrifuged at 3000 g for 10 min and then a clear supernatant was utilized as a substrate. Before use, stock solution (1 mL) was added to 0.05 N HCl to bring the volume to 100 mL. KI (6 g) and iodine (600 mg) dissolved in water (100 mL) to terminate the reaction. Absorption was measured at 620 nm upon adding distilled water (5.0 mL) to this reaction mixture. By measuring the amount of starch hydrolyzed per min per mg of protein, α-amylase activity

was determined. Bradford's technique was employed for determining protein content, employing bovine serum albumin (BSA) as a reference(7).

Dehydrogenase activity (optical density)

In four replicates, 25 seeds from each treatment were immersed in water for 18 hours. 0.2 % tetrazolium (TZ) (5 mL) was added to 10 embryos, which were then incubated in the dark for 4 hours. The extra solution was poured out after the incubation period and then embryos were appropriately cleaned with distilled water and eventually blotted dry. The formation was eluted after soaking labelled embryos in methyl cello solution (2 methoxy ethanol) (5 mL) for an entire night. Then, optical density (OD) was determined at 470 nm utilizing a UV-VIS spectrophotometer (Model SP-3000) (8).

Superoxide dismutase (SOD)

To prepare the extract, a one-gram leaf sample was frozen in liquid nitrogen and then crushed in extraction solution 10 mL (0.1M phosphate buffer pH 7.5, including 0.5 mM Ethylene diamine tetra acetic acid (EDTA)). Homogenates were centrifuged at 4 °C for 20 min at 15000Xg, followed by flattening it through 4-layer thicknesses of cheesecloth. The liquid supernatant had been collected and then utilized for enzymatic activity tests. At 4 °C, every step was performed to prepare the enzyme extract.

Reaction mixture consisting of 13.33 mM methionine, 75 µm NBT, 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.8), 50 mM sodium bicarbonate and 0.1 mL enzyme extract combined with 82.2 mM riboflavin (0.0 mL) for estimating superoxide dismutase activity. Then, tubes were exposed to two 15 W fluorescent lamps for 15 min to initiate the reaction. Compared to tubes without enzyme, the amount of enzyme that reduced absorbance reading to 50 % was considered 1 unit of enzyme activity. Absorbance was measured at 560 nm. Delta Optical Density (ΔOD) /min/g of fresh weight was utilized for expressing enzyme activity (9).

Statistical analysis

ANOVA was utilized to perform an analysis of variance based on various observations of the laboratory data that had been obtained (10). Applying Duncan's test determined significant differences ($p \leq 0.05$) between means. The percentage data was transformed into arcsine values and utilized for statistical analysis. Additionally, a correlation map was generated using SPSS version 21.

Results and Discussion

Seed dormancy breaking experiment in the wild moth bean

The results showed that soaking of moth bean seed germination, dry weight of the seedlings, seedling length and SVII and II were recorded highest in GA_3-treated seeds. The soaking time of half an hour recorded the highest germination and other seedling quality characters were recorded as higher. The maximum germination was recorded in moth bean seeds treated with 100 ppm for 30 min (94.0%) and it was statistically equivalent to seeds soaked in GA_3 (100 ppm) for an hour (93.0%). Both were significantly superior to other treatments. The lowest germination was recorded in untreated seeds at 47.0% (Fig. 5). Enhanced water absorption and metabolic changes, including the mobilization of storage proteins, likely contributed to these results (11-13).

Seeds treated with GA_3 (100 ppm) for an hour demonstrated longer seedling length (20.1cm), which is comparable to seeds treated with GA_3 (100 ppm) for half an hour (19.3 cm). The lowest seedling length (9.1 cm) was observed in the control. The higher dry weight of seedlings (17.7 mg) registered in the treatment of seeds soaked in GA_3 (100 ppm) for an hour was statistically comparable with the seeds soaked in GA_3 (100 ppm) for half an hour (17.3 mg). The lowest dry weight of the seedling (11.3 mg) was observed in the control. Seedling vigour I was recorded as significantly highest (1886) in the treatment of seeds soaked in (GA_3 100 ppm) for an hour, followed by seeds soaked in GA_3 (100 ppm) for half an hour (1791). The lowest seedling vigour I (391) was observed in control (Fig. 5). Seed priming catalysis the enzyme activity and enhanced vigour index as observed (14).

Compared to seeds soaked in GA_3 (100 ppm) for 30 min. (1623), the treatment of seeds soaked in GA3 (100 ppm) for an hour resulted in a higher SVI II (1643). The control had the lowest SVI II (528) (Fig. 5). The study showed that seeds soaked in GA_3 (100 ppm) for half an hour recorded higher seed germination rates, while seeds soaked in GA_3 (100 ppm) for an hour seedling resulted in enhanced seedling growth.GA_3 is widely used in agriculture, helping to break dormancy and increase seed germination and growth, flowering, fruit development and yield. However, its effectiveness varies depending on its concentration and duration of treatment. A similar result regarding GA_3 was reported (15). Research indicates the benefits of seed priming with varying GA_3 dosages of rapeseed, peanuts, sugar beet and sorghum (16-19).

Seed storage studies

The seed storage studies showed that the moth bean seeds treated with GA_3 (100 ppm) for half an hour recorded the highest seed germination, followed by GA_3 (100 ppm) for an hour. Among the various seed storage periods, germination was recorded as highest during 3 months, 6 months and 9 months after being stored in aluminium foil bags. The storage experiment results revealed that wild moth bean seeds can be stored for nine months without much reduction in seed germination (92 to 82 %) (Fig. 6), speed of germination (4.8 to 4.3) (Fig. 7). Seed priming with GA_3 0.2 g/ L in *Zea mays*, *Lathyrus sativus* and *Pisum sativum* raised final germination percentage (FGP) value compared to the control (unprimed treatment) as reported in another research (20).

There was a linear reduction observed in seedling vigour (2208 to 1648) (Fig. 8), dehydrogenase (0.94 to 0.63 OD) (Fig.10), alpha-amylase (12.20 mg to 11.20 mg of maltose per min. (Fig. 9) and superoxide dismutase (0.72 mg to 0.58 mg of protein per min.) (Fig. 11) from fresh seeds to storage period up to 9 months and a significant increase was recorded in moisture (12.50% to 13.20%) (Fig. 9), EC (0.25 to 0.44 dSm^{-1}) (Fig. 10) from fresh seeds to storage period up to 9 months.

Antioxidant enzyme activity, including glutathione reductase, peroxidase, catalase and superoxide dismutase, had decreased in aged seeds (21, 22). On the contrary, priming increases enzyme activity, which could result in better germination characteristics. As a result, the Seed's respiratory capacity is diminished, which lowers ATP while absorbing the germinating seed supply.

Correlation studies

Additionally, correlation studies demonstrated that electrical conductivity negatively correlated with seed germination, while seedling length, dehydrogenase, alpha-amylase and superoxide dismutase enzyme activity had a positive correlation. Correlation studies on fresh seeds showed that seed germination was positively correlated with seedling length (0.94), dehydrogenase (0.86), alpha-amylase (0.55) and superoxide dismutase (0.99) and it was negatively correlated with electrical conductivity (-0.340) (Fig. 1). At three months of storage, the correlation values with the seed germination for seedling length (0.94), dehydrogenase (0.92), alpha-amylase (0.65) and superoxide dismutase (0.97) were positively correlated. It negatively correlated (-0.95) with electrical conductivity (Fig 2). Seed germination was negatively correlated with electrical conductivity (-0.89) and positively associated with seedling length (0.89), dehydrogenase (0.94), alpha-amylase (0.92) and superoxide dismutase (0.98) through the course of six months of storage (Fig. 3).

Alpha-amylase (0.99), superoxide dismutase (0.98), seedling length (0.74), dehydrogenase (0.76) and electrical conductivity (-0.55) significantly demonstrated positive correlations with seed germination after nine months of storage (Fig. 4). Research results showed that while the activity of overall amylase decreased as the ageing period increased, GA invigoration of aged seeds improved α-amylase activity. Lower germination ability and seedling vigour in aged seeds were attributed to the rapid decline in alpha-amylase activity, which was inversely correlated with electrical conductivity (23).

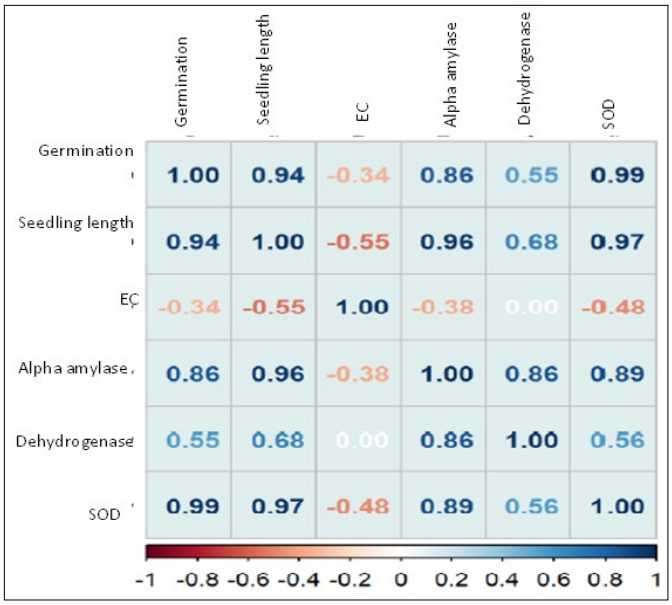

Fig. 1. Correlation coefficient between physiological and biochemical parameters of fresh seeds.

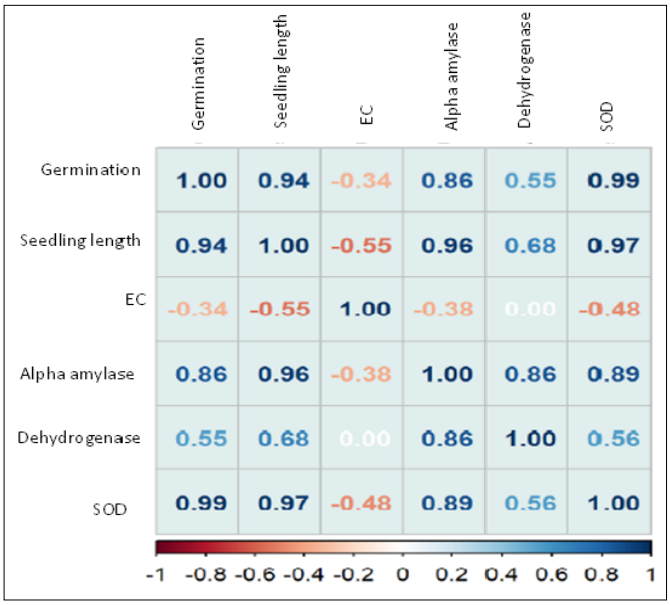

Fig. 3. Correlation coefficient between physiological and biochemical parameters of 6 months after storage of wild moth bean seeds.

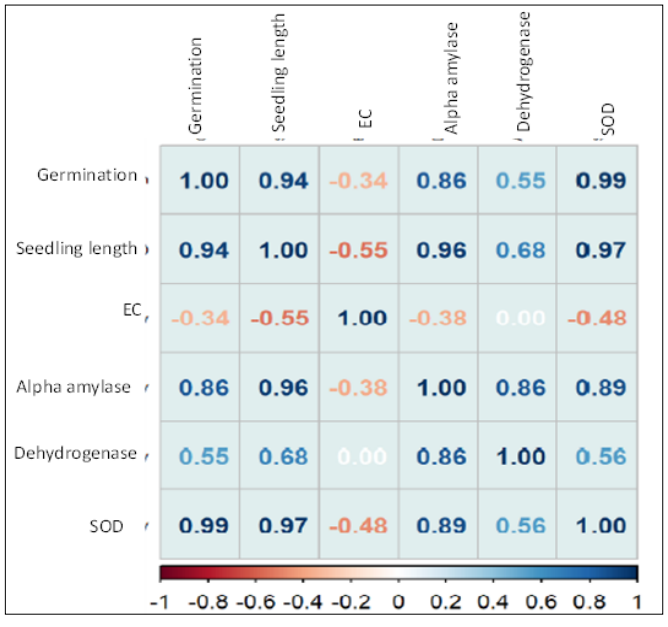

Fig. 2. Correlation coefficient between physiological and biochemical parameters of 3 months after storage of wild moth bean seeds.

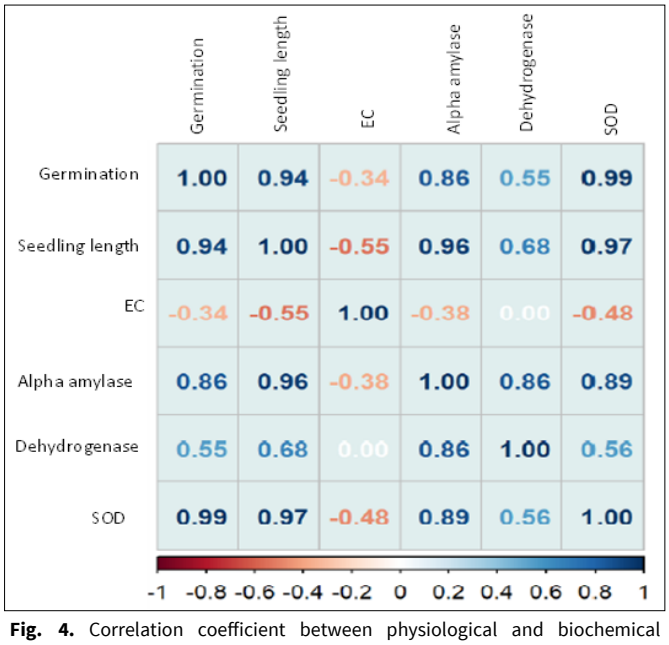

Fig. 4. Correlation coefficient between physiological and biochemical parameters of 9 months after storage of wild moth bean seeds.

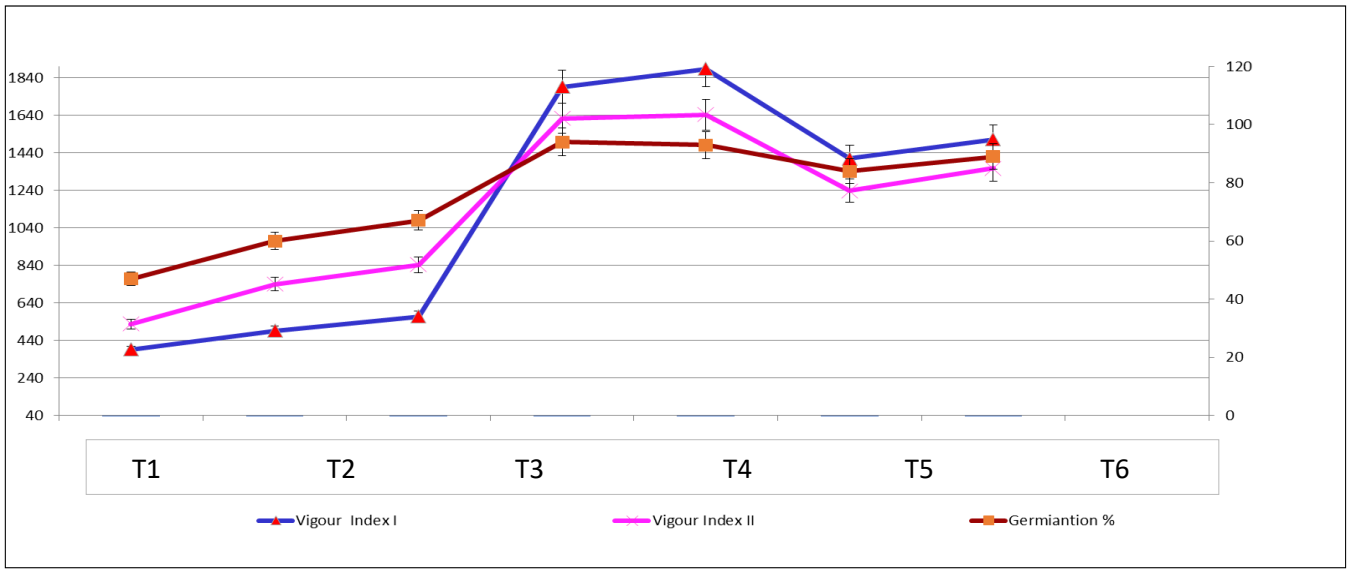

Fig. 5. Influence of seed priming on alleviation of seed dormancy in wild moth bean of fresh seeds.

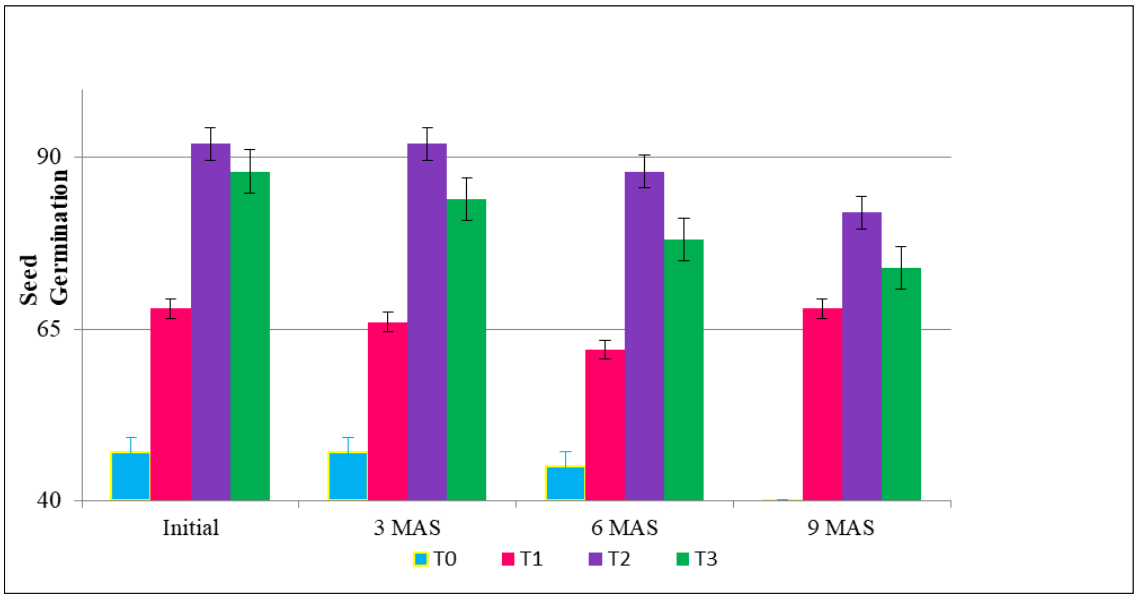

Fig. 6. Effect of seed treatments on seed germination (%) in moth bean during storage.

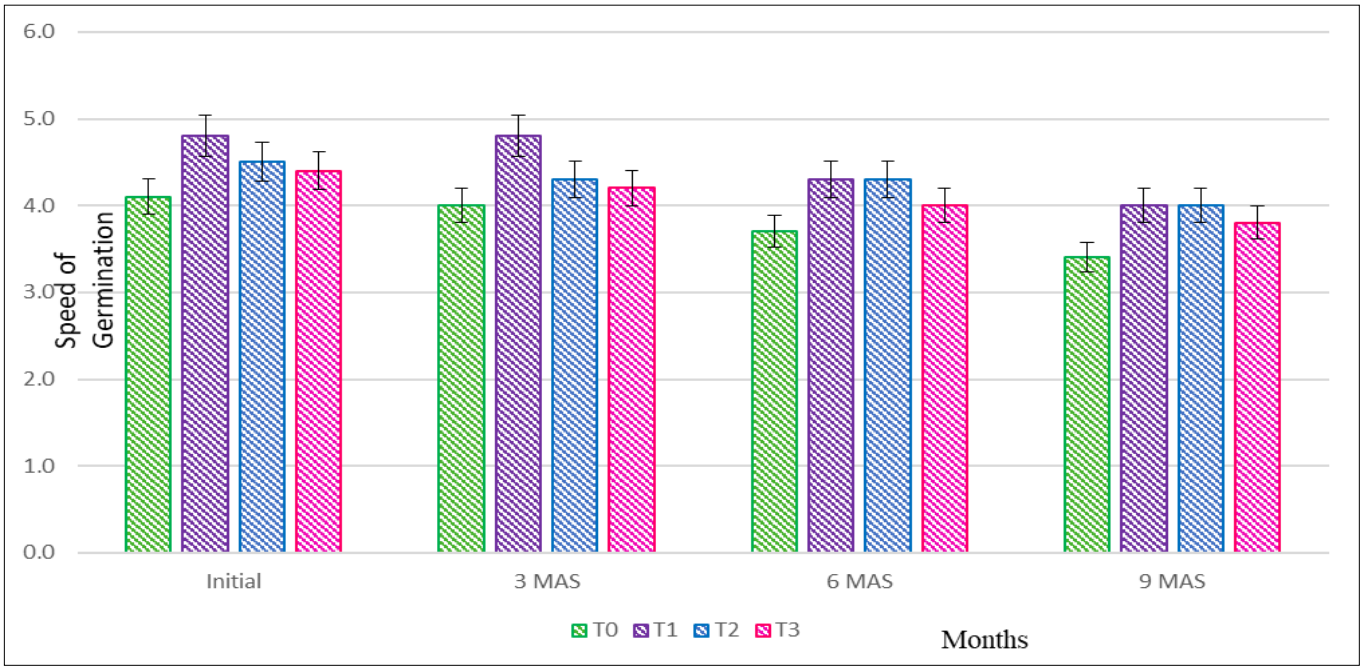

Fig. 7. Effect of seed treatments on speed of germination (%) in moth bean during storage.

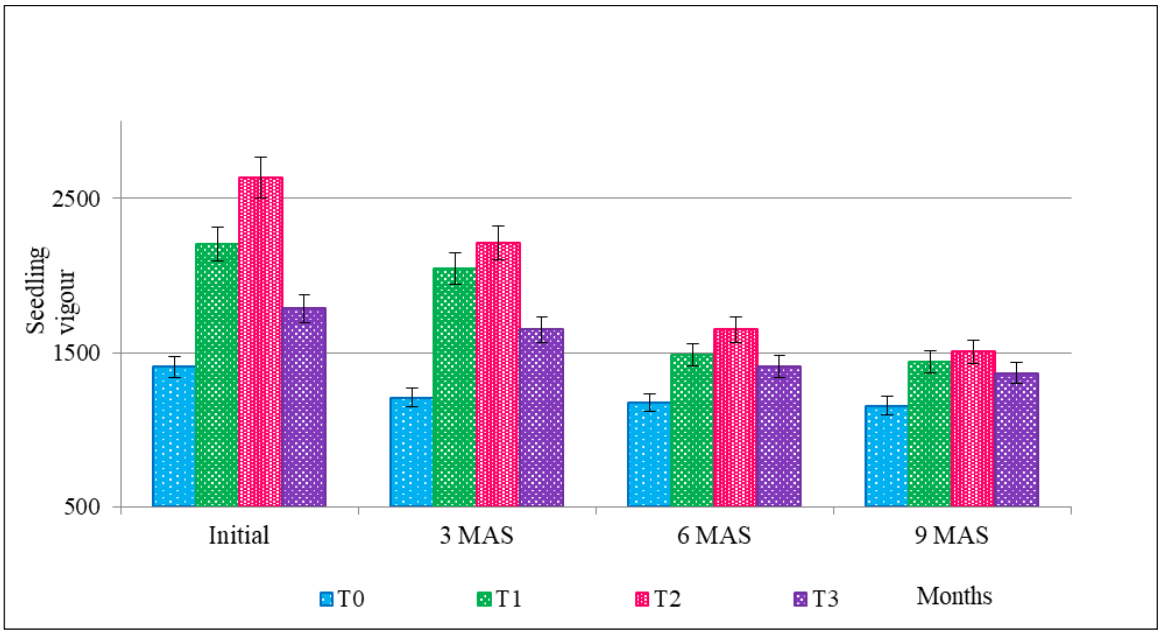

Fig. 8. Effect of seed treatments on seedling vigour of moth bean during storage.

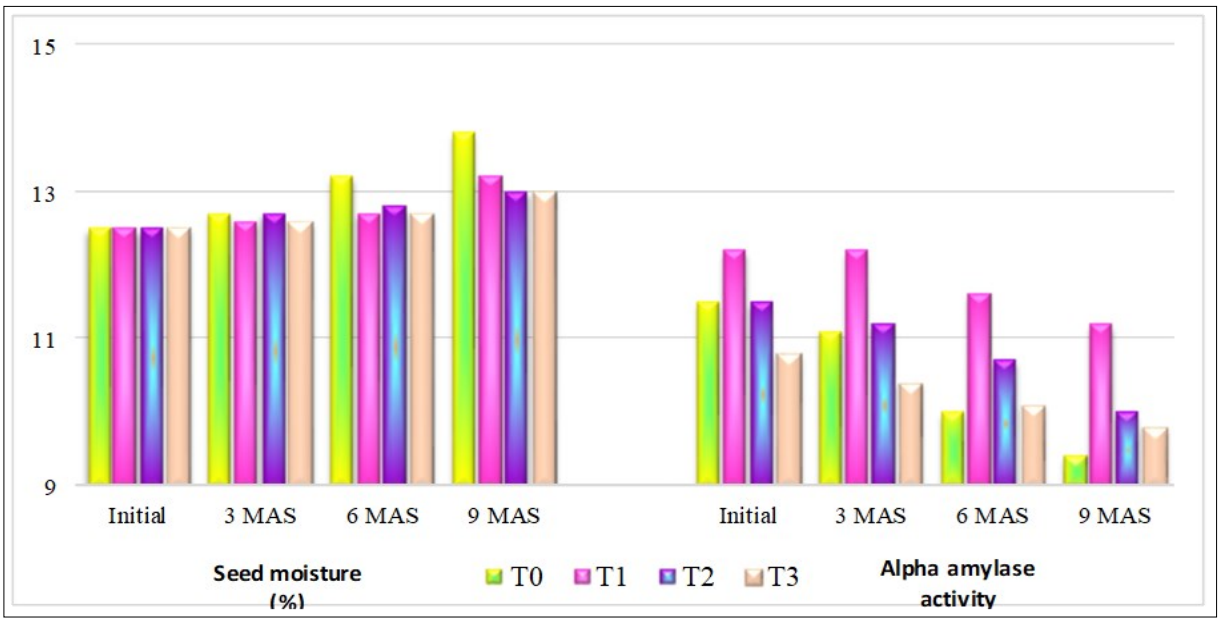

Fig.9. Effect of seed treatments on seed moisture (%) and alpha-amylase activity (mg of maltose min^{-1}) in moth bean during storage.

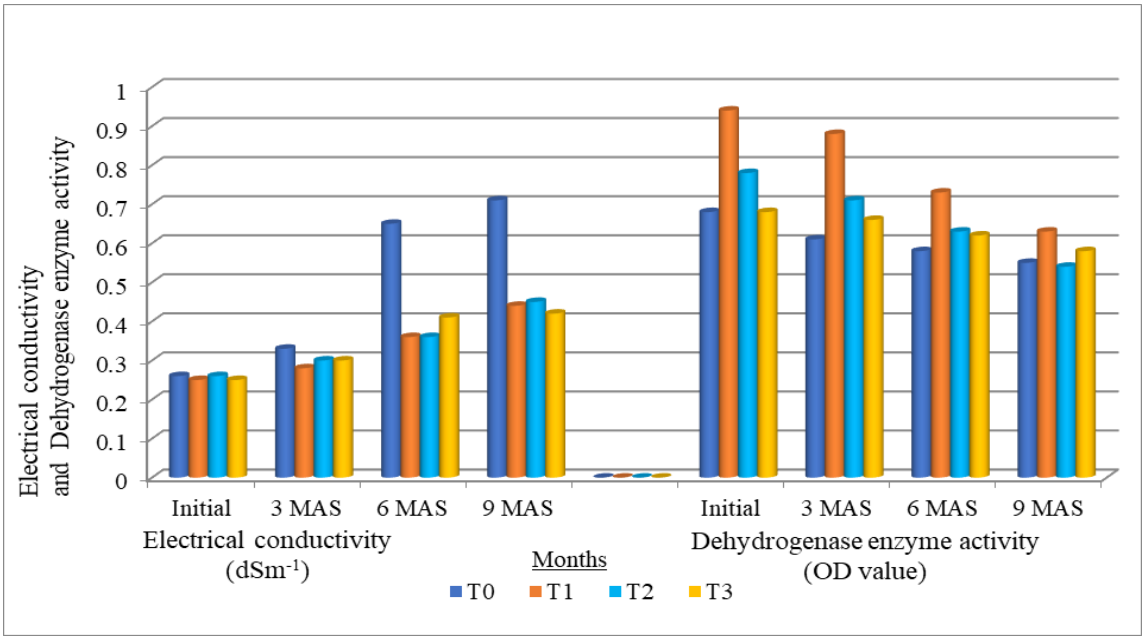

Fig.10. Effect of seed treatments on Electrical Conductivity (dSm^{-1}) and Dehydrogenase activity (OD) in moth beans during storage.

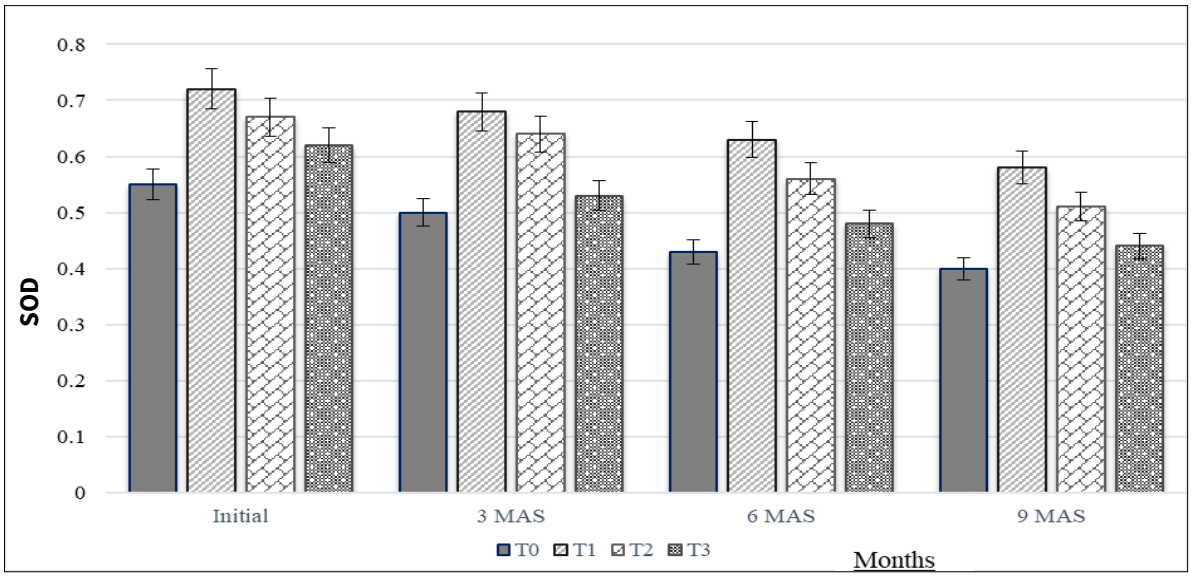

Fig. 11. Effect of seed treatments on super oxide dismutase enzyme activity in moth bean during storage.

Conclusion

Hence, it is concluded that soaking wild moth bean seeds in 100 ppm GA_3 for half an hour effectively breaks the seed dormancy, yielding results comparable to soaking for an hour when compared to unprimed seeds in fresh and aged conditions. Higher seedling vigour, seed quality and biochemical parameters of wild moth bean seeds were obtained by wild moth bean seeds soaked in GA_3 100 ppm for an hour than the other seed priming treatments.

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Authors' contributions

KV planned the study's design, conducted the experiments, performed the statistical analysis and drafted the manuscript. VV and KP helped summarize and revise the manuscript. PS made corrections in the manuscript. Meanwhile, KP, ES and RS alumni of M.S.S. AC&RI, Eachangkottai College, were assisted in conducting experiments. All the authors approved and read the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

Ethical issues: None

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