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Exogenous Melatonin Enhances Drought Tolerance in Pepino (*Solanum muricatum* L.) Through Morphological and Biochemical Adjustments

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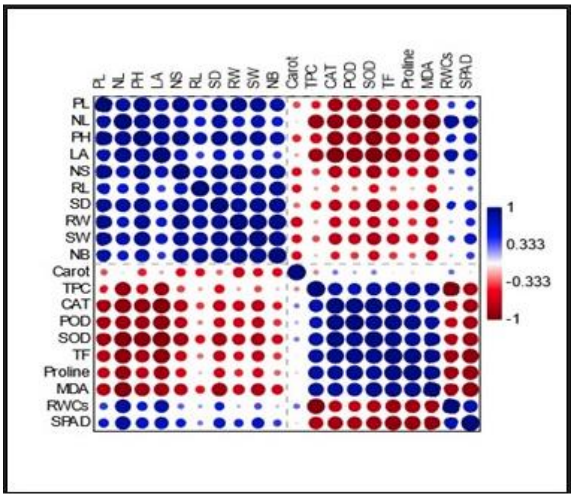
Graphical abstract



Abstract

Melatonin plays a vital role in supporting plant growth under stress conditions. This study assessed morphological and biochemical attributes of *Solanum muricatum* to foliar-applied melatonin under drought. Current study followed a completely randomized design (CRD) with three replications, with three levels of drought (100%, 75%, and 50% field capacity (FC)) and four levels of foliar applications of melatonin (MT) (0, 50, 100, and 150 µM). Several growth attributes are negatively affected through drought, which include plant growth, pedicle length, area of leaves, height of plants, and root length. Morphological traits such as pedicle length, leaf area, and root length improved particularly at 100 µM MT by 59.6%, 58.2%, and 41.3%, respectively. Melatonin reduced oxidative stress markers, with MDA levels decreasing by 61.5% and 55.8% under 50% and 75% FC, respectively. Antioxidant enzyme activities were enhanced; SOD was increased by by 28.2% under 50% FC at 100 µM, while CAT and POD activities were reduced to 44.7% and 58.9%, respectively under 50% FC at 100 µM. Proline content was increased by 20.5% under 50% FC at 50 µM. The correlation analysis, along with the heat map visualization, showed strong relationships between the melatonin application and growth, physiological traits, and biochemical responses. The study found that moderate melatonin concentrations (50–100 µM) significantly improved drought resilience, while a 150 µM concentration of melatonin showed lower improvements. These findings underscore melatonin's potential as a sustainable strategy to improve crop productivity under drought. Future studies should focus on long-term field studies and the molecular mechanisms of melatonin stress mitigation effects.

Keywords: melatonin, drought stress, climate change, antioxidant activity, water deficit.

1. Introduction

Pepino (*Solanum muricatum L.*) is a member of *Solanaceae* family. This cultigen originates from the Andean region of South America (Ahmad *et al.* 2014; Mahato *et al.* 2016). It has been poorly adopted in agriculture but has potential in both national and international markets. Pepinos are usually consumed fresh due to their low caloric value, mild sweet flavor, high water content, and aromatic fragrance (Rana and Brar 2017). A pepino plant can be morphologically different in many ways; the variation of the fruits in respect to weight, shape, and color is enormous. The taste of pepino includes elements of sweet and herbaceous acidity which makes it acceptable for fresh eating and for use in fruit salads. Pepino fruits also possess antioxidant, antidiabetic, anti-inflammatory, antitumor and other health-promoting properties (Chan *et al.* 2024). They show a close relationship to tomato and potato (Yang *et al.* 2023). Cultivation of pepino has a lot of possibilities in many regions, especially in temperate areas, still it is rather rare because of the negative effects of droughts and temperature changes (Pacheco *et al.* 2021). Recent efforts to cultivate pepino in arid regions such as the Mediterranean indicate the need for further research on its adaptation to low water levels (Pacheco Toabanda 2022). Knowing how this crop adjusts to the stress of drought is necessary for its effective cultivation and enhancement.

Drought stress is a chief constraint in productivity of agricultural crops as it impacts significant physiological and biochemical processes of plants. Pepino's response to salinity stress has been studied thoroughly (Ulas 2021), however, the response to drought (Duman and Sivaci 2015; Pacheco *et al.* 2021) still needs to be explored. Knowing its biochemical responses under such water deficit conditions is very important for improving crop management and breeding strategies for better drought tolerance (Pacheco *et al.* 2021). Drought response metabolites and enzymes like photosynthetic pigments, osmolytes and antioxidants play significant roles in cellular respiration and stress response mitigation (Elkelish *et al.* 2020). Reduction of chlorophyll and carotenoid content is a well-known phenomenon under drought conditions which adversely affects the cell's metabolism, while complete osmolyte accumulation aids in turgor maintenance (Ngamwonglumlert *et al.* 2020). Moreover, oxidative stress markers and the antioxidant defense response provide a clearer picture of how the stress of drought negatively impacts plant metabolism. Plant growth and productivity are severely compromised by drought and it is this challenge that necessitates a solution.

Recently, researchers unrevealed that melatonin (CAS no. 73-31-4) is effective for improvement of stress management in pepino plants through regulating growth and development (Dai *et al.* 2020; Tiwari *et al.* 2025). Melatonin acts as an effective antioxidant that helps neutralize cellular damage due to reactive oxygen species (ROS), thereby supporting cellular balance. Additionally, it plays a regulatory role in key physiological activities such as photosynthesis, root growth, and the aging process in plants (Omowumi *et al.* 2024). Application of melatonin exogenously can improve a plant's tolerance to stress by enhancing antioxidant enzymatic activity, protecting the photosynthetic system and limiting the absorption of toxic ions. Moreover, melatonin is involved in moderating plant stress responses through its dealing with hormones such as gibberellins and abscisic acid (Cano *et al.* 2024). Similar studies have been performed in case of rice (Yuan *et al.* 2025), maize (Wang *et al.* 2024; Li *et al.* 2025), and soybean (Zhao *et al.* 2025), and they have shown that melatonin alleviates oxidative stress and enhances drought tolerance. There is limited research on its activity on non-conventional crops such as pepino. Very limited information is available regarding the impact of melatonin on the morphological and biochemical characteristics of pepino under drought conditions which is a major gap in the literature. The present study assessed the mitigating effect of melatonin and determined what concentration of melatonin best aids in controlling stress and improving growth in pepino (*S. muricatum*).

2. Materials and Methods

The experiment was executed at the Botany Department of The Government Sadiq College Women University, Bahawalpur, under semi-arid conditions. Loamy pond soil was used to fill pots (pH 7.2, 1.5% organic matter and 35% field capacity). A completely randomized design with three replicates was applied to evaluate the effects of melatonin (0, 50, 100, and 150 µM) on *S. muricatum* under drought (100%, 75%, and 50% field capacity). Each treatment had 12 pots with one plant per pot.

2.1. Plant Material

Pepino shoot tip cuttings (3–4 inches long) were sourced from the experimental site of the Department of Horticultural Sciences, The Islamia University of Bahawalpur. These cuttings were placed in polyethylene bags containing a rooting medium and covered with polyethylene sheets to maintain high humidity for optimal rooting. Once rooted, they were transplanted into pots (25 cm wide, 30 cm deep) filled with pond soil. Soil field capacity (FC) was measured beforehand to ensure consistent conditions across all treatments.

2.2. Drought Stress Treatments

Drought stress was imposed by regulating soil moisture levels. The control group was maintained at 100% FC, while moderate and severe drought stress levels were maintained at 75% FC and 50% FC, respectively. Soil moisture levels were monitored using the weight method. Assessment of field capacity was made by drying soil samples (4 kg) in an oven at 103°C for 48 hours. The percentage of FC was calculated using the following formula:

$$FC(\%) = (\text{Wet soil weight}(WSW) - \text{Dry soil weight}(DSW)) \times 100$$

Water was added to the pots based on the calculated FC to maintain the desired stress levels. Drought stress treatments and melatonin applications were initiated simultaneously two weeks after transplanting.

2.3. Melatonin Application

Melatonin solution was prepared in deionized water by dissolving the following concentrations: 0 µM (control), 50 µM, 100 µM, and 150 µM, with 0.1% Tween-20, a surfactant, added to aid foliar absorption. Each plant received 30 mL of solution via manual spray, applied three times weekly. Uniform application conditions were maintained; control plants received a spray of only deionized water mixed with 0.1% Tween-20.

3. Data Collection and Measurements

3.1. Morphological Parameters

The height of plants was measured from the hypocotyl base to the shoot tip by a measuring tape. Leaf density was evaluated by counting all leaves per plant at fruiting stage. The leaf density was determined at the fruiting stage by averaging the total number of leaves from five randomly selected plants in each treatment. The leaf area was estimated using a combination of leaf discs and ImageJ® software. Similarly, root length was measured using a tape on five randomly selected plants per treatment, while the root area was calculated using the same method as leaf area with modifications. Stomatal density was evaluated by counting the number of stomata on young leaves collected from five plants per treatment. Additional parameters, including pedicle length, stem diameter, root weight, stem weight, and the number of branches, were also recorded. Post-harvest measurements involved uprooting plants, cleaning them with deionized water, and determining fresh weight using a digital balance. Samples were then oven-dried at 72°C for one week to obtain the dry weight. Root length and biomass were measured to assess root development.

3.2. Biochemical Analysis

The current study examined key biochemical attributes of pepino fruit plants grown under different stress levels, and various levels of melatonin were applied to mitigate stress phenomena including total phenolic content, antioxidant activity, vitamin C, flavonoids, titratable acidity and soluble solids. The assessment of total phenolics content was done by using a modified Folin-Ciocalteu method, while the activity of antioxidants was recorded using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging assay (Sugihartini *et al.* 2024). Flavonoid content was estimated by a colorimetric method with UV absorbance at 415 nm. Soluble solids (°Brix) were measured with a digital refractometer after juice extraction form ripened fruits. These analyses are helpful to assess the nutritional status of fruits for quality assessment.

Antioxidant enzymatic activities and other biochemical attributes were measured in pepino fruit grown under different treatments. The activity of superoxide dismutase (SOD) was evaluated through the nitro blue tetrazolium (NBT) test. The ctivity test of catalase (CAT) followed the method of Al-Qurainy *et al.* (2020) with small modifications. Similarly, the activity of Peroxidase (POD) was assessed by the guaiacol assay through tracking absorbance at 470 nm for 3 minutes in a 3 mL reaction mix composed of phosphate buffer (100 mM, pH 7.0), guaiacol (20 mM), H_2O_2 (10 mM) and enzymatic extract (0.1 mL). Quantification of total phenol content was done through the Folin-Ciocalteu reagent method (Riaz *et al.* 2021). However, extraction of carotenoids was carried out from 2 g of plant samples using a solvent mixture (hexane: acetone: ethanol = 1:1:2 by volume), mixed for 20 minutes, and centrifuged at 5,000 rpm for 15 minutes. In the hexane layer, the absorbance at 450 nm was measured, and carotene levels were determined using a β-carotene standard curve (0–24 µg/mL).

3.3. Statistical Analysis

Analysis of data was done through Statistica, version 8.1, applying two-way ANOVA to measure the influence of melatonin and drought on morphological and biochemical attributes. Tukey's test ($p < 0.05$) was applied for the comparison of treatment means. PCA (principal component analysis) radar plots and correlation analysis were performed in PAST software (Hammer and Harper 2001). The findings were described as mean ± SE (standard error).

4. Results

4.1. Morphological Parameters

The current study observed significant differences in morphological traits of *S. muricatum* under various drought stages (50% and 75% FC) under different concentrations of melatonin (0, 50, 100, and 150 µM) (**Table 1, Figure 1**). Analysis of variance (**Table 2**) depicted that melatonin application significantly affected these attributes with the greatest positive effect seen at 100 µM concentration.

Pedicle length exhibited a notable increase with melatonin application. Under 50% FC drought stress, 100 µM melatonin enhanced pedicle length by 59.6% compared to the untreated plants. Similarly, under 75% FC drought stress, pedicle length increased by 89.7% at 100 µM melatonin. The number of leaves in each plant also showed a positive response to melatonin treatment. Under 50% FC, leaf count increased by 30.1% at 100 µM compared to the control. However, a decline of 14.7% was observed at 150 µM, suggesting that higher melatonin concentrations may not further enhance leaf development. Under 75% FC, leaf production peaked at 100 µM with a 30.3% increase, while the lowest count was recorded in untreated plants. Plant height followed a similar pattern, with 78.9% and 82.5% increases observed at 100 µM under 50% FC and 75% FC, respectively. However, at 150 µM, plant height showed a decline. Leaf area was significantly expanded by melatonin treatment, with 44.5% and 58.2% increases at 100 µM under 50% FC and 75% FC, respectively. However, a reduction was observed at 150 µM, indicating a saturation point. Similarly, stomatal density peaked at 100 µM, showing 47.7% and 82.1% increases at 50% FC and 75% FC, respectively. Root length exhibited the highest increase of 41.3% at 100 µM under severe drought stress and 13.0% under moderate drought stress, highlighting its role in improving water uptake. Stem diameter followed a similar pattern, with 59.3% and 71.3% increases at 100 µM under 50% FC and 75% FC, respectively. Melatonin significantly enhanced root and stem weight. Root weight increased by 65.2% under 50% FC and 70.8% under 75% FC at 100 µM compared to untreated plants. Stem weight showed a similar trend, with 100.1% and 97.7% increases, respectively. The number of branches per plant also showed substantial improvement, with 138.5% and 116.7% increases at 100 µM under 50% FC and 75% FC, respectively. Plants grown in control treatment displayed fewest branches highlighting melatonin's effectiveness in enhancing shoot development during drought stress. **Figure 1 (a–j)** depicted that melatonin treatments affected several morphological attributes of *S. muricatum* in all drought levels (50% and 75% field capacity).

Table 1. Effects of melatonin applications on *S. muricatum* morphological parameters under drought stress.

Drought Stress Level	Melatonin Application	PL	NL	PH	LA	NS	RL	SD	RW	SW	NB
50% FC	**0 µM**	2.13 ± 0.12 e	225.00 ± 10.41 d	35.61 ± 1.16 f	24.35 ± 1.38 c	14.67 ± 0.88 cd	24.37 ± 0.45 e	2.43 ± 0.12 c	9.87 ± 0.42 e	42.17 ± 2.13 c	4.33 ± 0.33 d
	50 µM	2.33 ± 0.05 e	256.00 ± 3.79 bc	47.17 ± 1.30 d	28.80 ± 0.87 b	16.67 ± 1.45 c	28.77 ± 0.90 d	3.03 ± 0.07 b	13.53 ± 0.55 d	73.03 ± 1.63 b	7.67 ± 0.33 c
	100 µM	3.40 ± 0.05 a	292.67 ± 9.06 a	63.67 ± 1.01 a	35.18 ± 0.97 a	21.67 ± 0.33 a	34.43 ± 0.62 a	3.87 ± 0.12 a	16.30 ± 0.64 bc	84.33 ± 1.76 a	10.33 ± 0.88 a
	150 µM	3.07 ± 0.06 bc	249.67 ± 2.91 bc	52.37 ± 1.44 c	31.20 ± 0.85 b	20.00 ± 0.58 b	30.50 ± 0.52 bc	3.07 ± 0.09 b	15.77 ± 0.38 c	75.27 ± 0.96 b	9.00 ± 0.58 b
75% FC	**0 µM**	1.65 ± 0.06 f	201.67 ± 6.01 d	31.50 ± 1.15 g	18.23 ± 0.97 d	13.00 ± 0.58 d	29.63 ± 0.47 cd	2.37 ± 0.12 c	10.34 ± 0.29 e	44.03 ± 2.03 c	6.00 ± 0.58 c
	50 µM	2.60 ± 0.05 d	238.67 ± 1.86 bc	41.73 ± 0.91 e	21.80 ± 0.79 c	16.00 ± 0.58 c	31.57 ± 0.46 bc	3.17 ± 0.03 b	14.33 ± 0.43 d	74.50 ± 1.13 b	9.67 ± 0.33 b
	100 µM	3.13 ± 0.05 ab	262.67 ± 1.45 b	57.50 ± 1.95 b	28.83 ± 0.69 b	23.67 ± 0.88 a	33.50 ± 1.14 ab	4.07 ± 0.12 a	17.67 ± 0.61 a	87.07 ± 1.75 a	13.00 ± 0.58 a
	150 µM	2.89 ± 0.06 c	225.33 ± 2.60 c	49.50 ± 1.15 c	23.00 ± 0.91 c	20.00 ± 0.58 b	31.53 ± 0.70 bc	3.16 ± 0.09 b	16.73 ± 0.34 b	76.60 ± 0.76 b	10.33 ± 0.88 a

Table 2. Analysis of variance of melatonin and drought stress on morphological characteristics of Pepino

Source of Variation	df	PL	NL	PH	LA	NS	RL	SD	RW	SW	NB
L	1	0.1601**	3384.4**	129.36**	287.222**	0.0417 ns	25.031**	0.0477 ns	4.8690**	20.53 ns	22.0417 **
T	3	2.2090**	12763.5**	773.432**	118.552**	91.4861**	49.2779**	2.4662**	57.5241**	2037.86**	44.3750 **
L×T	3	0.1488**	121.1 ns	3.195 ns	1.297 ns	3.5972 ns	10.4241**	0.0193 ns	0.2086 ns	0.60 ns	0.4861 ns
Error	14	0.0168	105.98	4.461	2.683	1.3095	1.5436	0.03298	0.2784	7.9	0.6429
CV (%)	-	4.89	4.22	4.46	6.2	6.28	4.07	5.77	3.69	4.04	9.12

*Drought Stress (L) Melatonin (T), ns: Not significant, * Significant at P < 0.05, ** Significant at P < 0.01 df: Degree of Freedom, CV: Coefficient of Variation*

4.2. Biochemical Parameters

Under varying drought stress levels and melatonin (MT) applications, the biochemical responses of Solanum muricatum were significantly affected (**Table 3**). **Table 4** summarizes analysis of variance (ANOVA) outcomes for impact of various treatments on morphological attributes. Carotenoid content was highest in untreated plants under 75% FC (9.00%), while melatonin at 100 µM significantly reduced carotenoid levels by 44.4% and 48.8% under 50% and 75% FC, respectively. Similarly, total phenolic content (TPC) was highest in untreated 50% FC plants but declined with melatonin application, showing the lowest value at 150 µM (38.5% and 26.2% reduction in 50% and 75% FC, respectively).

Antioxidant enzyme activities varied across treatments. CAT activity peaked in untreated 75% FC plants (17.00%) but decreased with melatonin, reaching the lowest level at 100 µM (a reduction of 44.7% and 49% under 50% and 75% FC, respectively). A similar trend was observed for POD, where untreated 75% FC plants had the highest activity (16.00%), but melatonin application reduced it significantly, with the lowest value at 100 µM (a decrease of 58.9% and 70.8% under 50% and 75% FC, respectively).

Superoxide dismutase (SOD) showed a peak at 100 µM melatonin under 50% FC (63.67%), marking a 28.2% increase from untreated plants, whereas under 75% FC, melatonin at 150 µM reduced SOD activity by 13.7%.

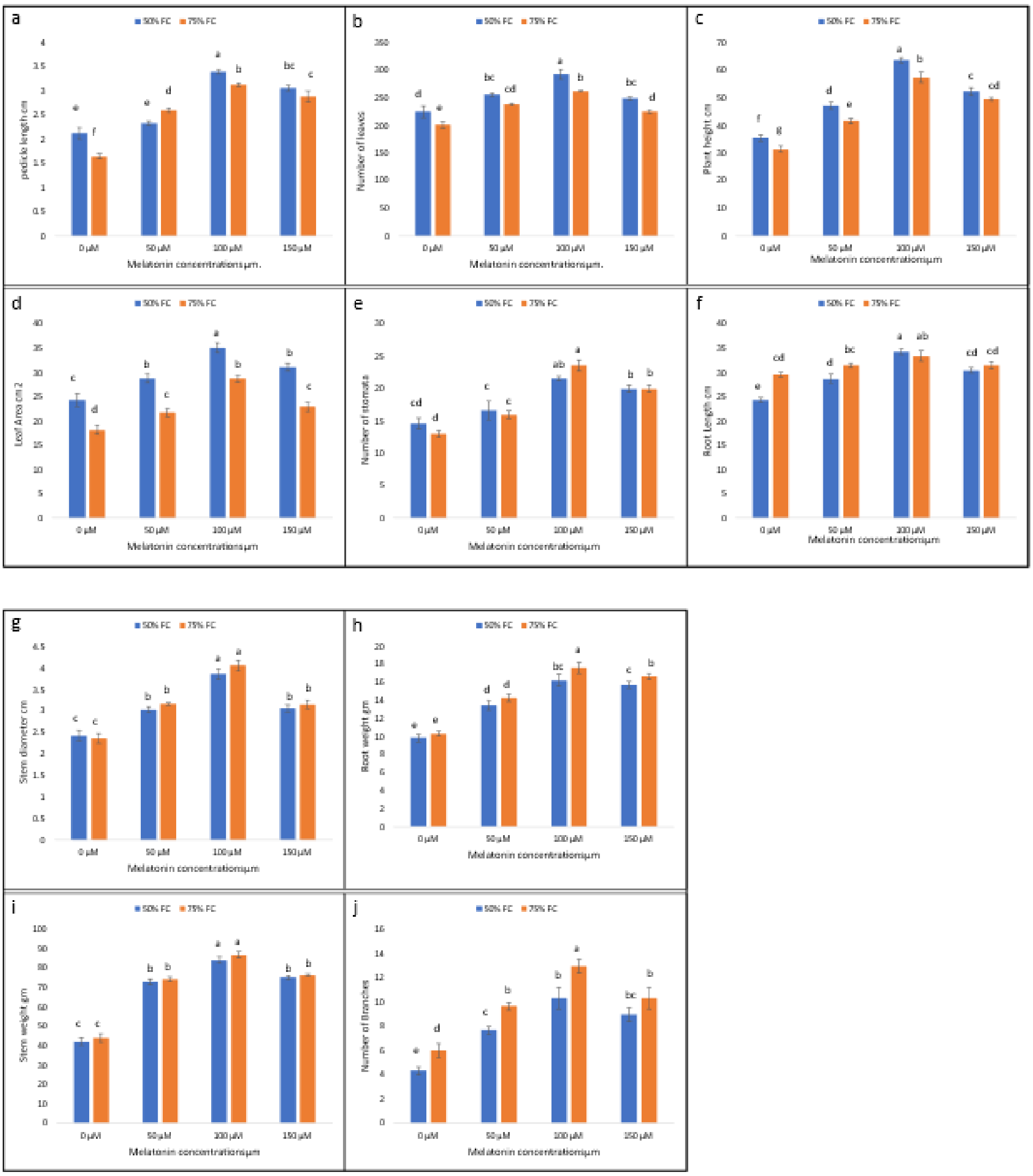


Figure 1. Impact of melatonin application on various morphological attributes of *Solanum muricatum* under different drought stress levels, Pedicle length (a), number of leaves (b), plant height (c), leaf area (d), stomatal density (e), root length (f), stem diameter (g), root weight (h), stem weight (i), and number of branches (j) all exhibited a progressive increase with melatonin application, peaking at 100 µM.

Total flavonoids (TF) content was highest in plants under 75% FC (0.93%), and melatonin application significantly reduced it, with the lowest value at 100 µM (a reduction of 61.3% and 53.8% under 50% and 75% FC, respectively). Proline content, a key stress marker, increased with melatonin application, reaching the highest value at 50 µM (47.0%, an increase of 20.5% over untreated plants under 50% FC). Malondialdehyde (MDA), an indicator of lipid peroxidation, was highest in untreated plants under 75% FC (1.20%), while 100 µM melatonin reduced it by 61.5% and 55.8% under 50% and 75% FC, respectively.

Relative water content (RWC) was highest in untreated 75% FC plants (0.51%), but melatonin significantly decreased it, with the lowest value at 100 µM (a reduction of 26.8% and 19.6% under 50% and 75% FC, respectively). Similarly, SPAD chlorophyll content was highest in untreated 75% FC plants (8.67%), and melatonin application at 100 µM significantly reduced it (a decrease of 42.0% and 53.8% under 50% and 75% FC, respectively). The graphs in **Figure 2(a–j)** illustrate the effects of melatonin (MT) application on various biochemical parameters in pepino plants under drought stress at 50% and 75% FC.

Table 3. Effects of melatonin applications on *S. muricatum* biochemical parameters under drought conditions.

Drought Stress	MT	Carotenoids	TPC	CAT	POD	SOD	TF	Proline	MDA	RWC	SPAD
50% FC	**0 µM**	6.33 ± 0.33 bc	0.57 ± 0.02 a	9.67 ± 0.33 c	5.67 ± 0.33 cd	49.67 ± 1.33 cd	0.70 ± 0.03 b	39.00 ± 1.00 c	0.70 ± 0.03 b	0.41 ± 0.01 c	6.33 ± 0.33 b
	50 µM	4.33 ± 0.33 d	0.39 ± 0.01 d	7.33 ± 0.33 d	3.33 ± 0.33 ef	56.67 ± 1.33 b	0.47 ± 0.03 d	47.00 ± 1.00 a	0.37 ± 0.03 de	0.38 ± 0.01 d	4.67 ± 0.33 cd
	100 µM	2.67 ± 0.33 e	0.44 ± 0.01 c	5.33 ± 0.33 e	2.33 ± 0.33 f	63.67 ± 1.33 a	0.27 ± 0.03 e	44.33 ± 1.33 b	0.27 ± 0.03 e	0.30 ± 0.01 e	3.67 ± 0.33 d
	150 µM	4.33 ± 0.33 d	0.35 ± 0.01 e	6.33 ± 0.33 de	5.33 ± 0.33 de	48.67 ± 1.33 d	0.50 ± 0.03 cd	39.67 ± 1.33 c	0.50 ± 0.03 cd	0.45 ± 0.01 b	6.00 ± 0.33 bc
75% FC	**0 µM**	9.00 ± 0.33 a	0.42 ± 0.01 c	17.00 ± 0.33 a	16.00 ± 0.33 a	39.00 ± 1.33 e	0.93 ± 0.03 a	34.00 ± 1.00 d	1.20 ± 0.03 a	0.51 ± 0.01 a	8.67 ± 0.33 a
	50 µM	7.00 ± 0.33 b	0.51 ± 0.01 b	11.00 ± 0.33 b	7.00 ± 0.33 c	48.33 ± 1.33 d	0.73 ± 0.03 b	40.00 ± 1.00 c	0.70 ± 0.03 b	0.46 ± 0.01 b	5.67 ± 0.33 bc
	100 µM	5.00 ± 0.33 cd	0.39 ± 0.01 d	8.67 ± 0.33 c	4.67 ± 0.33 de	52.00 ± 1.33 c	0.43 ± 0.03 d	43.00 ± 1.33 b	0.53 ± 0.03 c	0.41 ± 0.01 c	4.00 ± 0.33 d
	150 µM	5.67 ± 0.33 bc	0.31 ± 0.01 f	9.67 ± 0.33 c	10.00 ± 0.33 b	33.67 ± 1.33 f	0.63 ± 0.03 bc	39.00 ± 1.00 c	0.80 ± 0.03 b	0.51 ± 0.01 a	6.67 ± 0.33 b

Table 4. Analysis of variance of melatonin and drought stress on morphological characteristics of Pepino

Source of Variation	Df	Carot	TPC	CAT	POD	SOD	TF	Proline	MDA	RWCs	SPAD
L	1	0.00510**	0.04335**	30.3750**	117.042**	0.24000**	0.73500**	165.375**	7.0417**	782.042**	73.500**
T	3	0.09208**	0.05502**	46.4583**	139.458**	0.65667**	0.99000**	183.458**	44.4583**	1040.46**	216.833**
L×T	3	0.05761**	0.00195 ns	1.7917 ns	17.125**	0.01667 ns	0.04833 ns	55.792**	3.4583 ns	34.46*	40.833**
Error	14	0.00367	0.00316	8.25	7	0.09667	0.10917	11	9.6667	31.67	21.083
CV (%)	-	3.83	3.51	13.85	7.54	14.24	13.94	13.05	14.56	3.07	3.01

*Drought Stress (L) Melatonin (T), ns: Not significant, * Significant at P < 0.05, ** Significant at P < 0.01 df: Degree of Freedom, CV: Coefficient of Variation.*

4.3. Correlation matrix

The study revealed significant variations in the morphological traits of pepino in response to different drought stress levels and melatonin applications. The correlation matrix (**Figure 3**) shows the relationships among various parameters; plant phenological attributes (PL, NL, PH, LA) exhibited a strong positive correlation with plant physiological parameters such as RWCs and SPAD. Among these, leaf area (LA) showed the strongest correlation with RWCs and SPAD, showed that drought-resistant plants retained more water and sustained chlorophyll content under stress conditions.

Relative water content (RWC) displayed a strong positive correlation with plant biomass traits, including shoot and root weight, largely due to enhanced proline accumulation and antioxidant enzyme activity. Physiological and phenological traits also correlated closely with yield in *S. muricatum*, with shoot weight and branch number strongly linked to total biomass primarily driven by improved water use efficiency and photosynthesis under melatonin treatment.

Among the biochemical parameters, proline and MDA showed a strong negative correlation with plant physiological and yield parameters. As plants faced drought stress, they diverted photosynthates toward antioxidant and osmolyte production, reducing growth and yield efficiency. Stress indicators such as MDA and enzymatic antioxidants (CAT, POD, SOD) exhibited a strong negative correlation with phenological, physiological, and yield parameters across all treatments. Higher MDA levels were associated with lower plant growth and yield, reinforcing the negative effects of drought stress on plant metabolism. Results presented plant growth and yield showed a strong positive correlation with RWCs and SPAD but a negative correlation with MDA and stress-related biochemical markers.

4.4. Heatmap

The heatmap in **Figure 4** illustrates that how biochemical and physiological processes of plants under different drought stress levels (50% and 75% FC) and melatonin (0, 50, 100, 150 µM) treatments. PL, NL, and PH exhibited the highest intensity (blue), indicating a stronger response, whereas oxidative stress markers like MDA and Proline remained lower (red) under melatonin application (**Figure 4**).

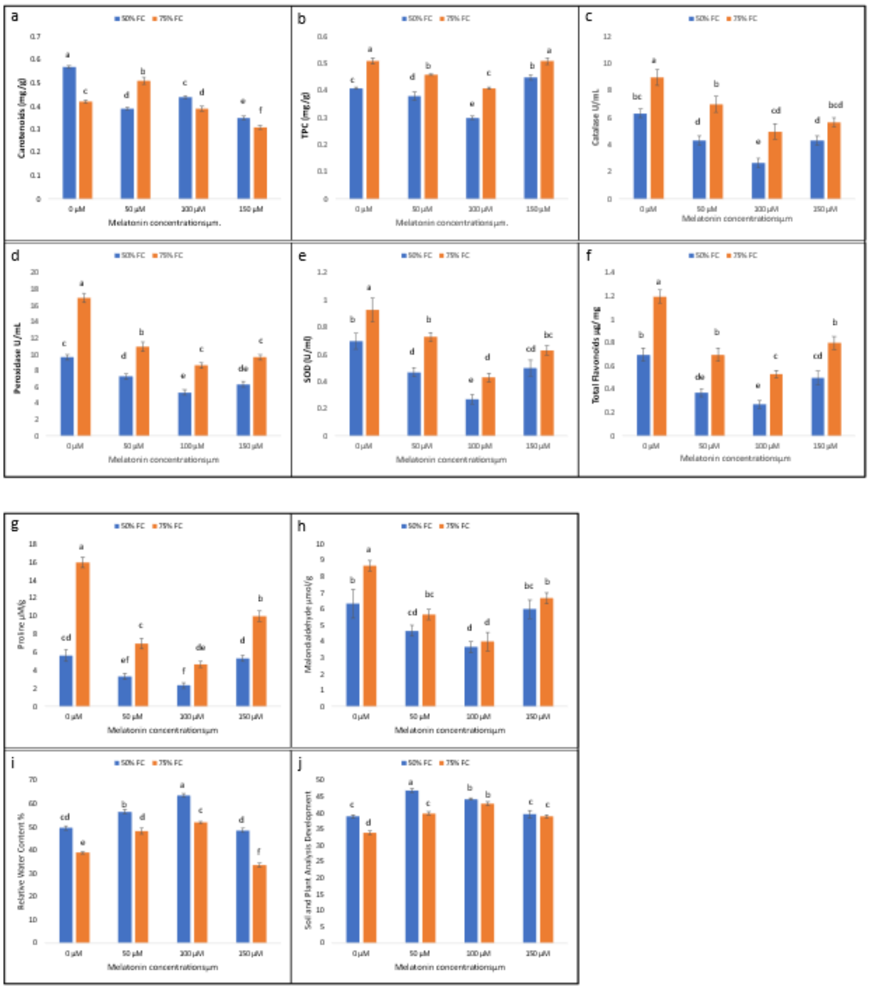


Figure 2. Effects of melatonin application on various biochemical parameters in pepino plants under drought stress, including In Proline accumulation (g) and malondialdehyde (MDA) levels (h) were significantly higher in the control, indicating increased stress levels, while MT-treated plants exhibited a dose-dependent reduction. Relative water content (RWC) (i) was highest at 100 µM MT under both drought conditions. SPAD chlorophyll index (j) also showed a declining trend with increasing MT doses.

A two-way clustered heatmap in **Figure 3** was generated to observe impact of melatonin applications on various parameters in pepino plant. The measurements were categorized based on their similarity at different treatment stages, with colored squares representing the intensity of relationships among parameters. The color gradient highlights correlation intensity, where red shades signify strong positive correlations, light red to pink indicate mild positive correlations, blue shades represent strong negative correlations, and light blue to white reflect weak negative or near-neutral relationships. The heatmap clustered the treatments into four groups: the first cluster (D50M0, D75M0) represented control conditions, where plants showed lower antioxidant enzyme activity (CAT, SOD, POD) and osmolyte accumulation (proline, MDA) but exhibited higher physiological parameters (RWCs, SPAD, LA, PH, NB), indicating minimal stress impact. The second cluster (D50M50, D75M50) included moderate melatonin applications, where plants demonstrated moderate antioxidant enzyme induction and proline accumulation while maintaining relatively stable water status and photosynthetic rates. The third cluster (D50M100, D75M100) showed increased antioxidant activity and stress markers (MDA, proline), resulted enhanced stress adaptation but reduced growth-related attributes such as NL, LA, and RWCs. The fourth cluster (D50M150, D75M150) linked to the highest melatonin doses, where biochemical responses were significantly upregulated, particularly SOD, POD, and proline, signifying an active stress response. However, this also led to a decline in growth and physiological parameters, revealed that extreme melatonin application may not be beneficial under extreme drought conditions.

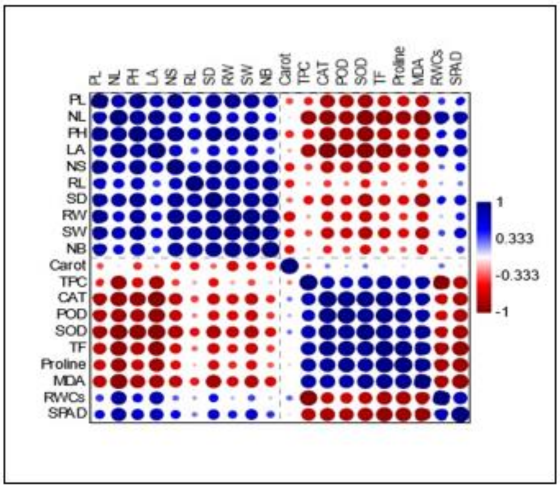


Figure 3. Correlation matrix for the effects of melatonin applications (0 µM, 50 µM, 100 µM, 150 µM) on S. muricatum (Pepino plant), morphological, and biochemical attributes under drought stress (50% FC and 75% FC). Plant length (PL), node length (NL), plant height (PH), leaf area (LA), number of shoots (NS), root length (RL), stem diameter (SD), root weight (RW), shoot weight (SW), number of branches (NB), carotenoids (Carot), total phenolic content (TPC), catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), total flavonoids (TF), proline, malondialdehyde (MDA), relative water content (RWCs), and SPAD chlorophyll content.

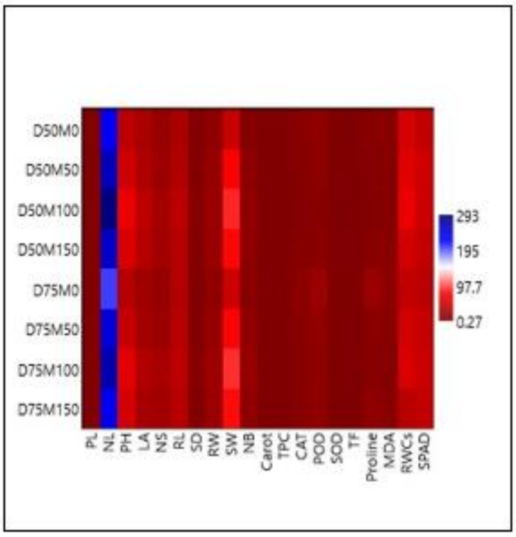


Figure 4. The heatmap for the biochemical and physiological responses of Pepino plant under different drought stress levels (50% FC and 75% FC) and melatonin (0 µM, 50 µM, 100 µM, 150 µM) treatments.

4.5. Correlation between various parameters

The PCA biplot shows a clear picture of how different treatments respond to drought stress with melatonin applications. The plot shows a distinct separation of treatments, with control groups (D50M0, D75M0) clustered at the negative end of Component 1, representing minimal variation in biochemical and physiological responses. Higher melatonin concentrations (D50M100, D75M100, D50M150, D75M150) shift towards the positive end, reflecting significant physiological and biochemical changes under drought. Moderate melatonin doses (D50M50, D75M50) fall near the center, signifying a balanced response to stress. Component 1 primarily differentiates treatments based on physiological traits (RWCs, SPAD, LA, PH) and biochemical markers (SOD, POD, TPC, Proline), while Component 2 captures variations in oxidative stress indicators (POD, SOD) and secondary metabolites (TPC, Proline), positioning high-dose treatments (D75M150, D50M150) in the upper quadrant. Carotenoid levels are negatively correlated with Component 1, suggesting lower pigment accumulation under high-stress conditions. Antioxidant enzymes (SOD, POD, CAT), osmolytes (Proline, MDA), and phenolic content (TPC) cluster together, showing a strong positive correlation in drought-stressed plants. Growth traits (RWCs, SPAD, LA) align with moderate melatonin doses. Higher melatonin levels (D50M150, D75M150) improve biochemical defenses (**Figure 5**).

The bar chart illustrates the correlation between various physiological and biochemical parameters, revealing key relationships between growth traits and stress responses. Strong positive correlations (above 0.8) were observed among plant length (PL), node length (NL), plant height (PH), leaf area (LA), and net photosynthesis rate (NS), indicating their close interdependence. Similarly, root length (RL), shoot dry weight (SD), root weight (RW), stem width (SW), and number of branches (NB) showed a strong positive correlation, suggesting that melatonin application positively influences plant growth. SPAD and relative water content (RWCs) exhibited moderate positive correlations, emphasizing their role in stress resilience. Stress-related factors, such as TPC, CAT, POD, SOD, and total flavonoids (TF) had strong negative relationships (-0.6 to -0.8) with growth traits. This shows that antioxidant activity is directly linked to plant growth in the opposite direction. Malondialdehyde (MDA) showed negative correlations with plant growth parameters as well, indicating the negative impact of oxidative damage on plant growth. Carotenoids (Carot) and proline showed weak negative correlations. Surprisingly, TF and proline showed moderate correlations.

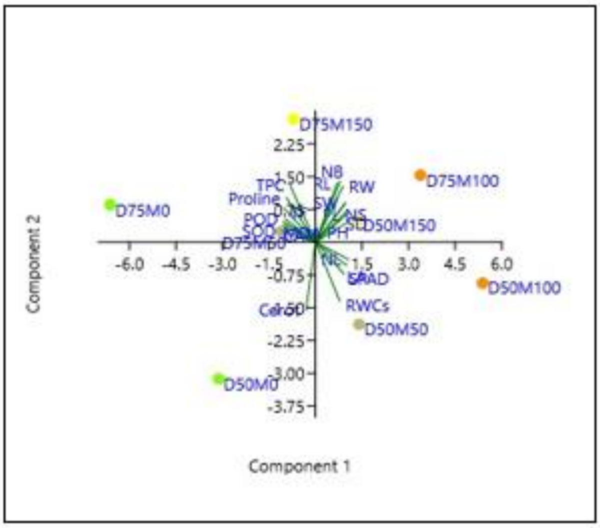


Figure 5. PCA biplot showing the interaction effects of melatonin and drought stress on morpho-physiological and biochemical traits in *Solanum muricatum*.

Radar chart gives a relative comparison of different physiological and biochemical parameters of *Solanum muricatum*. The horizontal and vertical axes represent different traits, and the colored lines indicate different treatment groups. The charts show the changes in root length, shoot dry weight, leaf area, and plant height that got a lot better with moderate amounts of melatonin (D50M50, D75M50). Relative water content (RWCs) and SPAD values follow a similar pattern. Antioxidant enzymes (SOD, POD, CAT) and total phenolic content (TPC) exhibit higher values in D50M100, D75M100, D50M150, and D75M150, with increased oxidative stress defense. Stress markers like proline and malondialdehyde (MDA) peak under severe drought (D75M150), reflecting melatonin's role in osmotic adjustment. While moderate melatonin concentrations (D50M100, D75M100) promote growth and physiological stability, higher doses (D50M150, D75M150) designate antioxidant and osmolyte accumulation over growth (**Figure 6**).

Discussion

Melatonin application significantly improved morphological characters of *S. muricatum* under drought, with strongest effects at 100 µM. These improvements were seen in characteristics like pedicle length, leaf number, plant height and root growth, supporting earlier results on melatonin's role in enhancing drought tolerance Khosravi *et al.* (2023), who conducted an experiment to display that melatonin application significantly supported shoot and root growth in hot peppers under drought and waterlogging conditions with notable increases in root length and biomass. Similarly, Imran *et al.* (2021) reported that exogenous melatonin enhanced root and shoot length in soybean plants, attributing these effects to increased auxin and ethylene production, which are critical for root initiation and development. These results support the present study, especially the marked improvement in root length and weight after exposure to melatonin, indicating a conserved mechanism among diverse plant species.

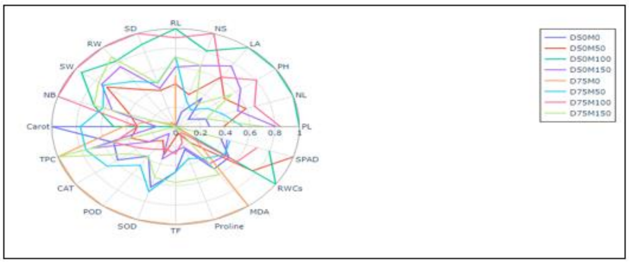


Figure 6. The Spider (Radar) Chart of various physiological and biochemical parameters under different melatonin treatments and drought stress conditions of Pepino, including plant length (PL), node length (NL), plant height (PH), leaf area (LA), number of shoots (NS), root length (RL), stem diameter (SD), root weight (RW), shoot weight (SW), number of branches (NB), carotenoids (Carot), total phenolic content (TPC), catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), total flavonoids (TF), proline, malondialdehyde (MDA), relative water content (RWCs), and SPAD chlorophyll content.

Studies from the past have also supported the current study's finding that morphological traits improved the most when melatonin was applied at a concentration of 100 µM. Zhang *et al.* (2023) reported that melatonin enhanced drought tolerance and improved lateral root formation in cucumber, and the most effective concentrations of the compound were determined. These align with the present study's observations in which high concentrations (150 µM) did not elicit further induction of traits (leaf number and plant height), suggesting a saturation point for melatonin's effectiveness. Yan *et al.* (2024) found that the excessive level of melatonin in transgenic rice delayed flowering and reduced the grain yield, highlighting the importance of dosage optimization.

The current study also provides evidence to enhance previous findings related to the stimulating role of melatonin in stomatal density and leaf area under drought stress conditions. Sun *et al.* (2023) found that melatonin application alleviated oxidative damage in *Malus hupehensis* via the activation of antioxidant enzymes, probably leading to the improved stomatal and leaf expansion. Similarly, Xian *et al.* (2024) concluded that melatonin could delay leaf senescence in apple trees, maintaining photosynthetic performance and leaf area under stress conditions. These results correlate with the current study's findings, in which melatonin treatment significantly enhanced leaf area and stomatal density, specifically at a 100 µM concentration.

When plants face drought, they adopt many changes inside. These changes are mainly due to stress by extreme reactive oxygen species (ROS). Melatonin has properties that fight these harmful types of ROS and can lessen stress effects (Naz *et al.* 2023; Sharma and Zheng 2019; Mehdi *et al.* 2025). Current findings displayed that, melatonin application in *Solanum muricatum* resulted in a significantly reduction in carotenoid content, which was in contrast to some studies that reported enhanced carotenoid biosynthesis by melatonin (Alharby and Fahad 2020; Dai *et al.* 2020). The difference indicates that melatonin affects carotenoids in a species-specific or concentration-dependent manner. In our study, melatonin decreased TPC levels in *Solanum muricatum*. The results of our study revealed that melatonin acted to modulate enzyme activity and there were significant differences depending on concentration and field capacity. CAT, SOD and POD activities decreased activity under the condition of 50% field capacity. Therefore, the melatonin-mediated regulation of oxidative stress has a dual character and is modulated by enzymatic reactions according to oxidative stress severity and concentration used. Enzymes engaged in antioxidant activity, including CAT, POD, and SOD, are essential to reducing oxidative stress induced by drought (Altaf *et al.* 2022; Amzeri *et al.* 2024). The current study's findings on *Solanum muricatum* showed that the application of melatonin increased proline levels, especially at 50 µM. According to Gul *et al.* (2022) study proline accumulation has been recognized as a typical response to drought stress, functioning as an osmo protectant stabilizing cellular structures and fighting ROS. Proline content has been shown to increase unequivocally in grape, okra (Yang *et al.* 2024; Saihood and Gerry 2024), soybean and pulses (Zou *et al.* 2019; Meti Kioko *et al.* 2024) in response to melatonin treatments.

In our study, MDA, RWC, Chlorophyll content (SPAD) showed reduced level results tested at various concentrations with melatonin in *Solanum muricatum.* Melatonin has been found to assist plants in retaining water and delaying leaf senescence in studies conducted on soybeans and cucumbers as well (Altaf *et al.*, 2022; Torres-Rodriguez *et al.*, 2024).

5. Conclusion

The current experiment indicated that spraying melatonin on leaves helps plants to control drought stress. It makes *S. muricatum* stronger by boosting its growth and health. During drought (50% and 75% FC), a moderate amount (100 µM) of melatonin worked best. It helped plants grow taller, have larger leaves, more pores, and longer roots while reducing stress. Enhanced relative water contents and chlorophyll stability indicate that melatonin supports to maintain hydration and photosynthetic functions during drought. Biochemical results showed reduction in oxidative stress, as evidenced by lower malondialdehyde (MDA) levels and increased activity of antioxidant enzymes like CAT, SOD, and POD. There was a strong positive correlation found to exist between plant growth parameters and water retention, indicating melatonin's role in enhancing drought resilience. However, excessive melatonin application (150 µM) negatively impacted plant performance, suggesting a threshold beyond which its benefits diminish. Further research is needed to determine the accurate mechanisms, how melatonin channelizes the biological processes in plants to mitigate drought responses in *Solanum muricatum*.

Data availability

Data will be made available on request.

Competing interest

All authors declare no potential conflict and no competing interest.

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