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RESEARCH ARTICLE

Thriving against the odds: Unveiling the role of melatonin in Okra germination and seedling traits under PEG-induced osmotic stress

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Abstract

Altered environmental conditions subject plants to abiotic stresses like salinity, drought, temperature fluctuations (heat and cold) and exposure to heavy metals. Frequent stresses prompt the plant defense system to consistently adapt. Biostimulants assist plants in refining their defense strategies, allowing more effective responses to the changing environment and challenges. Melatonin, a neurohormone and antioxidant in mammals, has extended its presence to the plant domain due to its pervasive nature as a signaling molecule. Melatonin acts as a bio-stimulatory molecule in confronting stress in plants by regulating its growth and development. The present study attempts to comprehend the role of melatonin in combating drought stress. Okra genotypes namely, CO 4, Arka Anamika, Arka Abhay and Arka Nikita were subjected to PEG-induced osmotic stress (15 % PEG) in Petri plates supplemented with different doses of melatonin (50, 100, 150, 200, 250 µM). The seedling response varied with respect to genotypes in a dose-dependent manner. Among the genotypes, Arka Anamika performed better followed by Arka Nikita in terms of seedlings traits recorded. Higher concentrations of melatonin exhibited an inhibitory effect on seed germination and seedling growth parameters. However, priming okra seeds with 100 µM of melatonin showed promising results in mitigating the adverse effect of osmotic stress by promoting seed germination and seedling characteristics.

Keywords

melatonin; drought; okra; germination indices

Introduction

The prevalence and severity of heat, salinity, and drought stresses in the environment are caused by global water scarcity and climate change (1). It is predicted that sustained global warming will worsen the variability of the global water cycle, the global monsoon precipitation, extreme wet and dry weather and climate events and seasons (2). Drought, a pivotal abiotic stressor in agriculture, adversely affects plant growth and development, posing a substantial challenge to overall productivity. More than 60 % of the geographical area of India is highly vulnerable to drought (3). About 80 % of the total area covering the southern zone of Tamil Nadu faces frequent droughts as indicated by the spectral indices (4). Improving water use efficiency (WUE) is a critical task for crop water management in order to maintain global crop output under changing climate conditions (5).

Drought stress poses a substantial constraint on the growth, development and yield of vegetables, which typically contain more than 90 % water (6). Even a slight decrease in plant water availability impairs photosynthesis, restricts metabolic processes, reduces CO_2 exchange and damages chloroplasts due to stress (7). Depending on the intensity of the drought and the various stages of the plant growth, each vegetable crop responds to it differently. Plants exhibit poor germination as their initial response to moderate drought stress (8, 9). On the other hand, plants under extreme drought stress grow more slowly and have smaller leaves, lesser stem girth, biomass and height (10). Apart from the morphological attributes, drought stress can also cause considerable impacts on physiological features such as photosynthetic rate, stomatal movement and chlorophyll fluorescence (9, 11). Thus, the organelle that performs photosynthesis, the chloroplast is impacted because of drought stress making it extremely susceptible. This elevates the production of reactive oxygen species (ROS), which reduces the quantum yield, efficiency and chlorophyll biosynthesis in photosynthetic antenna complexes I and II. As a result, the plant's health and viability are adversely affected (12).

Most vegetables exhibit sensitivity to drought when their water content drops to approximately 20 % (13). Okra (*Abelmoschus esculentus* L.), belonging to the family malvaceae is a vital vegetable crop grown in India. In India, okra cultivation spans an area of 554 thousand ha, yielding an annual production of 7252 thousand metric tons, while in Tamil Nadu okra is cultivated over an area of 22.29 thousand ha, with a total production of 215.60 thousand metric tons and a productivity of 9.67 MT/ha for the 2023–2024 period (14). Okra is well known for its nutritional value as it is rich in 2.10 g of protein, 1.70 g of fibre, 84 mg of calcium, 1.2 mg of iron, 185.00 µg of vitamin A, 0.08 mg of vitamin B2, 0.04 mg of vitamin B1, 0.60 mg of niacin and 47.00 mg of ascorbic acid (15).

As the cultivation of okra continues to rise, maintaining optimal irrigation levels and water availability becomes crucial to mitigate potential yield losses (16). Okra is vulnerable to drought stress, especially during the flowering and pod-filling stages, which can lead to a significant reduction in okra yields, ranging between 30 to 100 % (17). Enhancing the tolerance of vegetables to drought necessitates a multifaceted approach, integrating novel cultivation techniques and external regulatory technologies to enhance the growth and development of vegetables globally (12, 18).

Animals, plants and microbes all naturally produce melatonin (MT; N-acetyl-5-methoxy-tryptamine) (19). With a modest molecular weight and pleiotropic effects in the kingdom of plants, melatonin is a naturally occurring, multifunctional, non-toxic, regulatory and universal biomolecule (20). It has been demonstrated that melatonin increases tolerance to a variety of abiotic stressors, including heat, cold, salt, heavy metals, chemicals and phytopathogen infections. Melatonin alleviates the damaging effects of reactive oxygen and nitrogen species (ROS/RNS), thereby reducing damage from oxido-nitrosative stress (19). By controlling the transcription of essential genes for chlorophyll metabolism, enhancing the activity of enzymes in the Calvin cycle (21) and sustaining redox balance within plant cells (22), melatonin has the potential to enhance photosynthetic efficiency.

Melatonin (MT) exhibits growth-promoting effects in wheat under cold stress, leading to augmented fresh weight, root length and plant height attributed to reactive oxygen species (ROS) scavenging and chlorophyll preservation (23). Radicle length, fresh weight, germination rate and potential are all improved by treating cotton seeds with 100 µM melatonin (24). In a similar way, soaking cucumber seeds in melatonin beforehand reverses the growth and germination-delaying effects of 5 % PEG 6000, speeding up the process and increasing the lengths of the shoots and roots as well as the fresh and dry weights (25). The scientific exploration of the effects of externally applied melatonin on seed germination, seedling growth and overall plant development is relatively limited. This study attempts to unravel the potential of melatonin in counteracting the drought stress in okra by modulating the seedling growth attributes pertaining to enhanced tolerance under stress conditions thereby optimizing the melatonin concentration that contributes to drought tolerance in okra.

Materials and Methods

Planting material and source of melatonin

Okra genotypes namely, CO 4, Arka Anamika, Arka Abhay and Arka Nikita were purchased from Tamil Nadu Agricultural University, Coimbatore, Kerala Agricultural University, Thrissur and Indian Institute of Horticultural Research, Bangalore, respectively. Melatonin and polyethylene glycol (PEG 6000) were purchased from Sigma-Aldrich Pvt Ltd.

Experimental Layout

The okra seeds (CO 4, Arka Anamika, Arka Abhay and Arka Nikita) were sterilized using 75 % ethanol for 30 min, followed by 5 rinses with distilled water. The sterilized seeds were placed in Petri dishes with 3 layers of filter paper and treated with 5 %, 10 %, 15 % and 20 % PEG solutions, with distilled water as control. Petri dishes were incubated at 25 ± 1 °C for germination assessment on the 7th day to determine the ideal concentration of PEG 6000. Based on the germination rates recorded almost all genotypes showed tolerance to a drought level of 15 % PEG which is represented in Table 1. Further, the melatonin concentrations were optimized, wherein the surface sterilized seeds of okra were immersed in various melatonin (MT) concentrations (50, 100, 150, 200 and 250 µM) for 6 h at 25 °C. The control group comprised of sterilized seeds soaked in distilled water. Subsequently, 10 seeds from each treatment were placed on Petri dishes lined with three layers of filter paper, which were moistened with 10 mL of a 15 % PEG 6000 solution to simulate drought stress. The setup was then incubated at 25 °C for 7 days. Each treatment was replicated 3 times, with each replicate comprising 10 seeds. Germination rates were recorded on a daily basis whereas other seedling growth parameters were recorded on the 7th day after incubation to identify the most effective melatonin concentration for enhancing okra germination under drought stress conditions.

Table 1. Effect of PEG concentration on germination rate of different okra genotypes. df- degrees of freedom, F-test value, P - probability of significance at two-tailed test. Data in the table is expressed as mean ± SE.

PEG Concentration	CO 4	Arka Anamika	Arka Abhay	Arka Nikita
Control	79.15 (± 10.48)[a]	95.82 (± 4.17)[a]	70.80 (± 7.97)[a]	91.65 (± 4.82)[a]
5 % PEG	66.62 (± 6.79)[ab]	83.30 (± 6.81)[ab]	62.45 (± 10.48)[a]	70.80 (± 7.97)[ab]
10 % PEG	54.15 (± 10.48)[b]	74.95 (± 4.82)[b]	54.12 (± 12.49)[a]	58.30 (± 10.75)[b]
15 % PEG	58.3 (± 4.79)[b]	79.12 (± 4.17)[b]	58.30 (± 10.75)[a]	62.47 (± 7.96)[b]
20 % PEG	0.00 (± 0.00)	0.00 (± 0.00)	0.00 (± 0.00)	0.00 (± 0.00)
df	3			
F value	15.9			
P value	0.001			

Germination rate and germination potential

Seeds were considered germinated when the length of their radicle and hypocotyl exceeded half the length of the seed. The germination parameters, denoted as GP (Germination Potential) and GR (Germination Rate), were quantified on the 3rd and 7th days respectively, following the initiation of germination (24).

$$GR\ (\%) = \frac{\text{Seeds germinated on day 7}}{\text{Total seeds}} \times 100 \quad \text{(Eqn. 1)}$$

$$GP\ (\%) = \frac{\text{Seeds germinated on day 3}}{\text{Total seeds}} \times 100 \quad \text{(Eqn. 2)}$$

Vigour index (VI) and seedling length

VI was calculated by the formula (26), whereas seedling length is the sum of both shoot and root length. Shoot length was measured from the collar region to the cotyledon attachment point and root length was measured from the collar region to the root tip.

$$\text{Vigour index} = \text{seedling length} \times \text{germination rate} \quad \text{(Eqn. 3)}$$

Fresh and dry weight

The fresh and dry weights of seedlings were measured for three randomly selected seedlings from each replication. Fresh weights were initially recorded, followed by drying the samples in a hot air oven at 80 °C for 48 h to obtain dry weights, which were then expressed as grams per seedling.

Stress indices

The promptness index (PI) and germination stress tolerance index (GSI) were computed from the seedlings using the following formula (27),

P.I = nd2 (1.00) + nd4 (0.75) + nd6 (0.5) + nd8 (0.25) where n is the number of seeds germinated at day

$$GSI = \frac{\text{P.I of stressed seedling}}{\text{P.I of control seedling}} \times 100 \quad \text{(Eqn. 4)}$$

Shoot length stress index and root length stress index

Shoot and root length stress tolerance index was calculated using the given formula (28),

$$SLSI = \frac{\text{Shoot length of stressed plants}}{\text{Shoot length of control plants}} \times 100 \quad \text{(Eqn. 5)}$$

$$RLSI = \frac{\text{Root length of stressed plants}}{\text{Root length of control plants}} \times 100 \quad \text{(Eqn. 6)}$$

Statistical analysis

The experimental framework employed in this study was a Factorial Completely Randomized Design (FCRD) comprising four replications. The data were statistically analysed through SPSS software (version 16.0) using one way Analysis of Variance (ANOVA) for comparing the mean value among the genotype and melatonin concentration. Two-way ANOVA for studying the interaction effects. To assess distinctions among the group means, the Least Significant Difference (LSD) test was applied and the Critical Difference (CD) was computed at a significance level of 0.05 ($p \leq 0.05$). Graphs were visualized using GraphPad Prism (version 8.2.0).

Results

Selection of Melatonin concentration for seed treatment

A set of experiments was conducted to determine the effects of various melatonin dose combinations on seed germination under drought stress. Okra seeds treated with different concentrations of melatonin mitigated the detrimental impact of PEG-induced drought stress (15 % PEG) on germination. Seeds treated with 50 µM melatonin exhibited limited effects on germination indices during drought stress. Pre-soaking seeds with 100 µM melatonin proved more effective in alleviating drought stress than other melatonin treatments (150, 200, 250 µM) which can be substantiated by analysing the germination indices.

Influence of melatonin on germination indices under drought stress

Seeds subjected to absolute control conditions exhibited 80 to 100 % germination, whereas PEG-treated seeds exhibited a reduction in germination compared to absolute control. Among the treatment combinations, seeds supplemented with 100 µM of melatonin recorded a higher germination rate (79.13 %) compared to drought control (C). Arka Anamika outperformed other genotypes with a germination rate of about 70.21 % followed by Arka Nikita (62.66 %) (Fig. 1; Table S1). A similar trend was observed in the case of germination potential and vigour index under 100 µM of melatonin. Arka Anamika recorded higher germination potential (65.85 %),

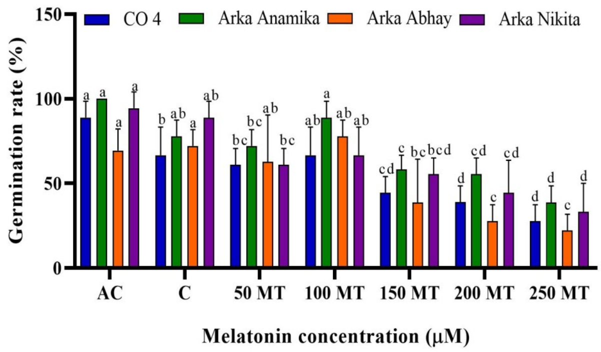

Fig. 1. Effect of melatonin on germination rate of okra seedlings under drought stress.

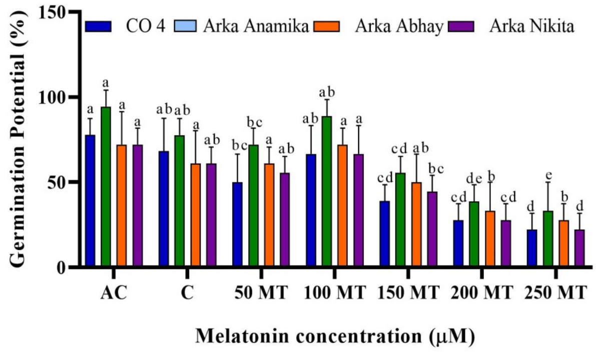

Fig. 2. Effect of melatonin on germination potential of okra seedlings under drought stress.

whereas the other 3 genotypes were on par (Fig. 2; Table S2). In the case of the vigour index, Arka Nikita (574.76) and Arka Anamika (568.99) recorded almost comparable vigour index followed by CO 4 (476.36) and Arka Abhay (471.25) (Fig. 3; Table S3).

Shoot length and root length were drastically reduced under drought control (C) compared to absolute control (AC). Under higher doses of melatonin, that is, 150 - 250 µM, the shoot and root length reduced significantly, whereas higher shoot (5.10 cm) and root length (3.38 cm) were recorded in the case of seeds treated with 100 µM of melatonin (Fig. 4 and Fig. 5; Table S4 and Table S5). Arka Nikita subjected to 100 µM of MT recorded a higher shoot length (5.44 cm) compared to other genotypes. Similarly, Arka Anamika and Arka Nikita treated with 100 µM of melatonin recorded a root length of about 4.43 cm and 4.23 cm respectively (Table 2).

Significant variations in fresh and dry weights of okra seedlings were observed among treatments. Drought controlled to a significant decrease in fresh and dry weights compared to the absolute control AC. Conversely, melatonin pre-treatment significantly increased the fresh and dry weights of seedlings compared to their respective drought controls. Within the melatonin treatments, seeds pretreated with 100 µM exhibited greater fresh (0.198 g) and dry weights (0.031 g) compared to the control seedlings. The lowest fresh (0.11 g) and dry weights (0.030 g) were observed at 50 µM of melatonin (Fig. 6 and Fig. 7; Table S6 and Table S7). The interaction analysis indicates that CO 4 seedlings subjected to 100 µM of melatonin demonstrated significantly higher seedling fresh weight and dry weight compared to other treatment and genotype combinations (Table 3).

Stress indices such as promptness index (PI), germination stress tolerance index (GSI), shoot length stress index (SLSI) and root length stress index (RLSI) were computed. The results revealed that promptness index (10.54) and germination stress index (90.04) of okra seedlings were higher in the melatonin pre-treatment of 100 µM compared to drought control (C) (Fig. 8 and Fig. 9; Table S8 and Table S9). The interaction effect of genotype and melatonin concentration resulted in a higher promptness index and germination stress index in the case of Arka Anamika seedlings supplemented with 100 µM melatonin (Table 3).

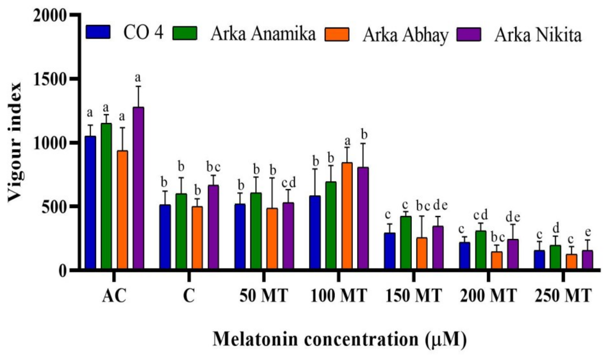

Fig. 3. Effect of melatonin on vigour index of okra seedlings under drought stress

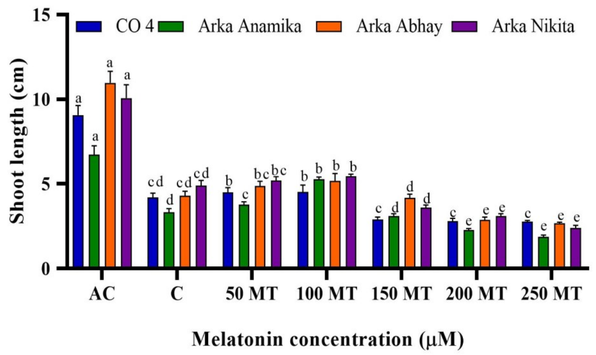

Fig. 4. Effect of melatonin on shoot length of okra seedlings under drought stress

Table 2. Effect of melatonin on germination and seedling growth parameters under drought stress on different okra genotypes. df- degrees of freedom, F-test value, *P* - probability of significance at two-tailed test. GR- Germination rate, GP- Germination potential, VI- Vigour index, SL- Shoot length, RL- Root length.

Factors	df	GR		GP		VI		SL		RL	
		F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Genotype	3	7.23	0.001	7.22	0.001	4.81	0.005	69.23	< 0.001	124.52	< 0.001
Melatonin Concentration	6	30.62	0.001	30.98	0.001	91.17	0.000	608.30	< 0.001	57.07	< 0.001
Genotype X Melatonin Concentration	18	0.77	0.71	0.28	0.99	1.12	0.350	12.32	< 0.001	18.653	< 0.001
Error	56										

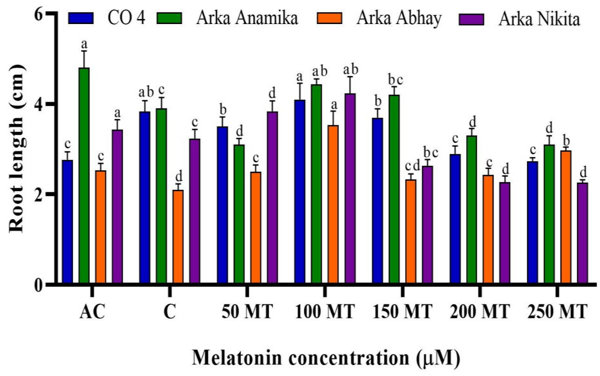

Fig. 5. Effect of melatonin on root length of okra seedlings under drought stress

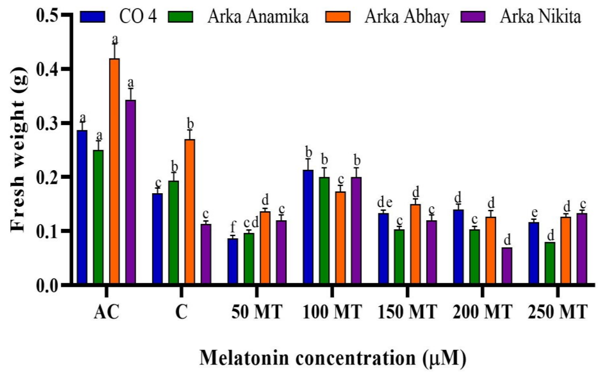

Fig. 6. Effect of melatonin on fresh weight of okra seedlings under drought stress

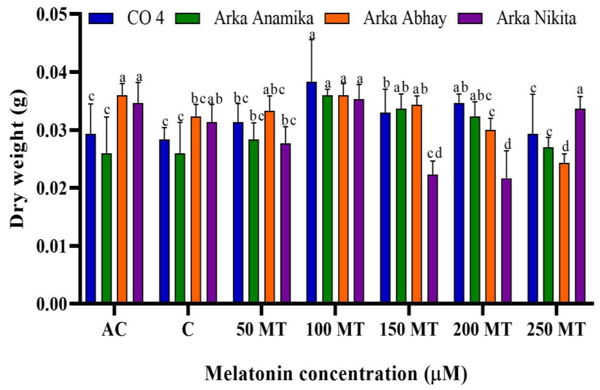

Fig. 7. Effect of melatonin on dry weight of okra seedlings under drought stress

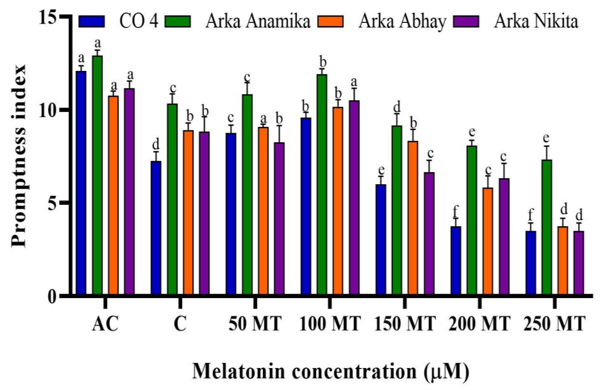

Fig. 8. Effect of melatonin on promptness index of okra seedlings under drought stress

Table 3. Effect of melatonin on seedling growth parameters under drought stress on different okra genotypes. df- degrees of freedom, F-test value, *P* - probability of significance at two-tailed test. FW- Fresh weight, DW- Dry weight, PI- Promptness index, GSI- Germination stress index, SLSI- Shoot length stress index, RLSI- Root length stress index.

Factors	df	FW		DW		PI		GSI		SLSI		RLSI	
		F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Genotype	3	73.55	< 0.001	5.94	0.001	113.73	< 0.001	61.186	< 0.001	11.46	< 0.00 < 0.00 < 0.00 <0.00	22.14	0.000
Melatonin Concentration	6	514.08	< 0.001	10.31	0.00	279.57	< 0.001	51.56	< 0.001	212.35	< 0.00	57.99	0.000
Genotype X Melatonin Concentration	18	28.98	< 0.001	6.73	0.00	6.044	< 0.001	6.06	< 0.001	8.56	< 0.00	2.24	0.011
Error	56												

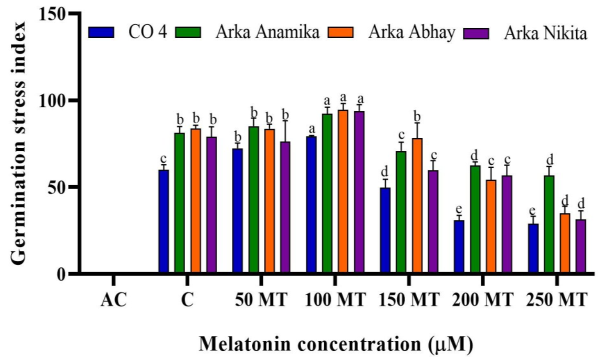

Fig. 9. Effect of melatonin on germination stress index of okra seedlings under drought stress

Under drought stress (PEG 6000), the control group exhibited a significant decrease in both shoot length stress index and root length stress index compared to the melatonin treatments. The highest shoot length stress index (56.33) and root length stress index (126.36) were observed in the 100 µM melatonin pre-treatments (Fig. 10 and Fig. 11; Table S10 and Table S11). The interaction effect of the genotype and treatment showed that Arka Anamika seedlings treated with 100 µM melatonin had higher plant height stress index in comparison to other combinations (Table 3). While CO 4 subjected to 100 µM melatonin pre-treatment exhibited a higher root length stress index followed by Arka Abhay.

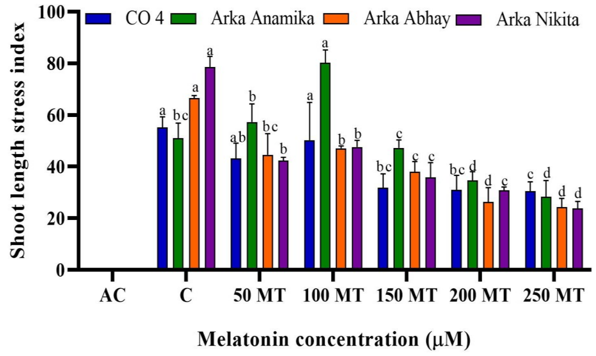

Fig. 10. Effect of melatonin on shoot length stress index of okra seedlings under drought stress.

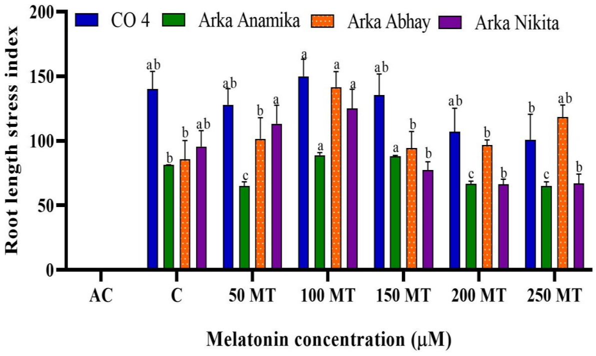

Fig. 11. Effect of melatonin on root length stress index of okra seedlings under drought stress.

Discussion

Drought stress significantly impedes plant growth by affecting seed germination and metabolism (29). The immediate plant response involves inhibiting germination rates. Impairment of seed germination under drought stress is primarily caused by reduced water uptake and decreased energy supply. This triggers metabolic changes, including ROS accumulation, modulation of antioxidant response mechanism, hormonal signaling pathway and production of osmolytes (30-32). In the present Petri plates experiment, PEG-induced drought stress significantly reduced the germination rate in okra seedlings compared to absolute control. Numerous studies have established that drought stress significantly inhibits seed germination across a range of plant species (33, 34).

Melatonin, recognized for its capacity to scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS) detrimental to plant systems, exhibits notable efficacy in ameliorating abiotic stresses, including salinity, drought and temperature extremes (35). It plays a crucial role in enhancing physiological processes during adverse conditions, contributing to improved seed germination and delayed senescence in plants. In the present study, 100 µM melatonin (MT) was identified as the optimal concentration for priming okra seeds under drought stress. In a study (36), melatonin was found to significantly enhance seed germination parameters and promote radicle growth in soybeans subjected to PEG-induced water stress. These effects were attributed to enhanced antioxidant enzyme activity, sustained cellular osmotic potential and reduced lipid membrane peroxidation.

Triticale treated with 20 µM melatonin showed improved seed germination, as evidenced by enhanced germination potential, rate and indexes, along with increased embryonic root (37). These findings align with prior research suggesting that melatonin (MT) priming promotes drought-impacted seed sprouting (38). The alleviating influence of melatonin on seed germination under stress conditions is concentration-dependent. For instance, it was determined that 50 µM was the ideal dosage for melatonin priming in waxy corn under cold stress (39). In rapeseed, it was concluded that 500 µM was the optimal concentration of melatonin priming under drought stress (40). Thus, the exact melatonin concentration for seed conditioning varies by species and environmental conditions (41).

Melatonin pre-treatment (100 µM) enhanced the vigour index of okra seedlings subjected to drought. The findings are in accordance with that confirmed in the case of cotton wherein lower doses of melatonin application resulted in improved vigour index (42). This can be substantiated by better germination and seedling growth, as the vigour index is an indicator of further growth of seedlings.

Melatonin treatment alleviates light energy absorption inhibition caused by water stress, increases sucrose content through carbon assimilation and enhances growth and productivity (43). In the present study, PEG-induced drought stress reduced both shoot and root length. Drought stress causes the extracellular pH to change from acidic to basic, which inhibits the growth of both shoots and roots, driven by ABA accumulation, resulting in the inactivation of cell wall loosening enzymes (44). Okra seedlings supplemented with 100 µM melatonin exhibited higher shoot and root length. Pre-treating seeds with melatonin may enhance the synthesis of endogenous phytohormones (GA biosynthesis) thereby promoting shoot development and increasing root density (45). Melatonin induces auxin, impacting root growth and water absorption by facilitating irreversible cell wall extension (46).

PEG-induced drought stress hampers the growth indicators such as seedling's fresh and dry weight. The current study reveals that 100 µM treatment of melatonin exhibited a consistent increase in both fresh and dry weights of seedlings. Melatonin's preservation of chloroplast integrity and functionality, crucial for photosynthesis, enhances the plant's ability to sustain elevated photosynthetic rates, ultimately leading to increased biomass production (47).

The promptness index quantifies the efficiency and speed of seed germination, reflecting the rapidity and uniformity of germination under specific conditions. Simultaneously, the germination stress tolerance index assesses the relative germination performance of seeds under drought stress, offering a metric to measure the impact of drought stress on seed germination. Additionally, it provides insights into the sensitivity or tolerance of seeds to drought conditions concerning various melatonin treatments. Under PEG-induced drought, stress indices were observed to decrease. Comparable findings were documented in soybean (48). The current results align with one report, indicating that melatonin seed priming accelerates and enhances maize germination under stress (49). Variations in promptness and germination stress tolerance index among melatonin-treated groups suggest concentration-dependent efficacy, with 100 µM proving most effective.

Compared to other treatments, the SLSI and RLSI values following the 100 µM melatonin pre-treatment were significantly higher, indicating that the optimal melatonin concentration of 100 µM effectively enhances both SLSI and RLSI. The reduction in intercellular pH and subsequent cell wall loosening induced by melatonin contribute to lupin's cell wall elongation and expansion, positively influencing the shoot and root growth (38).

The mechanism behind melatonin-mediated drought tolerance in okra involves an integrated response, including improved antioxidant defense, enhanced osmoregulation and regulation of hormonal pathways. These processes collectively contribute towards enhanced seedling traits like higher germination rate, increased germination stress indices and enhanced seedling length and vigour (Fig. 12).

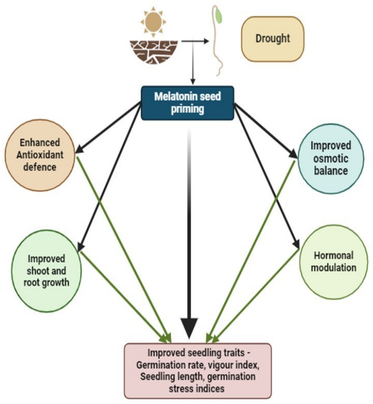

Fig. 12. Mechanism of melatonin-mediated drought tolerance in okra seedlings.

Conclusion

This study presents evidence of melatonin's substantial contribution in enhancing drought stress tolerance in plants. Priming okra seeds with 100 µM of melatonin enhanced seedling traits under drought stress. Melatonin pre-treatments reinforced drought defense in okra genotype Arka Anamika, resulting in enhanced germination rate, improved seedling development and enhanced stress tolerance indices. The transient water stress applied in our study aims to elucidate melatonin's regulation of plant water balance. Melatonin seed priming shows potential for enhancing seed emergence and seedling establishment of okra under drought stress. Further field evaluations are needed to explore the practicality of these findings. This can pave the way for exploring the signalling network of melatonin with other phytohormones.

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Authors' contributions

AG, VR, DV, LA, SR and RJ conceived the concept, designed the study and prepared the manuscript. AG carried out the experimental procedures and statistical analyses. All authors reviewed and endorsed the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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