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Anti-stress effects of melatonin pre-treatment on germination indicators in tomato (*Lycopersicon esculentum* L.) under polyethylene glycol induced drought stress

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Abstract

Tomato is a commercially grown vegetable crop that is susceptible to drought stress, which can be improved by understanding the tolerance mechanism. This study aims to evaluate the impact of melatonin pretreatment on alleviating PEG-induced drought stress on tomato. Initially, screening was performed with different polyethylene glycol (PEG) concentrations (-0.1, -0.2, -0.3, and -0.4 MPa), along with a control. The seeds treated with -0.3 MPa PEG exhibited lower germination percentage, seedling growth, and vigour index, while no germination was observed at -0.4 MPa. Therefore, -0.3 Mpa was selected as the sublethal osmotic concentration to assess the effect of melatonin to alleviate the osmotic stress. The seeds treated with various melatonin concentrations (20, 40, 60, 80, 100, and 120 µM) and untreated stress control were subjected to -0.3 MPa osmotic concentration, and absolute control was maintained. Seed germination parameters *viz.,* germination indicators, shoot and root length indicators, biomass indicators, stress indices, and germination speed, were recorded. The results showed that the germination parameters were significantly reduced in stress control; however, notable improvements were observed in melatonin pre-treated seeds. Pretreatment with 100 µM melatonin led to an increase in germination percentage (61%), germination index (150.5), vigour index (1010.1), promptness index (65), shoot length (6.28 cm), root length (7.38 cm), fresh weight (0.66 mg), dry weight (0.07 mg), shoot length stress index (86), root length stress index (112), dry matter stress index (87), rate of germination (1.5% per day), germination rate index (15.1% per day), and coefficient of the velocity of germination (20.6) under drought stress. The study evidenced that melatonin pretreatment could effectively enhance seed germination under PEG-induced drought stress in tomato, which, therefore, can be recommended for further research.

Key words: germination parameters, speed of germination, polyethylene glycol, melatonin, tomato

Introduction

Tomato (*Lycopersicon esculentum* L.) belongs to the Solanaceae family, and is widely recognized as one of the most valuable and economically grown vegetable crops worldwide. It is widely consumed as a staple food because of its health benefits and potential to treat various chronic diseases (Marti *et al.,* 2016). Tomato is the second largest vegetable crop, covering 4.85 mha after potato. Asia is leading in tomato production, accounting for 61.1% of the global output, surpassing the Middle East, Africa, the United States and Brazil (Faostat, 2019). In India, tomato cultivation spans an area of 8.41 lakh ha, with an annual production of 20 million metric tonnes (Indiastat, 2022). Climate change poses significant challenges to crop productivity, with abiotic stress emerging as a major constraint that adversely affects crop yield by 50% or higher. The 21st century has witnessed increased climatic variations, leading to more frequent drought occurrences, which are projected to rise by 3 to 8% (Tripathy *et al.,* 2023). Mainly, vegetable crops are susceptible to drought stress from germination to maturity. Insufficient soil moisture during sowing significantly hampers the germination process, leading to poor growth and development, ultimately reducing yield (Toscano *et al.,* 2023). To address the challenges posed by global warming, it is imperative to enhance seed germination and seedling growth under low moisture conditions (Tripathy *et al.,* 2023).

The successful establishment of crops is contingent upon the timing and duration of seed germination, particularly in low water conditions. Tomato seeds typically germinate within five days, while water stress can impede or delay germination due to various biochemical changes (Mohammadizad *et al.,* 2013). In this perspective, drought stress can be simulated using polyethylene glycol (PEG), and the application of plant hormones has proven to be an effective approach for regulating seed germination (Mahpara *et al.,* 2022). Melatonin, an amphiphilic indoleamine compound, is known for its environment-friendly properties and role in enhancing plant tolerance to abiotic stress. In addition to its neurohormonal functions, melatonin plays a multifaceted role in seed germination (Awan *et al.,* 2023), vegetative and reproductive development (Wang *et al.,* 2022). Several studies have shown that treating seeds with melatonin promotes germination and seedling growth in various crops, including wheat (Guo *et al.,* 2022), rice (Megala *et al.,* 2022), green gram (Anitha *et al.,* 2022) and cotton (Bai *et al.,* 2020). Moreover, previous research has demonstrated that exogenous melatonin application can alleviate oxidative stress under drought conditions (Liu *et al.,* 2015). However, understanding the physiological and biochemical mechanisms underlying drought tolerance in seed germination is essential, particularly for vegetable crops like tomato.

The changes observed in germination indicators depend on the concentration of melatonin and the intensity of drought stress. Nevertheless, pre-treating with melatonin can contribute to regulating biochemical and metabolic reactions involved in the germination process (Zhang *et al.,* 2020). Several studies have documented the positive effect of melatonin in mitigating drought stress (Annadurai *et al.,* 2023). However, little is known regarding the potential effects of melatonin concentrations on germination indicators in tomato. Consequently, our study aimed to examine the effects of melatonin pretreatment in the tomato variety 'PKM1', focusing on achieving consistent seed germination, which is crucial under drought stress conditions. The objectives of our investigation were twofold: (i) determining the lethal and sub-lethal doses of PEG for seed germination, (ii) identifying the optimal concentration of melatonin pretreatment for characterizing the seed germination, and seedling growth indicators. This study sets the dimension on how melatonin pre-treatment regulates seed germination, elucidating the underlying physiological mechanisms. Moreover, our research could explore the significance of melatonin seed treatment in aiding seedlings to overcome the negative effects of abiotic stress.

Materials and methods

Test Material: The tomato seeds used in the experiment were 'PKM1' variety, provided by the Department of Vegetable Science, Horticulture College and Research Institute, Coimbatore. Melatonin was obtained from Sigma-Aldrich Pvt. Ltd. India, stored at a temperature of -20 °C, and used when required.

Experiment and treatment details

Experiment I (Screening for optimum PEG concentration): The experiment was conducted during January 2022 in the Laboratory of the Department of Crop Physiology at Tamil Nadu Agricultural University, Coimbatore, India. The tomato seeds were surface sterilized with 5% sodium hypochlorite solution for 5 min, then washed three to four times with distilled water. The following treatments were used: Absolute Control with distilled water (without PEG) and four levels of PEG concentrations used to impose stress at -0.1, -0.2, -0.3 and -0.4 MPa, respectively. Each treatment had five replicates maintaining 25 seeds per replicate and the sterilized seeds were placed in a petriplate containing filter paper, to which 15 mL of different PEG solutions were added. To evaluate the sublethal dosage of polyethylene glycol, tomato seeds with 2 mm of radical were considered germinated (Florido *et al.,* 2018). On the 12th day, the germination parameters *viz.,* rate of germination, shoot length (SL), root length (RL), and vigour index (VI), were recorded. The germination percentage was recorded on 2nd, 4th, 6th, 8th, 10th and 12th day.

Experiment II (Screening for optimum melatonin concentration under PEG-induced drought): The melatonin stock solution is prepared by dissolving the required quantity of melatonin in 99.9% ethanol and made to a final volume of 1L with distilled water. The dilutions of different melatonin concentrations were prepared from the stock solution. The sterilized seeds were soaked in a beaker containing different melatonin concentrations (20, 40, 60, 80, 100 and 120 μM) for six h. To determine optimum melatonin concentration to alleviate the PEG-induced drought stress, 25 seeds were placed on a Petriplate containing filter paper, to which 15 mL of -0.3 MPa PEG 6000 (sublethal concentration) was added. Each treatment was replicated four times. Eight treatments were used in this experiment: absolute control (AC) with distilled water (without PEG), stress control (SC) with -0.3 MPa of PEG and the different concentrations (20, 40, 60, 80, 100 and 120 μM) of melatonin (Mel) treated seeds exposed to osmotic potential of -0.3 MPa. The germination indicators, shoot and root growth indicators, biomass indicators, stress indices, and the speed of germination were recorded on 12th day. The germination percentage was recorded on 2nd, 4th, 6th, 8th, 10th and 12th day.

Calculation of germination parameters:

Germination indicators: Germination percentage (GP) indicates the proportion of seeds germinating on the 2nd, 4th, 6th, 8th, 10th and 12th day out of the total number of seeds sown in Petriplates. The calculation of the germination percentage followed the method outlined in Nezar and Aldahadha, (2022).

$$\text{Germination } (\%) = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Vigour index (VI) was calculated according to the method mentioned in Hussain *et al.* (2015).

Vigour Index = Germination percent x Seedling length

Germination index (GI) was calculated as per the method described in Hussain *et al.* (2015)

GI = (12 x nl) + (11 x n2) 4- (10 x n3) ••• (1 x nl2)

where n1, n2, ..., n10 denote the number of seeds that germinated on the first, second, and subsequent days up to the twelfth day.

Promptness index (PI) calculates the percentage of seeds that germinate on the 2nd, 4th, 6th, 8th, 10th, and 12th day of observation, represented by nd2, nd4, nd6, nd8, nd10 and nd12, respectively, as described in Raza *et al.* (2012).

PI=nd2(1.20)+nd4 (1.0)+nd6(0.8)+nd8(0.6)+nd10(0.4)+nd12(0.2)

Germination stress index (GSI) was determined using the following formula:

$$\text{GSI } (\%) = \frac{\text{Promptness index (stress)}}{\text{Promptness index (control)}} \times 100$$

Shoot and root growth indicators: The average shoot length (SL) and root length (RL) were determined by measuring 25 seedlings from each replication within a treatment. The shoot length was measured from the base of the root to the tip of the shoot, while the root length was measured based on the length of the main root and expressed in cm.

Biomass indicators: Immediately after analyzing the growth indicators, the fresh weight (FW) of the seedlings was recorded. Subsequently, the seedlings were subjected to a hot air oven at 60 °C for 48 h and the dry weight (DW) was recorded.

Stress indices: The stress indices were calculated following the methodology outlined in Raza *et al.* (2012). The shoot length stress index (SLSI) and root length stress index (RLSI) were calculated from the measured shoot, root length of stress and control seedlings.

$$\text{RLSI } (\%) = \frac{\text{Root length (stress)}}{\text{Root length (control)}} \times 100$$

The dry matter stress index (DMSI) was calculated using the measured dry weight obtained after drying the samples in a hot air oven at 60 °C. The calculation of DMSI was based on the dry weight between the stress and control seedlings.

$$\text{DMSI (\%)} = \frac{\text{Dry matter (stress)}}{\text{Dry matter (control)}} \times 100$$

Speed of germination: Germination rate index (GRI) is determined by calculating the percentage of germination on the 2^{nd}, 4^{th}, 6^{th}, 8^{th}, 10^{th} and 12^{th} day, represented by G2, G4, G6, G8, G10 and G12, respectively using the following formula as mentioned in Rupal *et al.* (2020).

$$\text{GRI (\% per day)} = \frac{G2}{2} + \frac{G4}{4} + \frac{G6}{6} + \frac{G8}{8} + \frac{G10}{10} + \frac{G12}{12}$$

Rate of germination was calculated by counting the number of germinated seeds on the 12^{th} day of the germination process and expressed in days (Bakhshandeh *et al.,* 2017).

$$\text{MGT} = \frac{\text{Number of seeds germinated on } 12^{th} \text{ day}}{12}$$

Coefficient of velocity of germination (CVG) is a measure that determines the frequency at which the seeds germinate on each day (N), and the time took for seed N to germinate (T), starting from the day of sowing. It is calculated using the formula established by Talska *et al.* (2020).

$$\text{CVG} = \frac{N1 + N2 + N3 \cdots N_X}{100} \times (N1T1 + N2T2 + N3T3 \cdots N_X T_X)$$

Data Analysis: The study was carried out in a completely randomized block design (CRD). The germination parameters were analyzed using analysis of variance (ANOVA) using SPSS for Windows, version 16.0, developed by SPSS Inc., Chicago, USA. The data is presented as the mean of replications and standard error of means (SEM). The least significant difference test ($LSD_{5\%}$) was used to compare the means among different treatments. The significance level was denoted using small letters, given that the means with the same letters are not statistically significant at p = 0.05. High-resolution graphs representing the observed variables and the correlation among the germination parameters were assessed using Pearson correlation in GraphPad Prism software for Windows, version 9.0.0.

Results

Effect of PEG on germination parameters: The germination percentage (GP), shoot length (SL), root length (RL), vigor index (VI) and rate of germination was calculated on the 12^{th} day and had a significant difference ($P < 0.05$) among the treatments. The increased GP was observed in absolute control (90%), attaining its final germination on the 7^{th} day. However, higher concentrations of PEG -0.3 MPa showed decreased GP (40%) with a delay in germination time, attaining final germination on the 10^{th} day. No germination was recorded in -0.4 MPa (Fig. 1).

Effect of melatonin pretreatment on germination parameters under PEG induced drought

Germination indicators: The germination percentage (GP) was observed to be higher in absolute control (AC), attaining its final germination on the 7^{th} day, while a lower GP (49%) was observed in stress control (SC), reaching its final germination on 10^{th} day. However, seeds treated with different melatonin concentrations showed increased germination percentage in shorter germination time, attaining its final germination on 8^{th} day (Fig. 2). Among

Table 1. Effect of PEG-6000 on rate of germination, seedling growth (SL+RL) and vigor index (VI) in tomato

Treatments	Rate of germination	Shoot length (cm)	Root length (cm)	Vigor index
Absolute control	1.88 ± 0.020^a	8.54 ± 0.05^a	7.37 ± 0.096^a	1438.10 ± 20.52^a
-0.1 MPa	1.57 ± 0.031^b	7.22 ± 0.09^b	7.76 ± 0.092^a	1126.24 ± 21.97^b
-0.2 MPa	1.30 ± 0.020^c	5.22 ± 0.09^c	5.98 ± 0.106^b	698.48 ± 10.05^c
-0.3 MPa	0.83 ± 0.045^d	3.28 ± 0.08^d	3.94 ± 0.107^c	289.50 ± 18.44^d

*No germination was observed in -0.4 MPa. The data represent the mean of replications and standard error of means (SEM). The least significant difference test ($LSD_{5\%}$) was computed to compare the means among different treatments. The significance level was denoted using small letters, given that the means with the same letters are not statistically

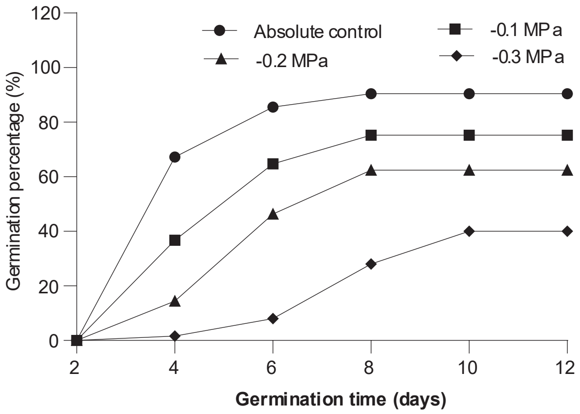

Fig. 1. Effect of PEG-6000 on germination percentage in tomato on 12^{th}

the treatments, -0.3 MPa (PEG) imposed seeds treated with 100 µM, 120 µM and 80 µM melatonin showed significant ($P < 0.05$) increase in germination percentage by 61%, 57% and 54% respectively than stress control. Notably, an increased concentration of melatonin (120 µM) exhibited an increased GP and showed a decreasing trend of GP. A direct correlation supports this data; under decreased water potential, when melatonin concentration increases, the GP increases with the same intensity (Fig. 5). In the present study, it is observed that GP showed a significant positive relationship with MGT (r2=1.00), PI (r^2=0.99), GRI (r^2=0.99), GI(r^2=0.98) and VI (r^2=0.98).

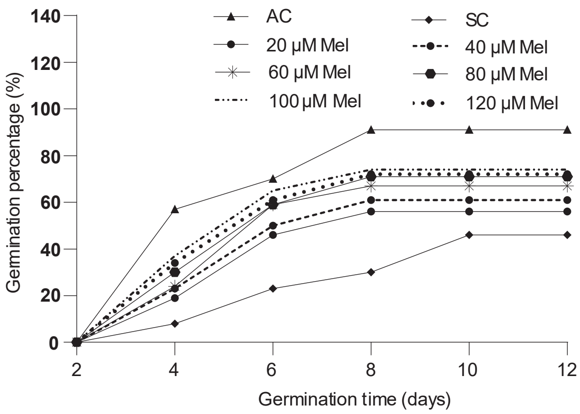

Fig. 2. Effect of melatonin pretreatment on germination percentage under PEG-induced drought in tomato on 12^{th} day of stress.

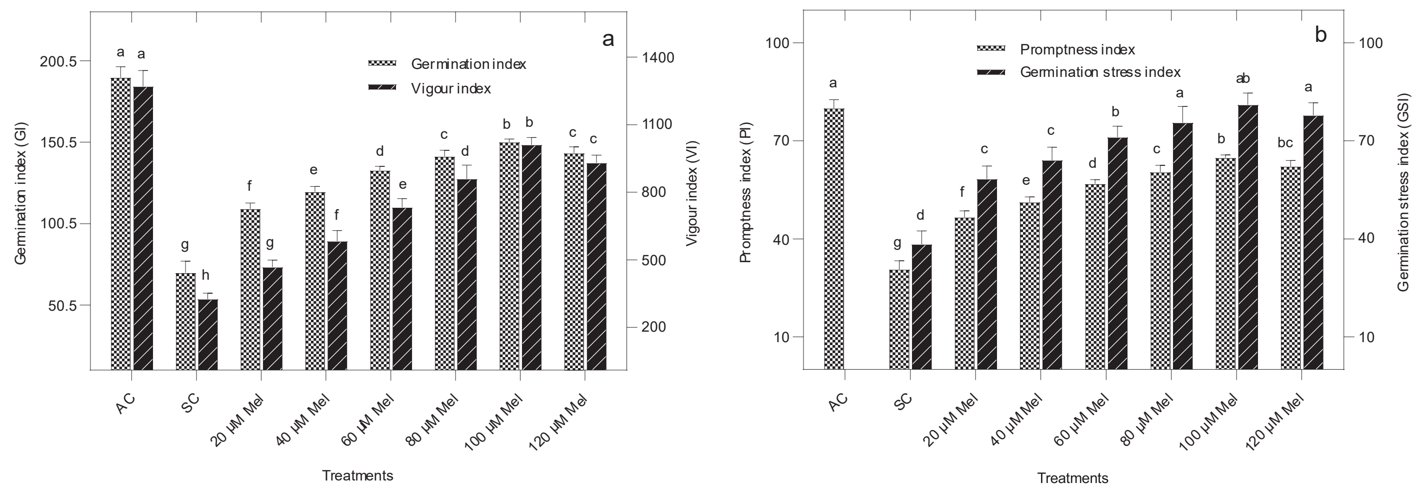

Fig. 3. Effect of melatonin pretreatment on germination indicators a) germination index and vigor index and b) promptness index and germination stress index under PEG-induced drought in tomato on 12th day of stress. The data is presented as the mean of replications and standard error of means (SEM). The least significant difference test ($LSD_{5\%}$) was computed to compare the means among different treatments. The significance level was denoted using small letters, given that the means with the same letters are not statistically significant at p = 0.05. (Absolute Control-AC, Stress

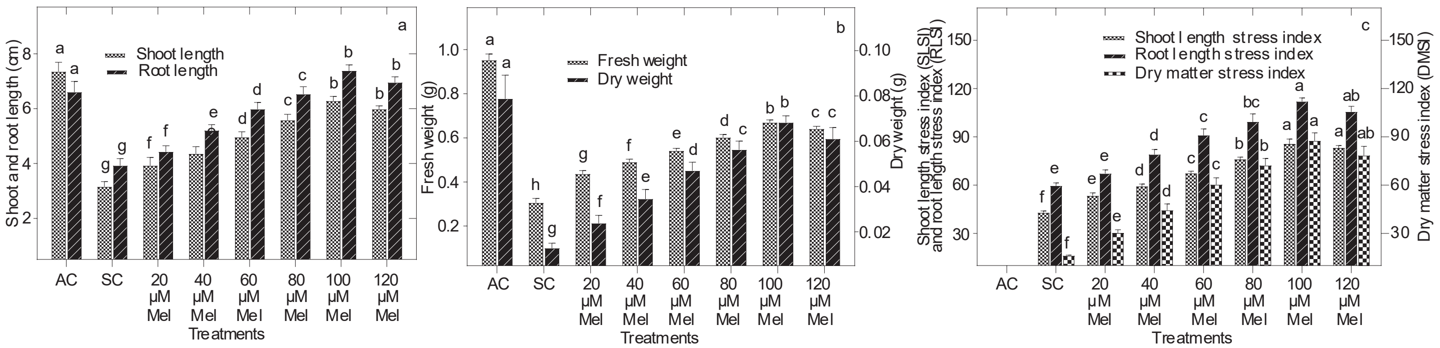

Fig. 4. Effect of melatonin pretreatment on growth and biomass indicators a) shoot length and root length and b) fresh weight and dry weight and c) stress indices under PEG-induced drought in tomato on 12th day of stress. The data is presented as the mean of replications and standard error of means (SEM). The least significant difference test ($LSD_{5\%}$) was computed to compare the means among different treatments. The significance level was denoted using small letters, given that the means with the same letters are not statistically significant at p = 0.05. (Absolute Control-AC, Stress Control-SC, Melatonin- Mel).

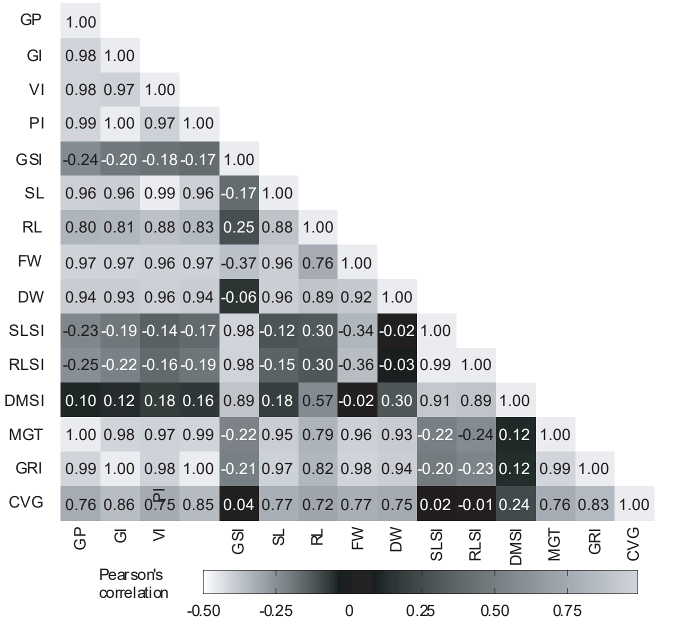

Fig. 5. The correlation matrix between all germination parameters in tomato was assessed using Pearson correlation at the significance of p = 0.05. Germination percentage (GP), Germination index (GI), Vigour index (VI), Promptness index (PI), Germination stress index (GSI), Shoot length (SL), Root length (RL), Fresh weight (FW), Dry weight (DW), Shoot length stress index (SLSI), Root length stress index (RLSI), Dry matter stress index (DMSI), Mean germination time (MGT), Germination rate index (GRI) and Coefficient of velocity of germination (CVG).

The germination index (GI), vigor index (VI), promptness index (PI) and germination stress index (GSI) were significantly (P < 0.05) decreased in stress control (SC) seedlings (Fig. 3a, 3b). Among the treatments, 100 µM melatonin pretreatment showed an increased GI, VI and PI of about 150.5 ± 0.9, 1010.1 ± 16.5, and 65 ± 0.4, respectively, that resulted in an increase in GSI compared to stress control. From the correlation matrix (Fig. 5), it is observed that GI is highly correlated with GRI (r^2=1.00), PI (r^2=1.00), vigor index with SL (r^2=0.99), PI with GRI (r^2=1.00) and GSI with SLSI (r^2=0.98) and RLSI (r^2=0.98).

Shoot and root growth, biomass, and stress indicators: The shoot length (SL), root length (RL), seedling fresh weight (FW) and dry weight (DW) were found to be significant among treatments (Fig. 4a, 4b). The results showed that SL, RL, FW and DW were significantly (P < 0.05) higher in absolute control (AC), while the lower SL, RL, FW and DW were observed in stress control (SC). Among the treatments, -0.3 MPa (PEG) treated with 100 µM melatonin showed an increased SL (6.28 ± 0.08 cm), RL (7.38 ± 0.22 cm), FW (0.66 ± 0.006 mg seedling^{-1} and DW (0.07 ± 0.001 mg seedling^{-1}. From the correlation analysis (Fig. 5), it is observed that SL had a significant (P < 0.05) positive correlation with GRI (r^2=0.97) and negatively correlated with SLSI (r^2=-0.12) and RLSI (r^2=-0.15). Similarly, RL strongly correlates with DW (r^2=0.89), while the FW and DW positively correlate with GRI (r^2=0.98; r^2=0.94), respectively. The shoot length stress index

(SLSI), root length stress index (RLSI) and dry matter stress index (DMSI) showed significant ($P < 0.05$) among the treatments (Fig. 4c). Among the treatments, SLSI, RLSI and DMSI were minimum in stress control (SC), while the maximum SLSI (86 ± 2.9), RLSI (112 ± 2.3) and DMSI (87 ± 4.8) were observed in 100 µM melatonin pre-treated seedlings. In this study, it is observed that SLSI, RLSI, and DMSI showed a significant ($P < 0.05$) positive relationship with RLSI (r^2=0.99), DMSI (r^2=0.89) and CVG (r^2=0.24), respectively (Fig. 5).

Speed of germination: The rate of germination, germination rate index (GRI) and coefficient of the velocity of germination (CVG) were significantly ($P < 0.05$) decreased by 49%, 63% and 32%, respectively, in stress control (SC) compared to absolute control (AC). Among the treatments, the increased rate of germination, GRI, and CVG by 61%, 115% and 41%, respectively, were recorded in 100 µM melatonin pre-treated seedlings, while the decreased rate of germination, GRI, and CVG by 22%, 51% and 33%, respectively were recorded in 20 µM melatonin pre-treated seedlings compared to stress control (Table 2). From the correlation analysis (Fig. 5), it is concluded that the speed of germination parameters such as MGT, GRI and CVG) is highly correlated with GRI, CVG and GI, respectively, having a correlation coefficient (r^2) value of 0.99, 0.83 and 0.86, respectively.

Table 2: Effect of melatonin pretreatment on the speed of germination under PEG-induced drought in tomato

Treatments	Rate of germination (% day^{-1})	Germination rate index (GRI) % day^{-1}	Coefficient of velocity of germination (CVG)
Absolute control	1.90 ± 0.021^{a}	19.04 ± 0.32^{a}	21.58 ± 0.40^{a}
Stress control	0.96 ± 0.024^{f}	6.98 ± 0.31^{g}	14.58 ± 0.57^{d}
20 µM Melatonin	1.17 ± 0.034^{e}	10.50 ± 0.22^{f}	19.38 ± 0.47^{c}
40 µM Melatonin	1.27 ± 0.021^{d}	11.63 ± 0.19^{e}	19.49 ± 0.09^{bc}
60 µM Melatonin	1.40 ± 0.021^{c}	12.83 ± 0.12^{d}	19.84 ± 0.25^{bc}
80 µM Melatonin	1.48 ± 0.040^{b}	13.83 ± 0.20^{c}	20.01 ± 0.43^{bc}
100 µM Melatonin	1.54 ± 0.024^{b}	15.04 ± 0.08^{b}	20.59 ± 0.35^{ab}
120 µM Melatonin	1.50 ± 0.034^{b}	14.38 ± 0.17^{c}	20.00 ± 0.43^{bc}

The data represents the mean of replications and standard error of means (SEM). The least significant difference test ($LSD_{5\%}$) was computed to compare the means among different treatments. The significance level was denoted using small letters, given that the means with the same letters are not statistically significant at $P = 0.05$.

Discussion

Drought significantly affects seed germination, crop growth, development and yield forming process. Germination is a physiological process influenced by regulating various enzymes. This process involves imbibition, seedcoat expansion, and enzymatic degradation. In the early stage of the life cycle, such as germination, polyethylene glycol (PEG) is used to induce drought stress (Khaeim *et al.,* 2022). Hence, the primary goal is to optimize the PEG concentration before setting an experiment. Drought influences seed germination ability, as evidenced in previous studies (Kaur *et al.,* 2023). Our research showed that tomato seeds imposed to PEG-induced drought resulted in a decreased germination percentage, seedling growth and vigor index than absolute control. Consequently, higher concentrations of PEG reduced the rate of germination, indicating a decline in seed germination capacity. This is due to reduced water absorption and hydrolysis of seed nutrient reserves. Comparable results were reported by Kintl *et al.* (2021) in clover.

To reduce the negative effects of drought, one of the phytohormones, like melatonin, is widely used and extensively reported in rice (Megala *et al.,* 2022) and maize (Ahmad *et al.,* 2022). However, the role of melatonin on seed germination indicators involved in stress alleviation has rarely been reported. The impact of melatonin on seed germination depends on the concentration, indicating that higher concentrations of melatonin may inhibit or not affect germination (Megala *et al.,* 2022). However, in our study, melatonin concentration exceeding 100 µM slightly declined. The increased negative osmotic potential significantly affected the germination indicators (Kumar *et al.,* 2017), indicating that enzymatic activities have a role in reducing the germination potential. Our study revealed that melatonin application ameliorated the negative effects, which may be due to enhanced mobilization of endosperm reserve, energy production in the respiration process, enzymatic activity, hormonal activity and dilution of protoplasm (Muscolo *et al.,* 2014). Consequently, more GSI indicates that seedlings withstand drought upon melatonin treatment, showing drought tolerance. Similar studies were reported comparable results in cucumber (Zhang *et al.,* 2013), and cotton (Bai *et al.,* 2020).

Crop productivity is assessed by evaluating seedling growth indicators such as shoot, root length and biomass indicators, including fresh and dry weight (Shahzad *et al.,* 2023). Root growth is constrained when crops experience drought stress, while shoot growth is significantly reduced. The decreased shoot length is due to reduced cell elongation and low water potential. Similarly, biomass indicators are also reduced under drought stress, indicating turgor loss (George *et al.,* 2013). In the present study, the application of melatonin enhanced the seedling growth and biomass indicators even under limited water availability by increasing the absorption capacity of water and nutrients, thus improving biomass. However, it should be noted that although these positive results were observed, they were insufficient to compensate for the damage caused by drought fully. Stress indices and speed of germination were found to be decreased under PEG-induced drought. Similar results were reported in soybean by Zou *et al.* (2019).

In contrast, the application of melatonin as pretreatment positively influences intercellular pH and cell wall modifications. This resulted in increased shoot length, root length and dry matter stress indexes. Comparable results were also reported in greengram (Anitha *et al.,* 2022). Regarding parameters related to germination speed, melatonin treatment led to an increase in rate of germination, substantiating a higher number of seeds germinated in a day. Additionally, there was an increase in GRI and CVG, representing faster germination in a shorter time. It is essential that CVG only focus on the time required to attain the final germination percentage. Similar studies were reported by Bai *et al.* (2020) . In the current study, correlation with seed germination parameters were found to be positive in most traits that, evidences the importance of melatonin pretreatment in mitigating drought stress.

In conclusion, treating tomato seeds with melatonin at varying concentrations (20-120 µM) improved germination and seedling growth. Notably, 100 µM melatonin showed the most significant enhancements in multiple growth and stress indicators. This study underscores melatonin's potential as a drought stress elicitor for enhancing commercial-scale yields. However, for broader applicability, standardized investigations into melatonin seed treatment across different crops and concentrations are essential for future research.

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