

Research Article

Melatonin mediated high-temperature tolerance at seedling stage in green gram (*Vigna radiata* L.)

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Abstract

Global warming is predicted to have a generally negative effect on food grain production. The emergence of seedlings, blooming, pod-filling stages and yield of the mung bean are affected by high-temperature stress. Melatonin is a multifunctional signaling molecule with antioxidant properties that plays a vital role in plant stress defense mechanism. With this knowledge, the experiment was conducted to identify the optimum melatonin concentration to mitigate the adverse effects of high temperature in green gram var CO 8 with a completely randomized design (CRD). The treatments consisted of soaking seeds with different melatonin concentrations, viz., 20, 40, 60, 80 and 100 μ M. Seeds were sown in a petridish and allowed to germinate. After 5 days, the seedlings were exposed to two different high-temperature stress following the temperature induction response (TIR) protocol in the growth chamber viz., Ambient + 2°C (40°C) and Ambient + 4°C (42°C). After stress period, the seedlings were allowed to recover at room temperature for 2 days. At the end of the recovery period, observations on temperature tolerance-related traits viz., survival percentage, per cent reduction of shoot and root growth, cell viability, mortality per cent, malondialdehyde content, superoxide dismutase and catalase activity of green gram seedlings were assessed. Seeds pre-treated with melatonin of 100 and 80 μ M exhibited higher survival percentage, shoot and root growth, cell viability and antioxidant enzyme activity (like superoxide dismutase and catalase) with reduced mortality per cent and malondialdehyde content under high-temperature stress at both 40°C and 42°C. The results revealed that seeds treated with different melatonin concentrations significantly improved green gram germination and seedling health.

Keywords: Green gram, High-temperature stress, Melatonin, Seedlings survival

INTRODUCTION

Greengram (Mung bean) is an imperative grain legume with high economic status and an excellent dietary protein source with nutritional health benefits. It increases soil fertility and plays a crucial role in major cropping

systems due to its short life span (Parihar *et al.*, 2017). Several abiotic factors like heat, drought, salt and water logging negatively impact greengram production and adaptation (Kaur *et al.*, 2015). Abiotic stresses affect crop growth and development by modifying physiological processes and the plant-water connection

(Zandalinas *et al.*, 2017). Mung bean production is altered by adverse environmental conditions, particularly by the rise in temperature due to climate change. The global air temperature is expected to reach 0.2°C every decade, raising temperatures from 1.8 to 4.0°C above the current level (IPCC, 2022). The optimum temperature ideal for the cultivation of greengram ranges from 30°C to 40°C (Zinn *et al.*, 2010). The maximum temperature during the summer month continually exceeds 39°C to 40°C in some places of Tamil Nadu, which is beyond the tolerance limit of the mung bean. Therefore, a rise in temperature beyond 40°C will severely affect the growth and yield of mung bean cultivation in summer (Karande *et al.*, 2018).

Moreover, most of the area under pulses is in the rain-fed ecosystem, which often encounters high temperatures during the reproductive stage of the crop. During the reproductive phase, extreme temperature causes flower drop, induces male sterility, impaired anthesis and shortens the grain-filling period in green gram (Basu *et al.*, 2019). The plant growth is severely reduced under heat stress by altering shoot net assimilation rates and the plant's total dry weight production (Fahad *et al.*, 2017). Several studies have found that high-temperature stress reduces crop growth and development of mung beans (Hanumantha Rao *et al.*, 2016; Sharma *et al.*, 2016; Sita *et al.*, 2017; Priya *et al.*, 2020).

Abiotic stress negatively impacts plant growth, photosynthetic pigments, water and nitrogen usage efficiency, cell structural changes and essential enzyme activities (Singh and Thakur, 2018). Extreme temperature shows impact on photosynthetic processes such as altered enzyme activity, photochemical reactions and disruption of the entire PSII protein complex due to increased membrane flexibility (Yamamoto *et al.*, 2016). High-temperature stress induces the production of reactive oxygen species that damages the membrane lipids and increases the membrane fluidity; therefore, metabolic activities are hindered (Yamamoto *et al.*, 2016). The coincidence of high-temperature stress at crucial growth phases of mung bean is one of the causes of decreased growth traits. Additionally, future means temperature trends in the areas where mung beans are grown are expected to vary significantly. Recently, the growth regulator hormone known as melatonin has been well recognized for its essential role in determining plant growth and development under adverse environmental conditions.

Melatonin (N-acetyl-5-methoxytryptamine) is an indolic substance synthesized from serotonin (5-hydroxytryptamine) and acts as a pleiotropic signaling molecule against various abiotic and biotic stresses (Zeng *et al.*, 2022). In higher plants, tryptophan, the precursor of indole-3-acetic acid (IAA), also serves as a

common precursor for the production of both serotonin and melatonin (Back *et al.*, 2016). Melatonin act as a promoter of seed germination (Cao *et al.*, 2019), lateral roots formation (Liang *et al.*, 2017), regulates blooming time (Sun *et al.*, 2021) and delays leaf senescence (Liang *et al.*, 2018) to manage and control plant growth and development under challenging environments. Hossain *et al.* (2020) suggested that exogenous melatonin improves plant growth by enhancing the enzymatic antioxidants like superoxide dismutase, peroxidase, catalase and ascorbate peroxidase and reduces the oxidative damage induced by ROS. Under stress, melatonin application reduces cell membrane lipids peroxidation and maintains higher plasma membrane integrity (Khan *et al.*, 2020). Buttar *et al.* (2020) reported that melatonin is a multifunctional signaling molecule that improves plants heat tolerance by modulating its antioxidant defense system. Exogenous melatonin can stimulate the biosynthesis of endogenous melatonin and reduce the generation of H₂O₂, O₂⁻, and malondialdehyde (MDA) (Chen *et al.*, 2021) under various abiotic stress, reducing cell membrane damage and enhances photosynthesis (Imran *et al.*, 2021). With this background, the present study thus examined the melatonin concentrations capacity on the enhancement of biochemical characteristics and development of green gram grown in high-temperature-stressed 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 standardize the optimum concentration of melatonin for mitigating the ill effects of high-temperature stress. Temperature induction response (TIR) is a promising tool for determining a seedlings' thermo-tolerance level based on its growth and recovery during the seedling stage. This approach involved exposing germinated greengram seedlings to an induced high temperature in a growth chamber for a short period to identify the optimum induction temperature (OIT) and challenging lethal temperature. The seeds of green gram variety CO 8 were used as source material. The uniform and healthy seeds were taken and the seeds were surface sterilized with 3% sodium hypochlorite for two mins and washed thrice with distilled water followed by the seeds were soaked in different concentrations of melatonin about 20, 40, 60, 80 and 100 µM for 6 h. The seeds treated with varying concentrations of melatonin were placed in petri plates (20 seeds per petri plate) separately for the imposition of high-temperature stress. About five days old, uniform seedlings were taken and subjected to two different high-temperature stress, *viz.*, Ambient + 2°C and Ambi-

ent + 4°C for 3 h without prior induction. The ambient temperature was recorded as 38°C. The treatment details consisted of T₁: Absolute control (no stress and melatonin), T₂: Control (exposure to high-temperature stress without melatonin), T₃: 20 µM of melatonin, T₄: 40 µM of melatonin, T₅: 60 µM of melatonin, T₆: 80 µM of melatonin and T₇: 100 µM of melatonin. All these treatments were subjected to two different high-temperature treatments, including 40°C (38°C + 2°C) and 42°C (38°C + 4°C). Three replications were maintained for each treatment. After the stress induction period, these seedlings were immediately allowed to recover in an incubator with the following conditions at 30°C and 60% relative humidity (RH) for 2 days. After the recovery period, various observations on seedling related traits were measured.

Measurement of seedling growth parameters

After the seedlings had recovered from the high-temperature stress for two days, observations were made. The temperature tolerance-related traits of green gram seedlings were assessed by the following methods

Survival percentage and shoot and root growth reduction percentage were calculated by the formula described by Rekha *et al.* (2016).

Percent survival of seedlings = (Number of seedlings survived at the end of recovery/ Total number of seeds sown) x 100Eq. 1

Percent reduction in shoot and root growth = $(C-T/C) \times 100$ Eq. 2

Where, C – Shoot and root growth of control seedlings

T – Shoot and root growth of treated seedlings

Mortality percentage = (Number of dead seedlings / Total number of seeds sown) X 100Eq. 3

Cell viability was determined according to the method described by Kheir *et al.* (2012). The green gram seedlings exposed to high-temperature stress were allowed to recover @ 30°C and 60% RH. After 3 h of recovery, the seedlings were incubated in 0.01% Evans blue dye solution for 2 h. After that, the excess dye was removed by washing it with distilled water. The stain from the seedlings was extracted in absolute alcohol and maintained at 50°C for 15 min, and the absorbance was measured at 600 nm using a UV visible spectrophotometer. The cell viability was calculated by the formula given below:

Cell viability = $(T/C) \times 100$ Eq. 4

Where, T – Absorbance @ 600 of dye extracted from induced seedlings.

C – Absorbance @ 600 of dye extracted from absolute control seedlings.

The malondialdehyde (MDA) content was assessed by the thiobarbituric acid (TBA) reaction according to the method illustrated by Karabal *et al.* (2003). The leaf

tissue of 0.5 g was homogenized with 5 mL of 0.1% trichloroacetic acid (TCA) and centrifuged at 10,000 rpm for 5 min. About 4 mL of 20% trichloroacetic acid (TCA) containing 0.5% thiobarbituric acid (TBA) was added to 1 mL of aliquot and heated at 95°C for 30 min. Immediately the tubes were cooled and centrifuged at 10,000 rpm for 10 min. The supernatant was collected and absorbance was measured at 532 and 600 nm and expressed as µmol g⁻¹ of fresh weight.

To estimate the antioxidant enzyme activity like catalase (CAT) and superoxide dismutase (SOD), the enzyme extract was prepared by weighing 0.5 g of leaf sample and ground into powder using liquid nitrogen. Enzyme extract was prepared by homogenizing the leaf powder with 0.1 M of phosphate buffer (pH 6.8) containing 0.1mM EDTA and 1% Polyvinylpyrrolidone (PVP) in a pre-chilled pestle and mortar. Homogenate was centrifuged at 10,000 rpm for 30 min at 4°C. Collected supernatant was used to estimate antioxidants enzyme activity *viz.*, CAT and SOD.

The CAT activity was analyzed using a method described by Aebi (1984). About 0.5 mL of 75 mM H₂O₂, 1.5 mL of 0.1 M phosphate buffer (pH 7) and 50 µL of enzyme extract were added; finally, the total reaction mixture volume was made up to 3 mL by adding distilled water. The addition of H₂O₂ started the reaction. The decrease in absorbance at 240 nm was recorded for 1 min for every 15 sec time intervals and enzyme activity was computed by calculating the amount of H₂O₂ decomposed. For catalase activity, the extinction coefficient is 39.4 mM⁻¹cm⁻¹ and expressed in terms of µg of H₂O₂ reduced mg protein⁻¹ min⁻¹.

The SOD activity was examined according to the method of Dhindsa *et al.* (1981). The reaction mixture consisted of 1.3µM riboflavin, 13 mM methionine, 63 µM nitro blue tetrazolium chloride (NBT), 0.05 M sodium carbonate, 1% Triton X-100, 50 mM sodium phosphate buffer (pH 7.8), enzyme extract and the final volume was made up to 3 mL by using distilled water. Test tubes were kept under illumination for colour development whereas the non-illuminated reaction mixture without enzyme extract served as a blank. The SOD activity was determined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT and expressed as units mg protein⁻¹ min⁻¹.

Statistical analysis

The design of the experiment was a completely randomized design (CRD) with three replications and the data collected from 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 a five percent probability ($P \leq 0.05$).

Figures were generated using Originpro 2019 software (Originlab Corp., USA).

RESULTS

A significant difference ($p \leq 0.05$) was recorded between the treatments of green gram seedlings exposed to two different high-temperature stress. The results revealed that absolute control (AC) showed 100 % survival of seedlings compared to control (C) treatment. While comparing the two temperature treatments, the seedlings exposed to 42°C exhibited a more reduced seedling survival percentage than 40°C (Fig. 1). Among the melatonin treatments, the seeds treated with 100 (77.67 %) and 80 (76.33 %) μM of melatonin concentration observed a higher survival percentage of green gram seedlings at 40°C, over the control (C).

The shoot and root growth were drastically reduced in control (C) under high-temperature stress conditions at both 40°C (28.74% and 37.29%) and 42°C (35.11% and 49.57%). Irrespective of the melatonin treatments, the maximum percent of the shoot (27.05% and 33.43%) and root growth (36.19% and 44.16%) reduction were noticed in seeds treated with 20 μM of melatonin concentration exposed to 40°C and 42°C, respectively (Fig. 2a and 2b). In contrast, the minimum percent reduction in shoot and root growth were noticed in 100 μM of melatonin-treated seeds subjected to 40°C (4.20 % and 9.73%) and 42°C (6.30% and 19.18%) than the control treatment.

High-temperature stress significantly affects the cell viability ratio and mortality percent of green gram seedlings. The mortality percent of seedlings exposed to 42°C was found to be higher than that of 40°C. Seedlings subject to high-temperature stress at 40°C and 42°C (51.00% and 60.00%) without any melatonin pre-treatment (C) showed higher mortality percent than the melatonin-treated seeds (Fig. 3a). A significant difference ($p \leq 0.05$) was observed between the melatonin treatments, melatonin @ 100 and 80 μM recorded the minimum mortality percent at both 40°C (22.33% and 23.67%) and 42°C (25.00% and 26.67%) over the control (C). The maximum mortality percent was observed in 20 μM of melatonin pre-treated seeds exposed to 40°C (32.67%) and 42°C (35.33%) when compared to other melatonin treatments. In contrast, the cell viability percent decreased significantly at 42°C than the seedlings exposed to 40°C. Under high-temperature stress conditions, a higher cell viability percent was recorded in seeds treated with 100 μM (87.67% and 80.00%) and 80 μM (85.67% and 76.67%) of melatonin concentration. In contrast, a lower percentage of cell viability was observed in 20 μM (74.00 % and 68.33 %) compared to control (C) treatment (70.00 % and 64.67 %) at both 40°C and 42°C, respectively (Fig. 3b).

The seedlings exposed to high-temperature stress recorded a significant ($p \leq 0.05$) variation between the control and melatonin treatments. The higher MDA content was observed in control (C) seedlings (1.28 $\mu\text{mol g}^{-1}$ and 1.38 $\mu\text{mol g}^{-1}$) around 80 % over absolute control (AC) at both 40°C and 42°C (Fig. 4a). Irrespective of the melatonin treatments, seedlings exposed to 40°C showed more malondialdehyde content than 42°C. Among the melatonin treatments, 100 μM (0.73 $\mu\text{mol g}^{-1}$) and 80 μM (0.76 $\mu\text{mol g}^{-1}$) of melatonin treatment produced significantly lower malondialdehyde content at 40°C than the control. The higher malondialdehyde content was observed in seeds pre-treated melatonin of 20 μM (0.89 $\mu\text{mol g}^{-1}$) at 40°C. Among the melatonin treatments, at both 40°C and 42°C temperature stress, the seeds treated with 100 μM melatonin showed a significantly increased production of the antioxidant enzyme CAT (6.89 and 6.79 $\mu\text{g of H}_2\text{O}_2$ reduced $\text{mg protein}^{-1} \text{min}^{-1}$) and SOD (1.89 and 1.93 units $\text{mg protein}^{-1} \text{min}^{-1}$) than the control (C) (Fig. 4b & 4c). In contrast, the lower antioxidant enzyme activity of CAT (5.38 and 5.68 $\mu\text{g of H}_2\text{O}_2$ reduced $\text{mg protein}^{-1} \text{min}^{-1}$) and SOD (1.73 and 1.75 units $\text{mg protein}^{-1} \text{min}^{-1}$) were recorded in 20 μM of melatonin pre-treated seeds exposed to 40°C and 42°C high-temperature stress. The production of the antioxidant enzyme like CAT (5.40 and 5.50 $\mu\text{g of H}_2\text{O}_2$ reduced $\text{mg protein}^{-1} \text{min}^{-1}$) and SOD (1.51 and 1.53 units $\text{mg protein}^{-1} \text{min}^{-1}$) was lower under high-temperature stress at both 40°C and 42°C compared to the melatonin treatments.

Principal component analysis

Principal component analysis was used to determine the impact of melatonin pre-treatment on the ability of seedlings to withstand high temperatures. (Fig. 5). The principal component analysis consists of two principal

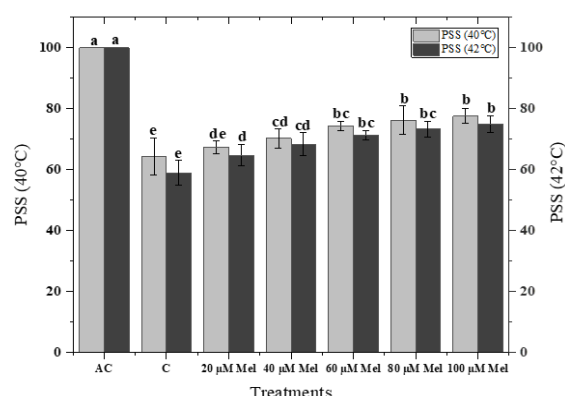


Fig. 1. Effect of melatonin on percent survival of seedlings (PSS) of greengram under two different (@ 40°C and 42°C) high-temperature stresses. AC: Absolute Control, C: Control, Mel: Melatonin (Different alphabet letters indicates that treatments are significantly different from each other)

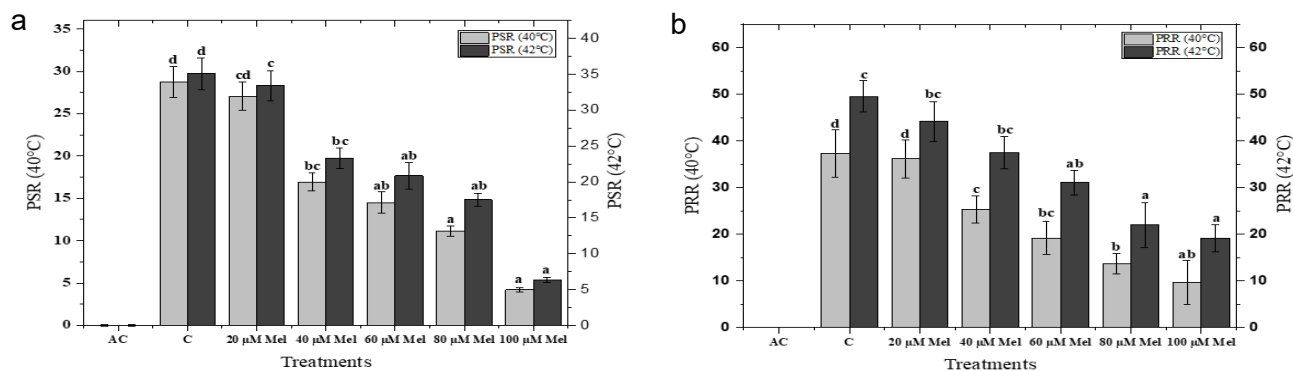


Fig. 2. Effect of melatonin on percent (a) shoot growth reduction (PSR) and (b) root growth reduction (PRR) of green gram seedlings under two different (@ 40°C and 42°C) high-temperature stresses. AC: Absolute Control, C: Control, Mel: Melatonin (Different alphabet letters indicates that treatments are significantly different from each other)

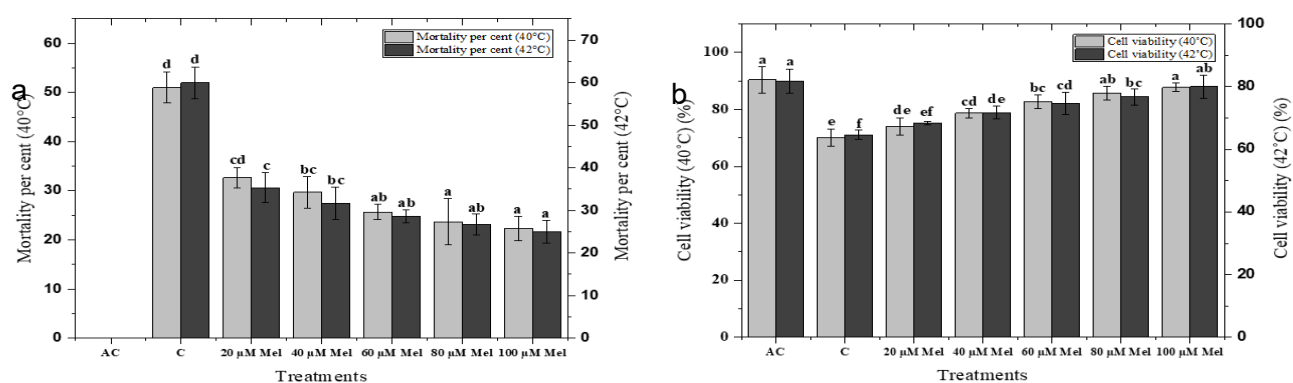


Fig. 3. Effect of melatonin on mortality percent (a) and cell viability (b) of greengram seedlings under two different (@ 40°C and 42°C) high-temperature stresses. AC: Absolute Control, C: Control, Mel: Melatonin (Different alphabet letters indicates that treatments are significantly different from each other)

components and the indices of different melatonin treatments were represented by PC1 and PC2, contributing 93.11 % of the total variations. Principal component 1 (PC1, X-axis) accounted for 68.70% of the variation and principal component 2 (PC2, Y-axis) explained 24.41% of the variation. From PCA analysis, it was observed that the indices of mortality per cent (MP), per cent survival of seedlings (PSS), per cent shoot growth reduction (PSR) and malondialdehyde (MDA) were grouped together and positively correlated, whereas cell viability (CV) and per cent survival of seedlings (PSS) were inversely correlated, meaning when CV and PSS increased the opposite MP, PSS, PSR and MDA were get decreased and vice versa. CAT and SOD were likely to be correlated with other parameters. The variation between absolute control (AC) and 100 μM of melatonin-treated seedlings showed 24.41% in response to high-temperature stress. Similarly, the variation between absolute control (AC) and control (C) treatment was explained by about 68.70%. Among the melatonin treatments, 20, 40, 60 and 80 μM of melatonin concentration were found close to the plot origin and showed no effect on seedlings tolerance to high-

temperature stress. However, 100 μM melatonin treatment showed the greatest deviation from the mean value, indicating that seedlings treated with 100 μM melatonin were significantly tolerant to high-temperature stress.

DISCUSSION

Plants are periodically exposed to various environmental factors. The plant metabolism, cellular homeostasis and major key enzymes involved in physiological and biochemical processes are altered by temperature as it increases over the optimal threshold (Hemantaranjan *et al.*, 2014). High-temperature stress affects the germination process (Essemine *et al.*, 2010), shoot and root growth (Luo *et al.*, 2020), membrane integrity (Narayanan, 2018), and antioxidant enzyme activity (Song *et al.*, 2014). The present study demonstrated the impact of melatonin on seedling tolerance to high-temperature stress.

The current study observed that seedlings exposed to high-temperature stress affected the seedling survival percentage, shoot and root growth, cell viability and

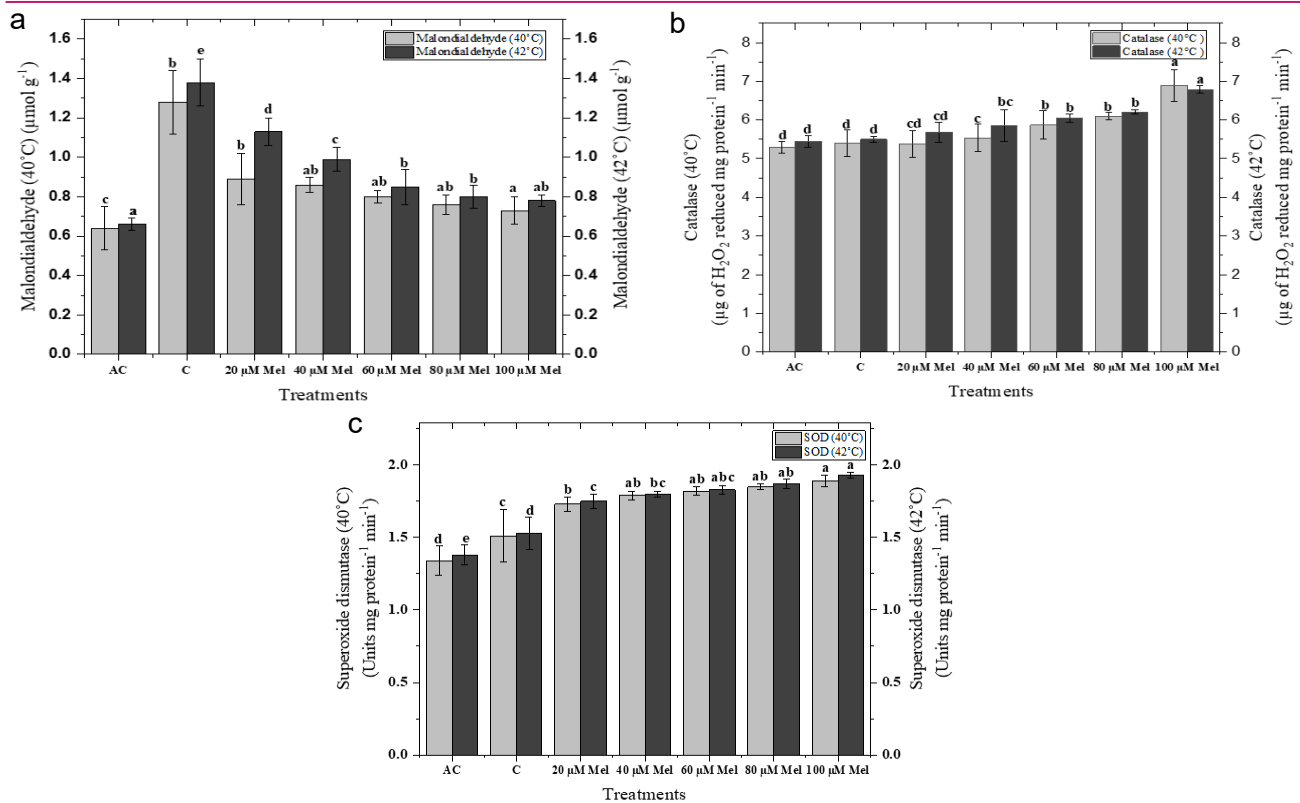


Fig. 4. Effect of melatonin on MDA (a), CAT activity (b) and SOD activity (c) of greengram seedlings under two different (@ 40°C and 42°C) high temperature stresses. AC: Absolute Control, C: Control, Mel: Melatonin (Different alpha-bet letters indicates that treatments are significantly different from each other)

antioxidant enzyme activity. The seeds treated with 100 and 80 µM of melatonin recorded nearly 21% and 19% enhanced survival percentage (Fig.1), 80% and 73% improved shoot and root growth (Fig.2a and 2b) and 28% and 16% higher cell viability ratio (Fig.3b) than the control treatment. Elevated temperature lowers the seeds' ability to germinate, which leads to poor germination and stands establishment (Fahad *et al.*, 2017). The application of melatonin counteracts these effects and improves the tolerance of seedlings to heat stress (Rajora *et al.*, 2022). Exogenous melatonin application enhances reserve mobilization (Lei *et al.*, 2021), upregulates the transcription of heat shock proteins (HSP) and activity of numerous antioxidant enzymes, which reduces the formation of reactive oxygen species (ROS) (Khan *et al.*, 2020). In addition, the level of other endogenous growth factors like IAA was increased by melatonin treatment, resulting in the formation of a denser root system (Zeng *et al.*, 2018). A similar effect was found in other crops like rice (Yu *et al.*, 2022), maize (Jiang *et al.*, 2016), cucumber (Zhang *et al.*, 2014), cherry (Sarropoulou *et al.*, 2012) and soybean (Imran *et al.*, 2021) that seeds pre-treated with melatonin had a positive effect on germination, seedling growth and establishment.

Cell membrane damage is the first phenomenon that occurs under conditions of extreme temperature stress

(Moradpour *et al.*, 2021). More membrane damage was observed under high-temperature stress and present results were corroborated with the previous finding of Sachdev *et al.* (2021), who articulated that elevated temperature stress leads to generation and accumulation of ROS like hydrogen peroxide (H₂O₂) and superoxide radical (O₂⁻) within the cell (Fig.3a) and create oxidative damage such as lipid peroxidation, which result in increased malondialdehyde (MDA) content. Heat stress tolerance is closely related to sustaining membrane stability and integrity of the cell (Bita and Gerats, 2013). The present study observed a positive effect of melatonin on membrane integrity when the seedlings were exposed to high-temperature stress. Seeds pre-treated with 100 and 80 µM of melatonin reduced the membrane damage by 12 % and 15 % to the control (Fig.4a). Huang *et al.* (2019) reported that melatonin has amphiphilic properties that can quickly diffuse and distribute in cytoplasm and lipid membranes. Yasmeen *et al.* (2022) observed that the hydrophilic side of the lipid bilayer was bounded with melatonin and protected the membrane against lipid peroxidation by directly neutralizing the harmful substances generated during stress conditions. The results of the current investigation concur with those indicated previously.

The antioxidant enzyme activity like SOD and CAT was remarkably reduced under high-temperature stress

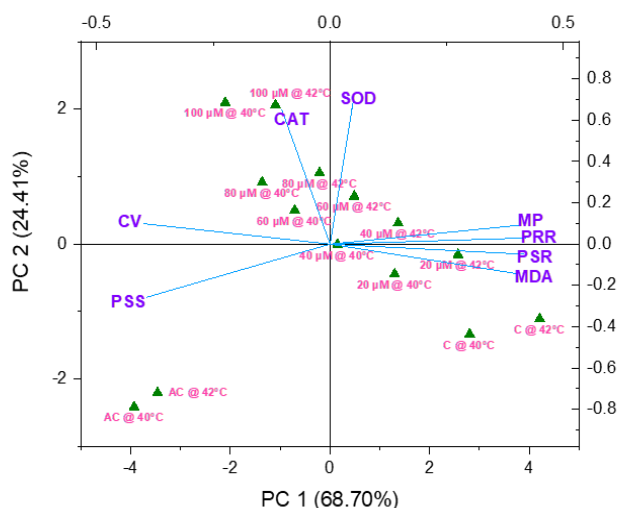


Fig. 5. Principal component analysis of various seedling growth parameters of greengram seedlings in response to high-temperature stress. Absolute control (AC), Control (C), Percent survival of seedlings (PSS), Percent shoot growth reduction (PSR), percent root growth reduction (PRR), Mortality percent (MP), cell viability (CV), Malondialdehyde (MDA), Catalase (CAT), Superoxide dismutase (SOD)

conditions. Similarly, in *Brassica oleracea*, Soengas *et al.* (2018), found that plants' antioxidant defense systems are significantly altered by heat stress. Experimental results of the present study demonstrated that seeds treated with 100 and 80 μM of melatonin significantly reduced ROS production through enhanced antioxidant enzymatic activities like CAT of nearly 28% and 23% (Fig. 4b) and SOD with 25% and 26% (Fig. 4c) than the seedlings subjected to high-temperature stress without melatonin treatment. The primary role of melatonin is to act as an antioxidant. It scavenges the reactive oxygen and nitrogen species produced during stressful conditions by enhancing the antioxidant enzyme activity, thereby maintaining cellular redox homeostasis (Arnao and Hernandez-Ruiz, 2019). ROS and RNS produced in response to stress conditions are detoxified by antioxidant enzymes like SOD and CAT (Di Meo *et al.*, 2016). The first line of defense and a key player in the plants' ability to detoxify ROS is the enzyme SOD, which removes O_2^- from ROS and transforms it into H_2O and O_2 . Further, the intermediary product H_2O_2 was detoxified by the enzyme POX and CAT, which converts H_2O_2 into O_2 and H_2O and protects the plant from oxidative damage (Hu *et al.*, 2016). The increased antioxidant enzyme activity and defense system by the exogenous application of melatonin under stress conditions have also been reported in rice (Yu *et al.*, 2022), wheat (Buttar *et al.*, 2020), tomato (Jahan *et al.*, 2019) and cabbage (Lee *et al.*, 2021).

Conclusion

In conclusion, high-temperature stress affected the germination of seedlings and other seedling growth parameters like survival percentage, shoot and root growth, cell viability and antioxidant enzyme activity like superoxide dismutase and catalase in green gram. Pre-treatment of green gram seeds with different concentrations of melatonin promoted seedling growth and enhanced the thermo-tolerance capacity by reducing oxidative damage. Among the different concentrations of melatonin treatment, 100 μM of melatonin showed the most significant impact on the germination of green gram seedlings. It improved shoot length, root length, cell viability and antioxidant enzyme activity with reduced malondialdehyde content, thereby alleviating the high-temperature stress inhibitory effect on green gram seedlings. Hence, melatonin can be used to enhance stress tolerance in plants. Further detailed studies on the molecular mechanism are needed to understand the interaction with other metabolites and growth-regulating hormones concerning plant tolerance to abiotic stress conditions.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Aebi, H. (1984). Catalase in vitro. *Methods Enzymol.* 105, 121–126. doi: 10.1016/S0076-6879(84)05016-3
2. Arnao, M. & Hernandez-Ruiz, J. (2019). Melatonin and reactive oxygen and nitrogen species: a model for the plant redox network. *Melatonin Research*, 2(3), 152-168. doi.org/https://doi.org/10.32794/11250036
3. Back, K., Tan, D.X. & Reiter, R.J. (2016). Melatonin biosynthesis in plants: Multiple pathways catalyze tryptophan to melatonin in the cytoplasm or chloroplasts. *Journal of Pineal Research*, 61, 426–437. doi.org/10.1111/jpi.12364
4. Basu, P. S., Pratap, A., Gupta, S., Sharma, K., Tomar, R. & Singh, N. P. (2019). Physiological traits for shortening crop duration and improving productivity of greengram (*Vigna radiata* L. Wilczek) under high temperature. *Frontiers in Plant Science*, 10:1508. doi: 10.3389/fpls.2019.01508
5. Bitar, C. E. & Gerats, T. (2013). Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. *Frontiers in Plant Science*, 4, 273. doi.org/10.3389/fpls.2013.00273
6. Buttar, Z. A., Wu, S. N., Arnao, M. B., Wang, C., Ullah, I. & Wang, C. (2020). Melatonin suppressed the heat stress-induced damage in wheat seedlings by modulating the antioxidant machinery. *Plants*, 9(7), 809. doi.org/10.3390/plants9070809
7. Cao, Q., Li, G., Cui, Z., Yang, F., Jiang, X., Diallo, L. & Kong, F. (2019). Seed priming with melatonin improves

- the seed germination of waxy maize under chilling stress via promoting the antioxidant system and starch metabolism. *Scientific Reports*, 9(1), 1-12. doi.org/10.1038/s41598-019-51122-y
8. Chen, Y., Li, R., Ge, J., Liu, J., Wang, W., Xu, M., Zhang, R., Hussain, S., Wei, H. & Dai, Q. (2021). Exogenous melatonin confers enhanced salinity tolerance in rice by blocking the ROS burst and improving Na⁺/K⁺ homeostasis. *Environmental and Experimental Botany*, 189:104530. doi.org/10.1016/j.envexpbot.2021.104530
 9. Dhindsa, R. S., Plumb-Dhindsa, P. and Thorpe, T. A. (1981). Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *Journal of Experimental Botany*, 32(1), 93-101. doi.org/10.1093/jxb/32.1.93
 10. Di Meo, S., Reed, T. T., Venditti, P. & Victor, V. M. (2016). Role of ROS and RNS sources in physiological and pathological conditions. *Oxidative Medicine and Cellular Longevity*. 1245049. doi: 10.1155/2016/1245049
 11. Essemine, J., Ammar, S. & Bouzid, S. (2010). Impact of heat stress on germination and growth in higher plants: physiological, biochemical and molecular repercussions and mechanisms of defence. *Journal of Biological Sciences*, 10(6), 565-572. doi: 10.3923/jbs.2010.565.572
 12. Fahad, S., Bajwa, A. A., Nazir, U., Anjum, S. A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., Saud, S., Ihsan, M. Z., Alharby, H., Wu, C., Wang, D. & Huang, J. (2017). Crop production under drought and heat stress: plant responses and management options. *Frontiers in Plant Science*, 1147. doi.org/10.3389/fpls.2017.01147
 13. Hanumantha Rao, B., Nair, R. M. & Nayyar, H. (2016). Salinity and high temperature tolerance in mungbean [*Vigna radiata* (L.) Wilczek] from a physiological perspective. *Frontiers in Plant Science*, 7, 957. doi: 10.3389/fpls.2016.00957
 14. Hemantaranjan, A., Bhanu, A. N., Singh, M. N., Yadav, D. K., Patel, P. K. & Katiyar, D. (2014). Heat stress responses and thermotolerance. *Advances in Plants & Agriculture Research*, 1, 62-70. doi: 10.15406/apar.2014.01.00012.
 15. Hossain, M. S., Li, J., Sikdar, A., Hasanuzzaman, M., Uzizerimana, F., Muhammad, I., Yuan, Y., Zhang, C., Wang, C. & Feng, B. (2020). Exogenous melatonin modulates the physiological and biochemical mechanisms of drought tolerance in tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn). *Molecules*, 25(12), 2828. doi: 10.3390/molecules25122828
 16. Hu, Z. R., Fan, J. B., Xie, Y., Amombo, E., Liu, A., Gitau, M. M., Khaldun, A. B. M., Chen, L. & Fu, J. (2016). Comparative photosynthetic and metabolic analyses reveal mechanism of improved cold stress tolerance in bermuda grass by exogenous melatonin. *Plant Physiology and Biochemistry*, 100, 94-104. doi: 10.1016/j.plaphy.2016.01.008.
 17. Huang, B., Chen, Y. E., Zhao, Y. Q., Ding, C. B., Liao, J. Q., Hu, C., Zhou, L. J., Zhang, Z. W., Yuan, S. & Yuan, M. (2019). Exogenous melatonin alleviates oxidative damages and protects photosystem II in maize seedlings under drought stress. *Frontiers in Plant Science*, 10, 677. doi: 10.3389/fpls.2019.00677.
 18. Imran, M., Aaqil Khan, M., Shahzad, R., Bilal, S., Khan, M., Yun, B. W., Khan, A. L. & Lee, I. J. (2021). Melatonin ameliorates thermotolerance in soybean seedling through balancing redox homeostasis and modulating antioxidant defense, phytohormones and polyamines biosynthesis. *Molecules*, 26(17), 5116. doi.org/10.3390/molecules26175116
 19. IPCC (2022). Climate Change Synthesis Report Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Geneva: IPCC, 151.
 20. Jahan, M. S., Shu, S., Wang, Y., Chen, Z., He, M., Tao, M., Sun, J. & Guo, S. (2019). Melatonin alleviates heat-induced damage of tomato seedlings by balancing redox homeostasis and modulating polyamine and nitric oxide biosynthesis. *BMC Plant Biology*, 19(1), 1-16. doi: 10.1186/s12870-019-1992-7
 21. Jiang, X., Li, H. & Song, X. (2016). Seed priming with melatonin effects on seed germination and seedling growth in maize under salinity stress. *Pakistan Journal of Botany*, 48(4), 1345-1352.
 22. Karabal, E., Yucel, M. & Oktem, H. A. (2003). Antioxidant responses of tolerant and sensitive barley cultivars to boron toxicity. *Plant Science*, 164(6), 925-933. doi.org/10.1016/S0168-9452(03)00067-0
 23. Karande, B. I., Patel, H. R., Yadav, S. B., Vasani, M. J. & Patil, D. D. (2018). Impact of projected climate change on summer mungbean in Gujarat, India. *International Journal of Current Microbiology and Applied Sciences*, 7(8), 4178-4189. doi:10.20546/ijcmas.2018.708.437
 24. Kaur, R., Bains, T. S., Bindumadhava, H. & Nayyar, H. (2015). Responses of mungbean (*Vigna radiata* L.) genotypes to heat stress. Effects on reproductive biology, leaf function and yield traits. *Scientia Horticulturae*, 197, 527-541. doi: 10.1016/j.scienta.2015.10.015
 25. Khan, A., Numan, M., Khan, A. L., Lee, I. J., Imran, M., Asaf, S. & Al-Harrasi, A. (2020). Melatonin: Awakening the defense mechanisms during plant oxidative stress. *Plants*, 9(4), 407. doi.org/10.3390/plants9040407
 26. Kheir, E. A., Sheshshayee, M. S., Prasad, T. G. & Udayakumar, M. (2012). Temperature induction response as a screening technique for selecting high temperature-tolerant cotton lines. *Journal of Cotton Science*, 16(3), 190-199.
 27. Lee, J., Lee, H., Wi, S., Yu, I., Yeo, K. H., An, S., Jang, Y. & Jang, S. (2021). Enhancement of growth and antioxidant enzyme activities on kimchi cabbage by melatonin foliar application under high temperature and drought stress conditions. *Journal of Horticultural Science and Technology*, 1, 583-592. doi.org/10.7235/HORT.20210052.
 28. Lei, K.Q., Sun, S.Z., Zhong, K.T., Li, S.Y., Hu, H., Sun, C.J., Zheng, Q.M., Tian, Z.W., Dai, T.B. & Sun, J.Y. (2021). Seed soaking with melatonin promotes seed germination under chromium stress via enhancing reserve mobilization and antioxidant metabolism in wheat. *Ecotoxicology and Environmental Safety*, 220, 112-241. doi.org/10.1016/j.ecoenv.2021.112241
 29. Liang, C., Li, A., Yu, H., Li, W., Liang, C., Guo, S., Zhang, R. & Chu, C. (2017). Melatonin regulates root architecture by modulating auxin response in rice. *Frontiers in Plant Science*, 8, 134. doi.org/10.3389/fpls.2017.00134
 30. Liang, D., Shen, Y., Ni, Z., Wang, Q., Lei, Z., Xu, N., Deng, Q., Lin, L., Wang, J. & Xia, H. (2018). Exogenous

- melatonin application delays senescence of kiwifruit leaves by regulating the antioxidant capacity and biosynthesis of flavonoids. *Frontiers in Plant Science*, 9, 426. doi.org/10.3389/fpls.2018.00426
31. Luo, H., Xu, H., Chu, C., He, F. & Fang, S. (2020). High temperature can change root system architecture and intensify root interactions of plant seedlings. *Frontiers in Plant Science*, 11, 160. doi.org/10.3389/fpls.2020.00160
 32. Moradpour, M., Abdullah, S. N. A. & Namasivayam, P. (2021). The impact of heat stress on morpho-physiological response and expression of specific genes in the heat stress-responsive transcriptional regulatory network in *Brassica oleracea*. *Plants*, 10(6), 1064. doi.org/10.3390/plants10061064
 33. Narayanan, S. (2018). Effects of high temperature stress and traits associated with tolerance in wheat. *Open Access Journal of Science*, 2(3), 177-186. doi:10.15406/oajs.2018.02.00067
 34. Parihar, A. K., Basandrai, A. K., Sirari, A., Dinakaran, D., Singh, D., Kannan, K., Kushawaha, K. P. S., Adinarayan, M., Akram, M., Latha, T. K. S., Paraniidharan, V. & Gupta, S. (2017). Assessment of mungbean genotypes for durable resistance to Yellow Mosaic Disease: Genotype × Environment interactions. *Plant Breeding*, 36, 94–100. doi: 10.1111/pbr.12446
 35. Priya, M., Pratap, A., Sengupta, D., Singh, N. P., Jha, U. C., Siddique, K. & Nayyar, H. (2020). Mungbean and high temperature stress: responses and strategies to improve heat tolerance. In *Heat stress in food grain crops: Plant breeding and omics research* (pp. 144-170). Bentham Science Publishers.
 36. Rajora, N., Vats, S., Raturi, G., Thakral, V., Kaur, S., Rachappanavar, V., Kumar, M., Kesarwani, A. K., Sonah, H., Sharma, T. R. & Deshmukh, R. (2022). Seed priming with melatonin: A promising approach to combat abiotic stress in plants. *Plant Stress*, 4, 100071. doi.org/10.1016/j.stress.2022.100071
 37. Rekha, K., Reddy, D. & Sudhakar, P. (2016). Standardization of optimum temperature conditions and screening of thermotolerant mungbean genotypes using thermo induction response (TIR). *Journal of Environmental Sciences*, 10(3&4), 485-489.
 38. Sachdev, S., Ansari, S. A., Ansari, M. I., Fujita, M. & Hasanuzzaman, M. (2021). Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. *Antioxidants*, 10(2), 277. doi: 10.3390/antiox10020277.
 39. Sarropoulou, V., Dimassi-Therious, K., Therios, I. & Koukourikou-Petridou, M. (2012). Melatonin enhances root regeneration, photosynthetic pigments, biomass, total carbohydrates and proline content in the cherry rootstock PHL-C (*Prunus avium* × *Prunus cerasus*). *Plant Physiology and Biochemistry*, 61, 162-168. doi.org/10.1016/j.plaphy.2012.10.001.
 40. Sharma, L., Priya, M., Bindumadhava, H., Nair, R. M. & Nayyar, H. (2016). Influence of high temperature stress on growth, phenology and yield performance of mungbean [*Vigna radiata* (L.) Wilczek] under managed growth conditions. *Scientia Horticulturae*, 213, 379-391. doi:10.1016/j.scienta.2016.10.033.
 41. Singh, J. & Thakur, J. K. (2018). Photosynthesis and abiotic stress in plants. In *Biotic and abiotic stress tolerance in plants* (pp. 27-46). Springer, Singapore.
 42. Sita, K., Sehgal, A., HanumanthaRao, B., Nair, R. M., Vara Prasad, P. V., Kumar, S., Gaur, P. M., Farooq, M., Siddique, K. H. M., Varshney, R. K. & Nayyar, H. (2017). Food legumes and rising temperatures: effects, adaptive functional mechanisms specific to reproductive growth stage and strategies to improve heat tolerance. *Frontiers in Plant Science*, 8, 1658. doi.org/10.3389/fpls.2017.01658
 43. Soengas, P., Rodriguez, V. M., Velasco, P. & Cartea, M. E. (2018). Effect of temperature stress on antioxidant defenses in *Brassica oleracea*. *ACS Omega*, 3(5), 5237-5243. doi.org/10.1021/acsomega.8b00242.
 44. Song, Y., Chen, Q., Ci, D., Shao, X. & Zhang, D. (2014). Effects of high temperature on photosynthesis and related gene expression in poplar. *BMC plant biology*, 14(1), 1-20. doi.org/10.1186/1471-2229-14-111
 45. Sun, C., Liu, L., Wang, L., Li, B., Jin, C. & Lin, X. (2021). Melatonin: A master regulator of plant development and stress responses. *Journal of Integrative Plant Biology*, 63 (1), 126-145. doi.org/10.1111/jipb.12993
 46. Yamamoto Y. (2016). Quality control of photosystem II: the mechanisms for avoidance and tolerance of light and heat stresses are closely linked to membrane fluidity of the thylakoids. *Frontiers in Plant Science*, 7:1136. doi.org/10.3389/fpls.2016.01136
 47. Yasmeen, S., Wahab, A., Saleem, M. H., Ali, B., Qureshi, K. A. & Jaremko, M. (2022). Melatonin as a foliar application and adaptation in lentil (*Lens culinaris* Medik.) crops under drought stress. *Sustainability*, 14(24), 16345. doi: 10.3390/su142416345.
 48. Yu, Y., Deng, L., Zhou, L., Chen, G. & Wang, Y. (2022). Exogenous melatonin activates antioxidant systems to increase the ability of Rice seeds to germinate under high temperature conditions. *Plants*, 11(7), 886. doi: 10.3390/plants11070886.
 49. Zandalinas, S. I., Sales, C., Beltran, J., Gomez-Cadenas, A. & Arbona, V. (2017). Activation of secondary metabolism in citrus plants is associated to sensitivity to combined drought and high temperatures. *Frontiers in Plant Science*, 7, 1954. doi: 10.3389/fpls.2016.01954.
 50. Zeng, L., Cai, J., Li, J., Lu, G., Li, C., Fu, G., Zhang, X., Ma, H., Liu, Q., Zou, X. & Cheng, Y. (2018). Exogenous application of a low concentration of melatonin enhances salt tolerance in rapeseed (*Brassica napus* L.) seedlings. *Journal of Integrative Agriculture*, 17, 328–335. doi: 10.1016/S2095-3119(17)61757-x.
 51. Zeng, W., Mostafa, S., Lu, Z. & Jin, B. (2022). Melatonin-mediated abiotic stress tolerance in plants. *Frontiers in Plant Science*, 13, 847175-847175. doi: 10.3389/fpls.2022.847175
 52. Zhang, H. J., Zhang, N. A., Yang, R. C., Wang, L., Sun, Q. Q., Li, D. B., Cao, Y. Y., Weeda, S., Zhao, B., Ren, S. & Guo, Y. D. (2014). Melatonin promotes seed germination under high salinity by regulating antioxidant systems, ABA and GA₄ interaction in cucumber (*Cucumis sativus* L.). *Journal of Pineal Research*, 57(3), 269-279. doi:10.1111/jpi.12167.
 53. Zinn, K. E., Tunc-Ozdemir, M. & Harper, J. F. (2010). Temperature stress and plant sexual reproduction: uncovering the weakest links. *Journal of Experimental Botany*, 61, 1959–1968. doi: 10.1093/jxb/erq053