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Research Article

Exogenous melatonin improves seed germination and seedling growth in greengram under drought stress

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Abstract
Drought stress diminishes seedling germination and vigor by reducing water uptake, inhibiting plant growth and development. Most of the pulse growing areas are under rainfed ecosystems, which significantly reduces crop yield. Melatonin, a growth-regulating compound, is widely used to mitigate the negative effects of abiotic stresses in pulses. With this background, a laboratory experiment was conducted to standardize the optimum melatonin concentration for seed treatment and foliar application in greengram, to minimize the ill effects of drought stress. The experiment was arranged in a completely randomized design (CRD) with three replications for each treatment. The treatments consisted of soaking seeds with different melatonin concentrations, *viz.,* 20, 40, 60, 80 and 100 µM. Seeds were sown in a perti dishes and the drought stress was imposed using poly ethylene glycol 6000 (PEG 6000) @ - 0.4 MPa, and plates were maintained at room temperature (24-30 °C). After the seedlings emerged, various seedling growth parameters like germination percentage, shoot length, root length, vigor index, promptness index, germination stress tolerance index, fresh and dry weight of the seedlings, plant height stress index and root length stress index were recorded. The experimental results showed that drought stress significantly reduced germination percentage and other growth-related parameters in greengram seedlings compared to the melatonin treatments. Among the melatonin treatments, seeds treated with @ 100 µM concentration recorded the highest germination percentage (99.67 %), promptness index (98.80), vigour index (1631.68), shoot and root length (8.9 cm and 7.5 cm), fresh and dry weight of the seedlings (3.249 and 0.147 mg seedling^{-1}) under PEG induced drought stress condition.

Keywords: Drought stress, Greengram, Melatonin, Seed treatment, Seedling tolerance

INTRODUCTION

Abiotic stresses are considered a major environmental factor that disturbs normal plant growth, and production is reduced below the optimal levels. Abiotic stress like drought, high temperature and salinity can occur sepa-

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rately or in combination, causing morphological, physiological, biochemical and molecular changes in plants that negatively influence plant development, productivity and yield (Bita and Gerats, 2013). According to the IPCC (2014) assessment, the increase in temperature and water scarcity causes a significant impact on agricultural productivity that affects the quality and yield of the crop (Zandalinas *et al.,* 2018). Pulses are considered a very important food crop, known for their health benefits (Arnoldi*et al.,* 2014) as they are rich in proteins, minerals and vitamins and an essential ingredient in most Indian diets. Among the pulses, greengram (*Vignaradiata* L.), known as mung bean rich in high protein, belongs to the family leguminaceaeand contributes 14% of the total pulses area and 7% to the total pulse production in India. Greengram, a short-duration legume crop, plays a major role in sustainable agriculture (Rambabu *et al.,* 2016). Mung bean is more likely to rely on an adequate and appropriate quantity of water with optimum temperature than on any other environmental factors. The soil moisture available to the crop throughout the growing season is the most critical factor, thus limiting agricultural productivity. However, Mung bean are grown in marginal soils with low inputs, where the crop periodically encounters various abiotic stresses that hinder crop productivity (Nair *et al.,* 2019). For example, drought, heat and salt stress have a detrimental effect on greengram production and adaptability, affecting crop growth and development by altering physiological processes and the plant-water status (Zandalinas*et al.,* 2017).Drought stress has been reported to affect around 68 per cent of India's net cultivated land (140 million hectares) (Rambabu *et al.,* 2016). It severely affects the germination of seedlings (Saima *et al.,* 2018), leaf area (Baroowa and Gogoi, 2016) and photosynthetic rate by damaging the photosynthetic pigments and altering the components and concentrations of chlorophyll pigments. The activity of several enzymes was also found to be declined in response to cell desiccation (Nadeem *et al.,* 2019).

Adaptation and mitigation strategies are required to cope with the adverse effects of drought stress. Recently, the application of plant growth regulators (PGRs) and nutrients has been adopted as one of the management strategies for improving plant tolerance to several abiotic stresses.Melatonin, a compound that acts as an anti-stress hormone, multifunctional and master regulator which plays a vital role in plant stress defencemechanisms mainly related to abiotic stresses (Huang *et al.,* 2022) such as drought, radiation, extreme temperature and chemical stresses and is also involved in plant growth and development (Moustafa-Farag*et al.,* 2020; Buttar*et al.*, 2020). Melatonin was structurally related to tryptophan (precursor), aromatic amino acid and its biosynthesis is analogous to that of auxin, serotonin, and their isoforms (Khan *et al.,* 2020).It acts as an antioxidant and detoxifies reactive oxygen and nitrogen species (ROS and RNS), by enhancing the antioxidant enzyme activity under drought conditions (Arnao and Hernandez-Ruiz, 2019).Melatonin modulates the electron transport chain (Khan *et al.,* 2020), maintains ion homeostasis (Zhang *et al.,* 2021) and protects the chlorophyll pigments from oxidative stress (Jahan *et al.,* 2021). The seedling growth was positively regulated by the exogenous application of melatonin by interacting with other phytohormones like, indole-3-acetic acid, gibberellic acidand abscisic acid (Rehaman*et al.,* 2021). Melatonin increases the growth-related attributes by stimulating the seed germination process, maintaining a robust root system and improving photosynthetic capacity by delaying leaf senescence which contributes to improved yield and yield characteristics (Sadak and Bakry, 2020). Several studies have reported that exogenous application of melatonin significantly improves the germination percentage, vigor index, fresh and dry weight of the seedlings in*Carthamustinctorius* (Akbari *et al.,* 2020), *Cucumissativus* (Zhang *et al.,* 2020), *Gossypiumhirsutum* (Bai*et al.,*2020), *Glycine max* (Imran *et al.,* 2021), *Beta vulgaris* (Liu *et al.,* 2022) and *Oryza sativa* (Li *et al.,* 2022) under various abiotic stress conditions.Based on the above scientific research findings, the present investigation on greengram was proposed to know the impact of different melatonin concentrations on germination and seedling growth characters under polyethylene glycol(PEG 6000) imposed drought stress conditions.

MATERIALS AND METHODS

Plant growth conditions and treatments

A laboratory experiment was conducted in the Department of Crop Physiology at Tamil Nadu Agricultural University, Coimbatore,to standardisethe optimum concentration of melatonin for alleviating the adverse effects of drought stress. The seeds of greengram, variety CO 8, were used as source material. The uniform and healthy seeds were taken, and the seeds were surface sterilised with 3 % sodium hypochlorite for two minutes and washed thrice with distilled water. The seeds were soaked in different melatonin concentrations *viz.,*20, 40, 60, 80 and 100 µM for 6 hours. Melatonin-treated seeds were placed separately in Petri plates (20 seeds per Petri plate) for drought stress imposition. Additionally, melatonin untreated seeds were used for absolute control (without PEG 6000) and control (with PEG 6000) treatments. Three replications were maintained for each treatment. The drought stress was imposed artificially by using polyethylene glycol (PEG 6000) of -0.4 MPa (Jincy*et al.,* 2021) and filter paper were damped periodically with PEG solution and kept at room temperature for germination when the seed radicle length attained its emergence of at least 2mm to determine as the germination criterion (Kaur *et al.,* 2017).

Measurement of seedling growth parameters

Observations on various seedling growth parameters were taken for each treatment. The traits recorded for assessing the drought stress tolerance of the greengram seedlings are described below.

Germination percentage

The percentage of germinated seed was counted on each day from the 2^{nd} to 10^{th} day. Seedlings with 2mm radical and plumule are counted as germinated seeds. Germination percentage was calculated by using the formula and expressed as a percentage.

Germination percentage = Number of seeds get generated / Total number of seeds kept for generation x 100 .....Eq.1

Shoot and root length

The shoot and root length was measured from randomly selected seedlings of every single replication on the 10^{th} day. Shoot length was measured from the collar region to the longest leaf tip and expressed in terms of cm. Similarly, the root length was measured from collar region to the longest root and expressed in cm.

Vigour index

The seedling vigour index was arrived as described by Abdul – Baki, (1973)

Vigour index = (Shoot length + Root length) x germination percentage .....Eq. 2

Promptness index and Germination stress tolerance index

Promptness index (PI) and germination stress tolerance index (GSTI) of the emerged seeds were estimated with the formula given by Sapra (1991) and Bouslama and Schapaug (1984), respectively.

PI = nd2 (1.0) + nd4 (0.8) + nd6 (0.6) + nd8 (0.4) + nd10 (0.2) .....Eq. 3

Where, nd2, nd4, nd6, nd8 and nd10 denote the percentage of seeds which germinate after 2, 4, 6, 8 and 10 days after sowing, respectively.

Germination stress tolerance index (GSTI) = (PIS/PINS) x 100 .....Eq. 4

Where,

PIS is PI under drought stress and PINS is PI under normal condition

Fresh and dry weight

The fresh and dry weight of the seedlings were recorded from the randomly selected three seedlings of every replication. The fresh weight was recorded and the samples were kept in a hot air oven at 70°C for 48 h, the dry weight was taken and expressed as mg seedling^{-1}.

Plant height stress index and root length stress index

Plant height stress index (PHSI) and root length stress index (RLSI) were estimated on the 10^{th} day and calculated by using the formula described by Ellis and Roberts (1981) and expressed as %.

PHSI= (Plant height stressed plants/Plant height control plants) x 100 ....Eq. 5

RLSI=Root length stressed plants/Root length control plants) x 100 .....Eq.6

Statistical analysis

The design of the experiment was a completely randomized design (CRD) with three replications and the data collected for various traits were statistically analyzed by using R software (version 4.1.2) with the analysis of variance (ANOVA). The least significant difference (LSD) test was used to compare the differences among group means and the critical difference (CD) was computed at five percent probability ($p \le 0.05$). Figures were generated by using Originpro 2019 software (Originlab Corp., USA).

RESULTS

Germination percentage

Under the ambient condition (AC), greengram seeds recorded 100% germination, whereas PEG imposed seeds (C) showed a drastic reduction in germination (43%) than the absolute control.The seeds treated with different concentrations of melatonin had a higher germination percentage than the control (Fig. 1a). Among the melatonin treatments, seeds treated with 100 (75%) and 80 (70%) µM of melatonin recorded a significantly higher germination percentage than control plants.

Shoot and root length and vigor index

Results indicated that shoot length, root length and vigor index of the greengram seedlings were substantially decreased under PEG-induced drought stress. However, melatonin pre-treated seeds had a significant ($p \le 0.05$) higher shoot and root growth and vigor index under PEG stress conditions than its respective control. Among the melatonin treatments, seeds pretreated with 20 µM of melatonin had the lowest shoot length (7.1 cm), root length (4.5 cm) and vigor index (1007.59),respectively(Fig. 1b). At a higher concentration (100 µM) the shoot (8.9 cm) and root length (7.5 cm) and vigor index (1631.68) increased by 77.5%, 80% and 87.9 %, respectively as compared to PEG induced drought-stressed seedlings.

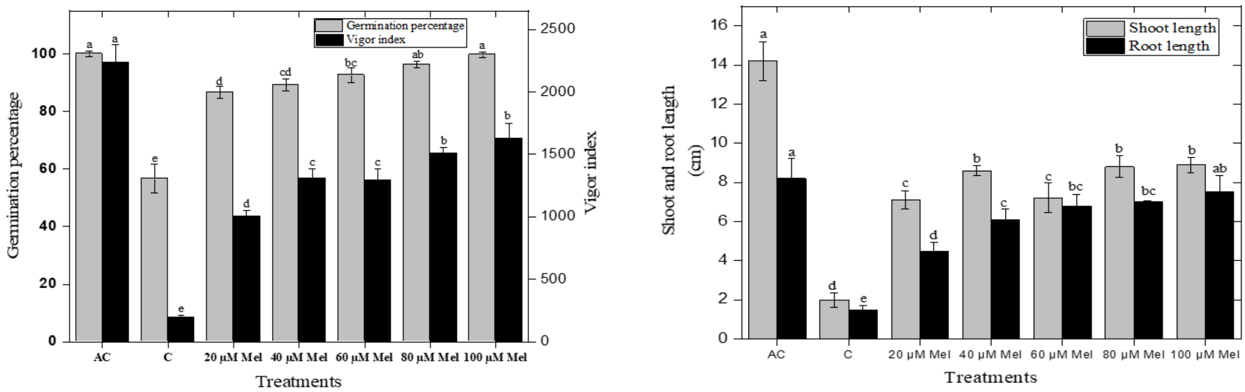

Fig. 1. *Effect of melatonin on (a) germination percentage and vigor index, (b) shoot and root length of greengram seedlings under PEG-induced drought stress condition.AC: Absolute Control, C: Control, Mel: Melatonin (Least significant difference test was used to compare the differences among group means and the critical difference was computed at $p \leq 0.05$; Values with different letters are significantly different)*

Promptness index and germination stress tolerance index

The promptness index of greengram seedling was observed to be higher in the melatonin pre-treatment at 100 µM (79%) and 80 µM (77%) compared to control treatments. Similarly, the germination stress tolerance index (79%) was higher in all melatonin treatments than PEG-induced drought-stressed (C) seedlings (Fig. 2).

Fresh and dry weight of seedlings

The fresh and dry weight of greengram seedlings varied significantly between the treatments. The absolute control showed a significant ($p \leq 0.05$) decrease in fresh and dry weight over the control treatment. However, pre-treatment melatonin showed a significant ($p \leq 0.05$) increase in fresh and dry weights of seedlings than in its control. Among the melatonin treatments, seeds pretreated with 100 µM of melatonin had higher fresh and dry weight (3.249 and 0.147 mg seedling^{-1}, respectively) over control seedlings (Fig. 3). The minimum fresh and dry weight of the seedlings were observed in 20 µM (1.496 and 0.119 mg seedling^{-1}, fresh and dry weight respectively) and 60 µM (1.870 and 0.124 mg seedling^{-1}, fresh and dry weight respectively) of melatonin pre-treatments.

Plant height stress index and root length stress index

Plant height stress index and root length stress index were significantly ($p \leq 0.05$) decreased due to drought stress (PEG 6000) in control (14.43 % and 17.58 %) compared with melatonin treatments. There was a significant ($p \leq 0.05$) variation among the melatonin treatments (Fig. 4) except for 80 and 100 µM of melatonin pre-treatments. The maximum plant height stress index and root length stress index were noticed in 100 (62.96 % and 61.76 %) and 80 (91.70 % and 85.79 %) µM of melatonin pre-treatments.

DISCUSSION

Climate change is predicted to have a deleterious effect on plant growth and development. Germination, vegetative growth, reproductive organ development, grain filling rate and quality were severely affected by the various abiotic stresses (Sehgal *et al.,* 2017). Plant growth and development begin with the seed germination process. The occurrence of drought stress during germination poses a major impact on the seed germination process. Water stress is the key environmental factor that affects the seed germination process (Donohue *et al.,* 2010).

In the laboratory experiment, PEG-induced drought-stressed seedlings showed a drastic reduction in germination percentage compared to the control seedlings. Several studies reported the inhibitory effect of drought stress on seed germination which was alleviated by the melatonin treatments (Cao *et al.,* 2019; Chen *et al.,* 2021; Yu *et al.,* 2021;Guo*et al.,* 2022; Li *et al.,* 2022). Under abiotic stress conditions, melatonin acts as a signalling molecule and positively regulates the germination process by upregulating the genes involved in gibberellin (GA) biosynthesis (*CsGA20ox* and *CsGA3ox*) and abscisic acid (ABA) catabolism (*CsCYP707A1* and *CsCYP707A2*) in cucumber (Zhang *et al.*, 2014). In the present study, from the correlation analysis, it was observed that germination percentage showed a significant ($p \leq 0.05$) positive relationship with vigor index (r^2=0.96) (Fig. 5). Similar studies on the impact of melatonin on pre-treated seeds under different abiotic stresses were reported in corn (Jiang *et al.,* 2016) and soybean (Wei *et al.,* 2015).

The shoot and root lengths were reduced under the PEG-induced drought stress. Similarly, Geilfus (2017) showed that drought stress inhibited shoot and root growth by changing the apoplastic pH from acidic to alkaline due to the accumulation of ABA, which further

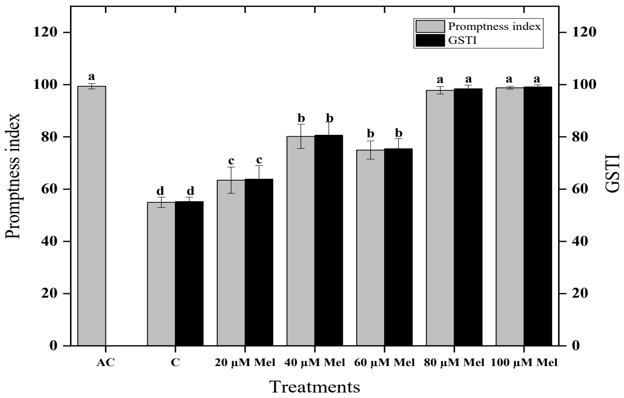

Fig. 2. *Effect of melatonin on promptness index (PI) and germination stress tolerance index (GSTI) of greengram seedlings under PEG induced drought stress condition.AC: Absolute Control, C: Control, Mel: Melatonin (Least significant difference test was used to compare the differences among group means and the critical difference was computed at p≤ 0.05)*

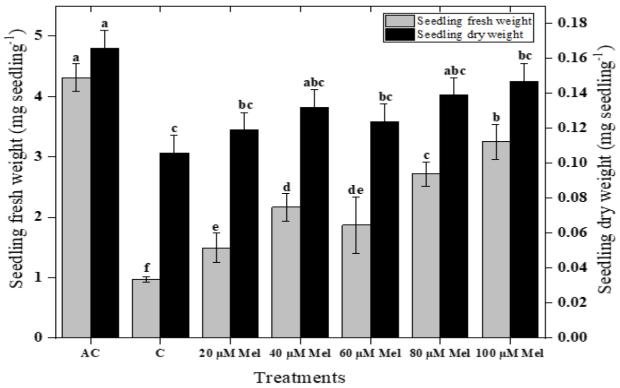

Fig. 3. *Effect of melatonin on the fresh and dry weight of greengram seedlings under PEG induced drought stress condition.AC: Absolute Control, C: Control, Mel: Melatonin (Least significant difference test was used to compare the differences among group means and the critical difference was computed at p≤ 0.05)*

inactivates cell wall loosening enzymes. Hence, melatonin, an indoleamine compound, is involved in the cell elongation process (Nawaz *et al.,* 2016). Therefore, pre-treatment of melatonin with seeds may enhance the seed germination by involving the GA biosynthesis, which is involved in the activation of H^+-ATPase causes a reduction of intercellular pH to acidic nature and activates the cell wall loosening enzymes which in turn to induces cell elongation. A significant ($p \le 0.05$) positive correlation of vigor index with shoot length (r^2=0.98) and root length (r^2=0.96) was observed (Fig. 5).The positive effect of melatonin treatment was consistent with the findings of Huang *et al.* (2019) in maize and Li *et al.* (2022) in rice.

The promptness index and germination stress tolerance index showed higher in the pre-treated melatonin treatments than in the PEG-induced drought stress condition. Based on studies, melatonin has been found to have a stimulatory impact on growth and development. From the correlation analysis, it was observed that the promptness index showed a significantly ($p \le 0.05$) positive correlation with germination percentage (r^2=0.85). Similarly, the germination stress tolerance index significantly ($p \le 0.05$) had a positive correlation

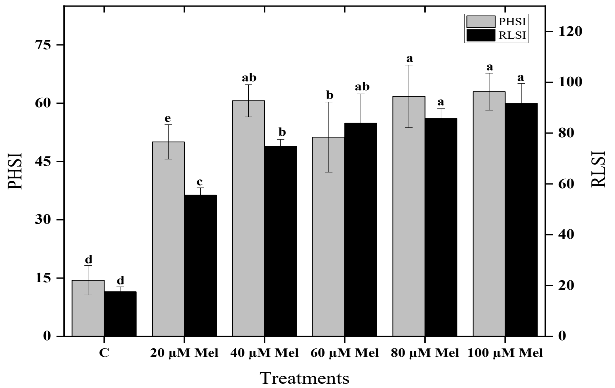

Fig. 4. *Effect of melatonin on plant height stress index (PHSI) and root length stress index (RLSI) of greengram seedlings under PEG induced drought stress condition.AC: Absolute Control, C: Control, Mel: Melatonin (Least significant difference test was used to compare the differences among group means and the critical difference was computed at p≤ 0.05)*

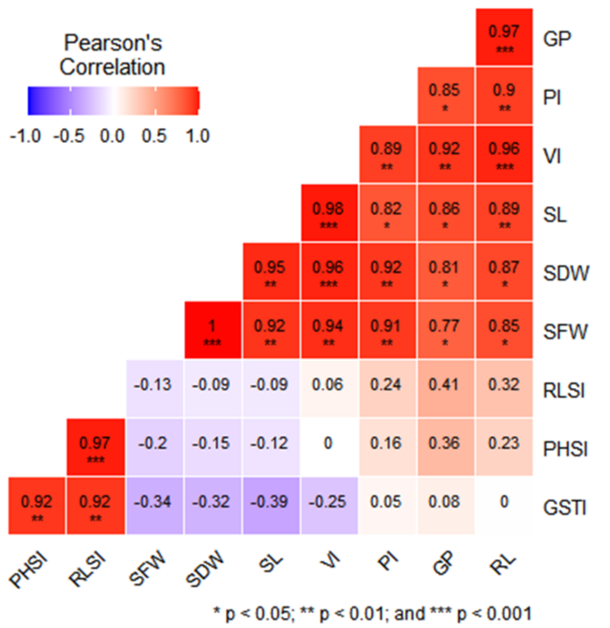

Fig. 5. *Pearson's correlation between seedling growth parameters of greengram under PEG induced drought stress condition.Germination percentage (GP), Promptness index (PI), Vigor index (VI), Shoot length (SL), Root length (RL), Shoot fresh weight (SFW), Shoot dry weight (SDW), Root length stress index (RLSI), Plant height stress index (PHSI), Germination stress tolerance index (GSTI.*

with plant height stress index and root length stress index (r^2=0.92) (Fig. 5). The present results were found to be consistent with the findings of Jiang *et al.* (2016)in that the priming of seeds with melatonin improves and fastens the seed germination process in maize under abiotic stress conditions. Seed priming activates hydrolytic enzymes and alters embryo physiology to accelerate germination metabolism (Farooq *et al.,* 2019). Therefore, seed germination and seedling growth were improved as a result of seed priming by synthesizing the stress-related proteins and activating signalling pathways under water deficit conditions in wheat (Abid *et al.,* 2018).

Correlation analysis revealed that both the fresh (r^2=0.94) and dry weight (r^2=0.96) of the seedlings have a significant ($p \leq 0.05$) positive correlation with vigor index (Fig. 5). The present result was in accordance with Zhang *et al.* (2013), the inhibitory effect of PEG induced drought stress on seed germination and root/ shoot ratio was alleviated by exogenous melatonin application in cucumber. Melatonin enhances the fresh and dry weight of the seedlings more than the drought-stressed plants. It has been reported that the application of melatonin might be attributed to the increases in the volume of pre-existing cell or the expansion of the newly formed cells through the cell division process (Sarropoulou*et al.,* 2012). Water absorption by the seed during the seed germination process has shown a positive effect on the fresh weight and biomass accumulation (Xiao *et al.,* 2019). Similar effects of melatonin on biomass accumulation are reported in lupin cotyledons (Hernandez-Ruiz and Arnao, 2008), rapeseed (Zeng *et al.,* 2018) and maize (Ahmad *et al.,* 2019).

The PEG-induced drought stress significantly ($p \leq 0.05$) reduced the plant height stress index and root length stress index more than the seeds treated with melatonin. The data indicated that a significant ($p \leq 0.05$) positive correlation of both plant height stress index and root length stress index with germination stress tolerance index (r^2=0.92) was observed (Fig. 5). The application of melatonin stimulates the production of endogenous growth factors, which could lead to the production of shoots and denser roots (Zeng *et al.,* 2018). Melatonin activates the auxin-related genes, indicating that the auxin signal pathway is required for melatonin-mediated root growth (Liang *et al.,* 2017). The melatonin treatment increases the shoot and root length, leaf area and biomass accumulation and enhances the plant tolerance to drought stress conditions (Ahmad *et al.,* 2021).

Conclusion

The present study concluded that seed germination and early seedling growth characteristics were adversely affected by drought stress. In general, green gram seeds pre-treated with different concentrations of melatonin (20, 40, 60, 80 and 100 µM) significantly enhanced the seed germination and other growth characteristics compared to untreated under drought conditions. Seed treatment with 100 µM melatonin recorded the highest percentage of seed germination and seedling vigour under PEG induced drought stress conditions.

Conflict of interest

The authors declare that they have no conflict of interest.

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