A-6147
[1-8]

RESEARCH ARTICLE   Indian Journal of Agricultural Research, Volume Issue : ()

Evaluation of Melatonin Concentrations on Germination and Seedling Traits of Rice against Drought Stress

K. Arun Kumar[1], M.K. Kalarani[1], D. Vijayalakshmi[1], V. Geethalakshmi[2], M. Raveendran[3], V. Anandhi[4]

10.18805/IJARe.A-6147

ABSTRACT

Background: Rice is the world's third most important food crop, particularly in Asia. Drought stress has varying degrees of impact on its production. New plant growth regulators have been studied in recent years as a way to overcome physiological limitations and increase productivity. Melatonin has been investigated for its potential advantages in boosting plant resistance to environmental stresses. In this study, the precise processes and functions of melatonin with respect to germination and early seedling establishment during drought were investigated.

Methods: Rice seeds were subjected to different pretreatment of melatonin (0, 50, 100, 150, 200 and 250 µM) prior to experiencing drought stress characterized by a water potential of -0.4 MPa. Germination and physiological traits, including germination speed, shoot length, root length, promptness index, vigor index, stress index, fresh and dry weight were assessed.

Result: These findings demonstrated that pretreatment of melatonin effectively mitigated the detrimental impacts of drought stress by enhancing germination percentage, seedling growth and physiological traits. Among the various concentration, 200 µM of melatonin proved to be the ideal concentration that has the ability to improve rice seed germination, early seedling growth and minimize the negative consequences of drought stress.

Key words: Drought, Germination, Melatonin, Rice.

INTRODUCTION

Rice (*Oryza sativa* L.) a member of the Poaceae family, is the most extensively consumed cereal. It is found throughout Asia and Africa's tropical and subtropical regions. Water scarcity is expected to affect 15-20 million acres of rice-growing areas by 2025 (Nawaz *et al*., 2022). Rice is susceptible to moisture stress and exhibits distinct morphological changes when subjected to the drought stress at various development stages. Seed germination is a vital and intricate process that requires the protrusion of the radicle through the seed coat. This complicated process is governed and regulated by an array of physiological, metabolic and molecular actions (Yan *et al*., 2014). For early plant establishment, germination and seedling growth are crucial. Seed germination and the early phases of seedling development are most vulnerable to water stress (Swain *et al*., 2014). The establishment of young seedlings significantly impacted by uneven germination under water-stress circumstances. Thus, it is of utmost importance to understand the physiological and growth mechanisms behind drought resistance upon seed germination. The excessive formation of reactive oxygen species during drought stress results in oxidative damage and cell death which impedes the development of seedlings (Pandey and Sukla, 2015). Therefore, it is becoming more and more crucial to create diverse drought tolerance and adaptation techniques to dwindling the impact of water scarcity.

Melatonin, chemically referred to as N-acetyl-5-methoxytryptamine, is an indolic compound that is produced from serotonin. Various factors, including the plant's species, stage of growth and climatic circumstances might affect the amounts of melatonin in plants. Melatonin concentrations in plants tend to rise during the time of stress, indicating that it could be linked to the plant's reaction to unfavorable circumstances. Light, temperature and other stresses from the edaphic and climatic conditions can all have an impact on the melatonin levels in plants. Melatonin acts as an antioxidant by scavenging harmful reactive oxygen species (ROS), such as superoxide radicals, hydroxyl radicals and singlet oxygen. It also indirectly enhances the activities of

[1]Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.
[2]Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.
[3]Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.
[4]Department of Physical Sciences and Information Technology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.

Corresponding Author: M.K. Kalarani, Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India. Email: kalarani.mk@tnau.ac.in

How to cite this article: Kumar, K.A., Kalarani, M.K., Vijayalakshmi, D., Geethalakshmi, V., Raveendran, M. and Anandhi, V. (2023). Evaluation of Melatonin Concentrations on Germination and Seedling Traits of Rice against Drought Stress. Indian Journal of Agricultural Research. DOI: 10.18805/IJARe.A-6147.

Submitted: 02-08-2023 **Accepted:** 05-10-2023 **Online:** 12-10-2023

antioxidant enzymes like superoxide dismutase, catalase and peroxidases that help detoxify ROS (Sadak and Bakry, 2020). The germination of seeds in a variety of plant species may be aided by melatonin (Liu *et al*., 2019). The possible impact of exogenously administered melatonin on seed germination, seedling growth and overall plant development has been less examined.

To move forward with the concept of grasping mechanisms behind plant responses to water deficit situations with various melatonin concentrations, comparative evaluation of various melatonin concentrations on germination and seedling traits of rice var. CO 51 under PEG induced drought stress was carried out with the following objectives. i. To investigate the ideal melatonin concentration that has the ability to improve rice seed germination, early seedling growth and mitigate the negative effects of drought stress. ii. To evaluate the potential of melatonin in mitigating drought stress by assessing stress indices.

MATERIALS AND METHODS

Plant growth conditions and treatments

A laboratory experiment took place at the Department of Crop Physiology, Tamil Nadu Agricultural University, located in Coimbatore. The experiment utilized the CO 51 seed material, which is a prominent rice variety. The drought stress was induced using polyethylene glycol (PEG 6000). -0.4 MPa (PEG 6000) was chosen as the concentration to cause drought stress. To ensure cleanliness, healthy seeds underwent surface sterilization by treating them with a 0.1% mercuric chloride ($HgCl_2$) solution for a duration of 2-3 minutes. Following this, the seeds were carefully rinsed using distilled water to remove any remnants of the sterilizing agent. Melatonin concentrations ranging from 50 µM (MT 50), 100 µM (MT 100), 150 µM (MT 150), 200 µM (MT 200) and 250 µM (MT 250) were used to soak the seeds overnight. Two control groups were also maintained: one without PEG 6000 (Absolute control - AC) and another without melatonin treatment (Control - C). The Petri dishes were cleaned with distilled water after being sterilised with 70% ethanol. Blotting papers, moistened with PEG 6000 (-0.4 MPa), were placed in each petri dish and pre-soaked seeds (15 seeds) of each melatonin concentration were placed on these papers. The petri dishes were placed in the laboratory under ambient temperature conditions of 25-30°C. The seeds were let to germinate within the petri dishes and were routinely moistened with a PEG solution of -0.4 MPa at specified intervals (10 ml initially and afterwards according to the needs). The experiment was replicated four times to ensure reliable results.

Germination percentage

The daily count of germinated seeds (from 2^{nd} day of sowing) were recorded throughout a period of 14 days, with measurements taken at 24-hour intervals. A seed was considered germinated when both the root (radical) and shoot (plumule) reached a minimum length of 2 mm. The germination percentage was obtained by dividing the count of germinated seeds by the total number of seeds used for germination and then multiplying the quotient by 100.

Shoot and root growth indices

Seedlings from each replication were selected on the 14^{th} day after sowing to assess the growth of both the shoot and root. Shoot length was calculated by determining the distance from the collar region to the growth point, while root length was measured based on the length of the primary root and given in cm.

Vigor index (VI)

Abdul-Baki and Anderson (1973) formula was used to determine the vigor index of the seedlings. This formula computes the vigor index as the multiplication of the sum of shoot length and root length with the germination percentage.

Promptness index (PI)

Sapra *et al*. (1991) approach was used to determine the promptness index. The formula for calculating the PI involves summing the products of the percentage of germinated seeds at specific time intervals (2, 4, 6, 8, 10, 12 and 14 days after sowing) and corresponding weighting factors (1.4, 1.2, 1, 0.8, 0.6, 0.4, and 0.2, respectively).

$$PI= [(nd2\ (1.4) + (nd4\ (1.2) + (nd6\ (1) + (nd8\ (0.8) + (nd10\ (0.6) + (nd12\ (0.4) + (nd14\ (0.2)]$$

Germination stress tolerance index (GSI)

The germination stress index was determined using the method developed by Bouslama and Schapaugh (1984). The calculation of GSI involves dividing the promptness index (PI) obtained under drought stress conditions (PIS) by the promptness index obtained under normal conditions (PINS) and multiplying the result by 100.

Plant height stress index (PHSI) and root length stress index (RLSI)

Using the Ellis and Roberts (1981) formula, the plant height stress index (PHSI) and root length stress index (RLSI) were calculated on the 14^{th} day. The PHSI was determined by dividing the plant height of the stressed plant by the plant height of the control plant and then multiplying the result by 100. Similarly, the RLSI was calculated by dividing the root length of the stressed plant by the root length of the control plant and multiplying the outcome by 100.

Fresh and dry weight

Seedlings were selected at random from each replication and their weights were measured when they were freshly harvested. These seedlings were then placed in a hot air oven with a set temperature of 70°C for a duration of 48 hours. Subsequently, their weights were recorded again after drying and the results were reported in terms of milligrams per seedling.

Statistical analysis

The statistical analysis of the collected data on various germination traits was conducted in completely randomized design by following the guidelines proposed by Gomez and Gomez (1984). Analysis of variance (ANOVA) was employed to analyze the data, and the results were presented in terms of means and standard errors. To perform the statistical analysis, SPSS 13.0 software (Version 133, LEAD Technologies Inc.) was utilized. Furthermore, the germination percentage, shoot and root length, as well as fresh and dry weight data, were visualized by creating graphs using GraphPad Prism 8 software.

RESULTS AND DISCUSSION

Germination percentage (%)

Water is necessary for the activation of enzymes that break down stored reserves in the seed and allowing the embryo to emerge from the seed coat. Insufficient water availability can delay or prevent this process and leads to poor germination. When compared to absolute control, the current study's control group's rice CO 51 germination was considerably reduced by drought stress. PEG inhibits germination by creating osmotic stress, while melatonin treatment mitigates germination stress and promotes better germination under drought conditions (Li *et al*., 2021). It has been determined that melatonin is a potential agent for improving seed germination. Every concentration of melatonin considerably excelled over the control in terms of seed germination under drought stress (Fig 1a). Melatonin controls a number of physiological and biochemical processes in plants, acting as a signaling molecule. It control the amounts of reactive oxygen species (ROS) and antioxidant enzymes, which would lessen oxidative stress and encourage germination during drought stress (Liu *et al*., 2019). Within the treatments involving different

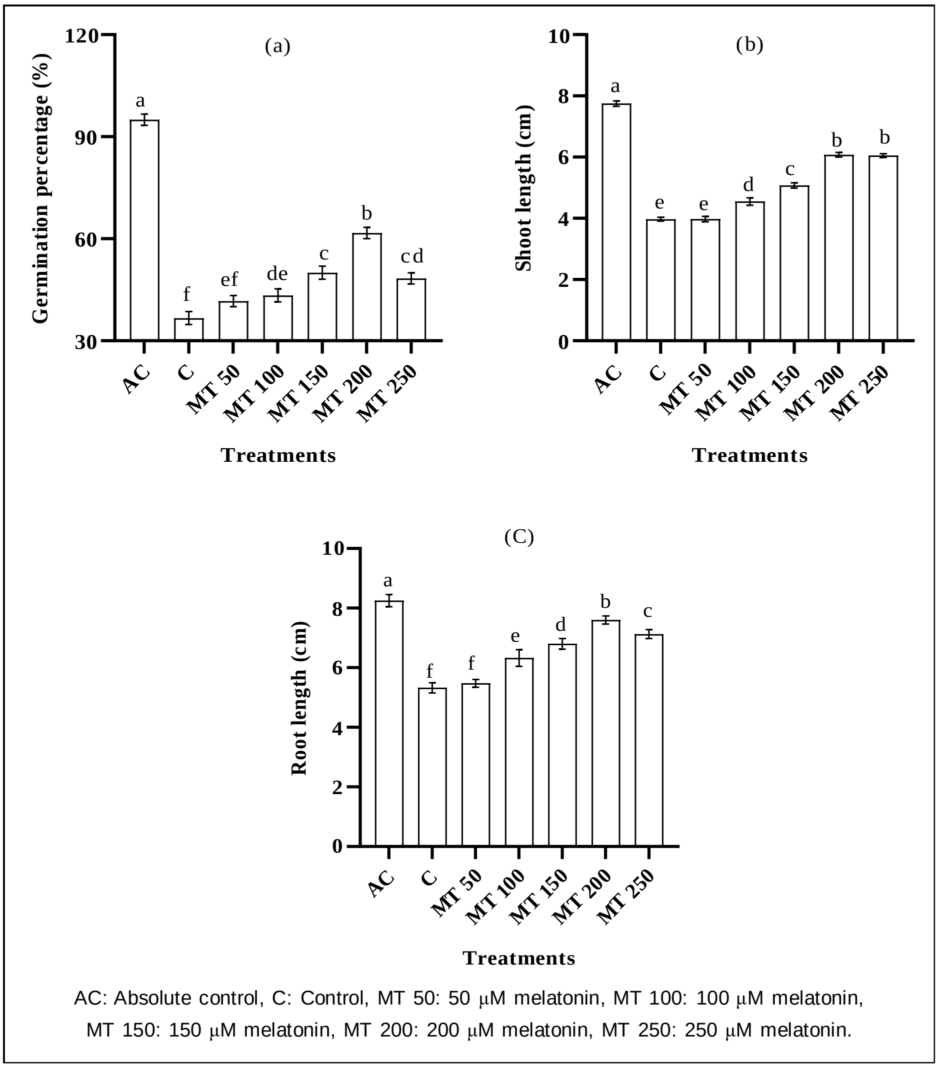


Fig 1: Comparative evaluation of melatonin concentrations on germination percentage (a) Shoot length; (b) Root length; (c) of seedlings under drought stress.

concentrations of melatonin, it was observed that seeds treated with 200 µM of melatonin exhibited the highest impact on germination percentage, reaching 61.67% (Plate 1). However, the germination percentage began to drop, reaching 48.33%, when the melatonin dosage was 250 µM. Melatonin has been shown to increase the activity of a number of germination-related enzymes, including -amylase, which induce starch hydrolysis during seed germination. It may help to mobilize stored reserves and counteract the negative effects of drought stress by boosting enzyme activity (Wang *et al*., 2022).

Shoot length

Drought stress shortened the shoot length of rice seedlings significantly. The shoot length varied substantially across the treatments and ranged from 3.98 to 7.75 cm (Fig 1b). There is evidence suggesting that melatonin can have positive effects on seedling growth and aboveground plant parts (Kołodziejczyk *et al*., 2021). In this study, it was an evident that the treatment of 200 µM had the most noticeable promoting effect in comparison to the control treatment (Plate 1). The shoot length increased by 52.83% under the influence of the 200 µM melatonin treatment followed by 52.20% in the 250 µM treatment. Melatonin enables plants to conserve water during drought, this regulatory feature can help to maintain the water status of plants and improve seedling height. Moreover, melatonin's physiological action corresponds to that of auxin, which stimulates seedling growth in drought-stressed conditions (Huang *et al*., 2019). Melatonin aids in maintaining cell membrane integrity by lowering oxidative stress, defending proteins and DNA from injuries, thereby encouraging seedling growth. Similar outcomes were seen by Sadak and Bakry (2020), applying melatonin as a foliar treatment has been found to have advantageous outcomes on foliage yield, plant height, the number of leaves per plant, as well as the fresh and dry weight of leaves particularly in drought-induced conditions.

Root length

In the current study, based on the pre-treated melatonin concentration, significant variation in the root length of the rice seedlings (Fig 1c) was recorded. Murch and Erland (2021) who reviewed the previous 20 years of melatonin research in plants, claim that this PGR induces the development of lateral and adventitious roots through its interaction with auxins. Melatonin interacts with other phytohormones like ABA or cytokinins that are important in root formation. Strong correlations were found between high melatonin levels during germination. Among various melatonin pretreatment levels, the highest root length of 7.60 cm was observed in the seedlings subjected to a melatonin concentration of 200 µM. These findings were in line with Zhang *et al*. (2019) who pretreated seeds with melatonin and reported that during PEG stress, soybean seed's radicle length, surface area, volume, dry weight and fresh weight all went up dramatically.

Vigor index

The impact of drought stress on crop vigor and productivity is typically detrimental, resulting in reduced growth. The pretreatment of melatonin exhibited a noticeable influence on both root and shoot length, which was evident in vigor index. Consequently, the melatonin-treated seeds with a concentration of 200 µM exhibited the highest vigor index, reaching 843.17. In contrast, the control plants had a relatively lower vigor index of 340.67 (Table 1). Hanci (2019) conducted an experiment where carrot seeds were subjected to soaking in melatonin solutions with a low concentration and found that treated seeds exhibited significantly higher vigor index when exposed to stress conditions. In this study, it was shown that melatonin pretreated seeds grew more quickly, had better germination capacities at the initial and final counts and had fewer aberrant seedlings than control. These findings showed that melatonin has an impact on seed germination and seedling vigor. Simlat *et al*. (2018)

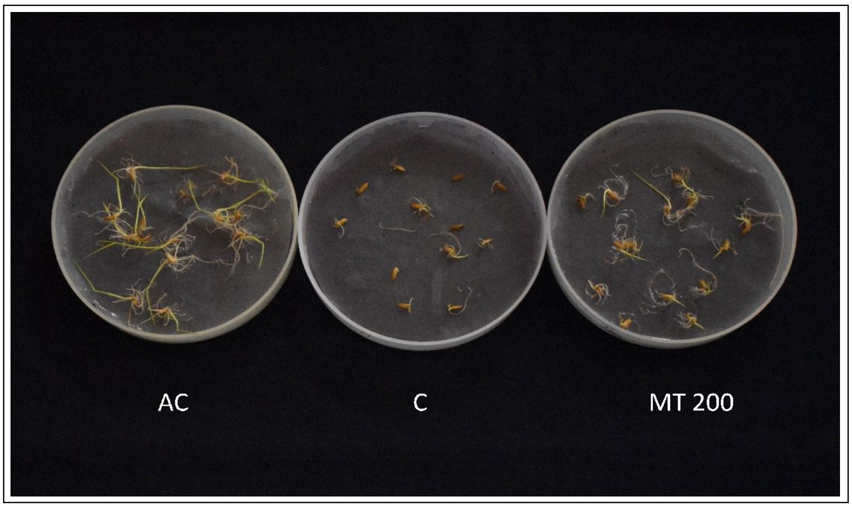


Plate 1: Impact of melatonin on germination under drought stress.

conducted a study that yielded similar results, supporting the notion that melatonin, specifically at low concentrations of 5 and 20 µM, had a significant positive impact on the seed vigor and properties of *Stevia rebaudiana* seedlings. Melatonin has been suggested to play a role in regulating relative water content, seedling height, photosynthesis and enhancing the efficiency of this process which probably improved seedling vigor. Melatonin administration in maize affected a number of morphological traits significantly and led to appreciable gains in comparison to the control group by altering elements such as plant height, root length, as well as fresh and dry weight (Muhammad *et al*., 2022).

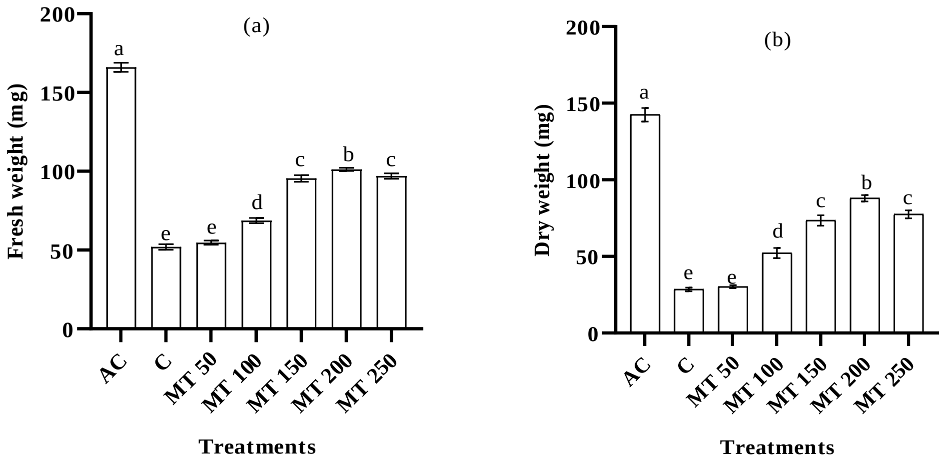
Promptness index and germination stress tolerance index

The promptness index is a metric for evaluating the effectiveness and speed of seed germination. It shows how rapidly and consistently seeds germinate under particular circumstances. The germination stress tolerance index provides a measure of the relative germination performance of seeds under drought stress conditions. It helps to quantify the impact of drought stress on seed germination and provides insights into the relative sensitivity or tolerance of seeds to drought conditions with respect to various melatonin treatments. Those seeds that were exposed to 200 µM melatonin showed a better promptness index of 81.33 and higher germination stress tolerance index (62.17) (Table 1). The current findings were found to be in agreement with Jiang *et al*. (2016), that priming seeds with melatonin enhances and speeds up seed germination in maize during stress. The difference in promptness and germination stress tolerance index between the melatonin-treated groups suggests that the effectiveness of melatonin on seed germination may vary with concentration, among the treatments, 200 µM being the most effective in this instance.

Drought stress affects seed germination by limiting the availability of water for imbibition and the hormones connected with seed germination. ABA, which is normally

Table 1: Evaluation of melatonin concentrations on stress indices.

Treatments	Vigor index	Promptness index	Germination stress index	Plant height stress index	Root length stress index
T1: Absolute control	1519.83^{a}	131.00^{a}	-	-	-
T2: Control	340.67^{e}	41.67^{e}	31.85^{d}	51.30^{d}	64.57^{e}
T3: 50 µM melatonin	393.17^{e}	47.00^{de}	35.98^{cd}	51.33^{d}	66.37^{e}
T4: 100 µM melatonin	471.67^{d}	51.00^{d}	38.90^{c}	58.76^{c}	76.68^{d}
T5: 150 µM melatonin	593.83^{c}	62.67^{c}	47.91^{b}	65.53^{b}	82.43^{c}
T6: 200 µM melatonin	843.17^{b}	81.33^{b}	62.17^{a}	78.42^{a}	92.16^{a}
T7: 250 µM melatonin	636.67^{c}	61.67^{c}	47.16^{b}	78.11^{a}	86.41^{b}
Mean	685.57	68.05	51.99	69.06	81.23
SEd	29.808	2.979	2.415	1.973	1.595
CD (P=0.05)	61.990	6.196	5.023	4.104	3.317



AC: Absolute control, C: Control, MT 50: 50 µM melatonin, MT 100: 100 µM melatonin, MT 150: 150 µM melatonin, MT 200: 200 µM melatonin, MT 250: 250 µM melatonin.

Fig 2: Comparative evaluation of melatonin concentrations on fresh weight (a) and dry weight (b) of seedlings under drought stress.

present in greater amounts during dry circumstances and inhibits seed germination. Melatonin improves the percentage and speed of germination during PEG-induced drought stress. These alterations may be due to the overexpression of genes involved in the breakdown of ABA and genes involved in the synthesis of GA, which led to a sharp decline in ABA and rising levels of GA, respectively (Zhang *et al*., 2014). The similar impact of melatonin under various abiotic conditions has been studied in both maize (Jiang *et al*., 2016) and soybean (Wei *et al*., 2015).

Plant height and root length stress index

The plant height stress index (PHSI) and root length stress index (RLSI) is a measurements used to assess the impact of stress on the height of plants. A higher PHSI and RLSI value indicates less impact on plant height due to stress, while a lower value suggests a greater reduction in shoot and root length. Melatonin treatment has been found to mitigate the negative effects of drought on plant height and root length, resulting in a higher stress index compared to untreated plants. During drought stress, the application of melatonin has been shown to positively influence the plant height and root length stress index by acting as a signaling molecule, antioxidant and regulating various physiological processes (Megala *et al*., 2022). The outcomes of our study showed that the treatments differed significantly from one another (Table 1). In comparison to other treatments, the 200 µM melatonin pre-treatment's PHSI (78.42) and RLSI (92.16) values were considerably greater. In case of (PHSI) the values of 200 µM were on par with 250 µM, however maximum influence was recorded in 200 µM. This implies that the ideal melatonin concentration of 200 µM can efficiently improve both the PHSI and RLSI. The fall in intercellular pH and cell wall loosening caused by melatonin is responsible for the lupin's cell wall elongation and expansion, which creates favorable impacts in terms of shoot and root growth (Arnao and Hernandez-Ruiz, 2007). The current results were shown to be compatible with the above findings, by reducing the negative impact of drought on plant height, melatonin treatment can enhance the plant height and root length stress index under stress conditions.

Fresh and dry weight of rice seedling

In this study, the fresh and dry weight of the seedlings were measured and the data is depicted in Fig 2a and b. The findings indicated a significant ($p \leq 0.05$) distinction between the control group and the melatonin-treated groups. Melatonin regulates seed germination and energy metabolism. The application of melatonin gradually diminishes the hampering effect of PEG-induced drought stress on germination and this effect was reflected in the fresh and dry weight. The seedlings in the control group had the lowest fresh weight (52 mg) and dry weight (29 mg) of all the treatments. In parallel with these findings, Li *et al*. (2021) found that melatonin pretreatment increased the shoot dry weight and leaf area of maize seedlings subjected to drought stress. The seedling's fresh and dry weight steadily rose up in 200 µM treatment and showed the most influential fresh weight (101 mg) and dry weight (88 mg). Melatonin can maintain the integrity and functionality of chloroplasts, which are responsible for photosynthesis. This preservation of chloroplast function can contribute to the plants' ability to maintain a high photosynthetic capacity, leading to increased biomass production (Muhammad *et al*., 2022).

In the current study, melatonin-treated plants retained larger biomass levels, which may indicate that melatonin improves root length and its capacity to absorb more water thereby reduced the growth-inhibiting effects of drought. By modulating the relative water content, activity of enzymes, melatonin may enhance photosynthetic efficiency and positively influence various aspects of photosynthesis and carbon assimilation which ultimately contribute to increased biomass production (Qiao *et al*., 2020). Pearson correlation analysis also confirmed that (Table 2) that melatonin treatments positively influenced the germination traits of

Table 2: Correlation coefficient (Pearson) between melatonin and variables associated with germination.

	MTC	GP	SL	RL	VI	PI	GSI	PHSI	RLSI	FW	DW
MTC	1										
GP	0.718**	1									
SL	0.950**	0.772**	1								
RL	0.916**	0.831**	0.931**	1							
VI	0.838**	0.965**	0.901**	0.933**	1						
PI	0.778**	0.961**	0.842**	0.888**	0.973**	1					
GSI	0.767**	0.952**	0.824**	0.891**	0.966**	.990**	1				
PHSI	0.939**	0.756**	0.994**	0.918**	0.887**	.829**	.804**	1			
RLSI	0.912**	0.818**	0.916**	0.986**	0.919**	.875**	.879**	.890**	1		
FW	0.931**	0.820**	0.930**	0.951**	0.907**	.878**	.867**	.917**	.947**	1	
DW	0.926**	0.851**	0.947**	0.975**	0.938**	0.900**	0.887**	0.937**	0.965**	0.986**	1

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed); MTC (Melatonin concentration), GP: Germination percentage, SL: Shoot length, RL: Vigor index, PI: Promptness index, GSI: Germination stress index, PHSI: Plant height stress index, RLSI: Root length stress index, FW: Fresh weight, DW: Dry weight.

seedlings (GP; r^2 = 0.718), (SL; r^2 = 0.950), (RL; r^2 = 0.916), (VI; r^2 = 0.838) (PI; r^2 = 0.778), (GSI; r^2 = 0.767), (PHSI; r^2 = 0.939), (RLSI; r^2 = 0.912), (FW; r^2 = 0.931) and (DW; r^2 = 0.926).

CONCLUSION

This study provided evidence of the significant role played by melatonin in enhancing drought stress tolerance in plants. This study shows that the rice CO 51 cultivar's defenses against drought stress were strengthened by melatonin pretreatments, which boosted germination rate, seedling development and various stress tolerance indices under drought. Since the water stress in our current study is only temporary to determine how melatonin controls plant water balance. More research should focus on how melatonin's signaling pathways affect plant's hydraulic conductance during germination.

ACKNOWLEDGEMENT

We are most grateful to the Ministry of Science and Technology, Government of India for offering DST-INSPIRE Fellowship and financial support to complete this research work. We would like to thank all our colleagues and students who have helped this research.

Conflict of interest

The authors declare that they have no competing interests.

REFERENCES

Abdul-Baki, A.A. and Anderson, J.D. (1973). Vigor determination in soybean seed by multiple criteria. Crop Science. 13(6): 630-633.

Arnao, M.B. and Hernández-Ruiz, J. (2007). Melatonin promotes adventitious- and lateral root regeneration in etiolated hypocotyls of *Lupinus albus* L. Journal of Pineal Research. 42(2): 147-152.

Bouslama, M. and Schapaugh, W. (1984). Stress tolerance in soybeans. I. Evaluation of three screening techniques for heat and drought tolerance. Crop Science. 24(5): 933-937.

Ellis, R. and Roberts, E. (1981). The quantification of ageing and survival in orthodox seeds. Seed Science and Technology (Netherlands). 9(2): 373-409.

Gomez, K.A. and Gomez, A.A. (1984). Statistical Procedures for Agricultural Research, John Wiley and Sons.

Hanci, F. (2019). The effect of L-tryptophan and melatonin on seed germination of some cool season vegetable species under salinity stress. Düzce Üniversitesi Bilim ve Teknoloji Dergisi. 7(3): 1879-1891.

Huang, B., Chen, Y.E., Zhao, Y.Q., Ding, C.B., Liao, J.Q., Hu, C., Zhou, L.J., Zhang, Z.W., Yuan, S. and Yuan, M. (2019). Exogenous melatonin alleviates oxidative damages and protects photosystem ii in maize seedlings under drought stress. Front Plant Sci. 10: 1-16.

Jiang, X., Li, H. and 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.

Kołodziejczyk, I., Kaźmierczak, A. and Posmyk, M.M. (2021). Melatonin application modifies antioxidant defense and induces endoreplication in maize seeds exposed to chilling stress. International Journal of Molecular Sciences. 22(16): 8628.

Li, Z., Su, X., Chen, Y., Fan, X., He, L., Guo, J. and Yang, Q. (2021). Melatonin improves drought resistance in maize seedlings by enhancing the antioxidant system and regulating abscisic acid metabolism to maintain stomatal opening under PEG-induced drought. Journal of Plant Biology. 64: 299-312.

Liu, J., Hasanuzzaman, M., Wen, H., Zhang, J., Peng, T., Sun, H. and Zhao, Q. (2019). High temperature and drought stress cause abscisic acid and reactive oxygen species accumulation and suppress seed germination growth in rice. Protoplasma. 256: 1217-1227.

Megala, R., Kalarani, M.K., Jeyakumar, P., Senthil, N., Pushpam, R. and Umapathi, M. (2022). Standardization of optimum melatonin concentration for drought tolerance at germination and early development stage in rice (CO-54). Journal of Applied and Natural Science. 14(3): 1022-1030.

Muhammad, I., Yang, L., Ahmad, S., Mosaad, I.S., Al-Ghamdi, A.A., Abbasi, A.M. and Zhou, X.B., (2022). Melatonin application alleviates stress-induced photosynthetic inhibition and oxidative damage by regulating antioxidant defense system of maize: A meta-analysis. Antioxidants. 11(3): 512.

Murch, S.J. and Erland, L.A. (2021). A systematic review of melatonin in plants: An example of evolution of literature. Frontiers in Plant Science. 12: 683047.

Nawaz, A., Rehman, A.U., Rehman, A., Ahmad, S., Siddique, K.H. and Farooq, M. (2022). Increasing sustainability for rice production systems. Journal of Cereal Science. 103: 103400.

Pandey, V. and Shukla, A. (2015). Acclimation and tolerance strategies of rice under drought stress. Rice science. 22(4): 147-161.

Qiao, Y., Ren, J., Yin, L., Liu, Y., Deng, X., Liu, P. and Wang, S. (2020). Exogenous melatonin alleviates PEG-induced short-term water deficiency in maize by increasing hydraulic conductance. BMC Plant Biology. 20: 1-14.

Sadak, M.S. and Bakry, B.A. (2020). Alleviation of drought stress by melatonin foliar treatment on two flax varieties under sandy soil. Physiology and Molecular Biology of Plants. 26: 907-919.

Sapra, V.E., Savage, A.A. and Beyl, C. (1991). Varietal differences of wheat and triticale to water stress. Journal of Agronomy and Crop Science. 167(1): 23-28.

Simlat, M., Ptak, A., Skrzypek, E., Warcho³, M., Morañska, E. and Piórkowska, E. (2018). Melatonin significantly influences seed germination and seedling growth of *Stevia rebaudiana* Bertoni. Peer Journal. 6: e5009.

Swain, P., Anumalla, M., Prusty, S., Marndi, B.C. and Rao, G.J.N. (2014). Characterization of some Indian native land race rice accessions for drought tolerance at seedling stage. Australian Journal of Crop Science. 8(3).

Wang, J., Lv, P., Yan, D., Zhang, Z., Xu, X., Wang, T. and Li, R. (2022). Exogenous melatonin improves seed germination of wheat (*Triticum aestivum* L.) under salt stress. International Journal of Molecular Sciences. 23(15): 8436.

Wei, W., Li, Q.T., Chu, Y.N., Reiter, R.J., Yu, X.M., Zhu, D.H., Zhang, W.K., Ma, B., Lin, Q., Zhang, J.S. and Chen, S.Y. (2015). Melatonin enhances plant growth and abiotic stress tolerance in soybean plants. Journal of Experimental Botany. 66(3): 695-707.

Yan, D., Duermeyer, L., Leoveanu, C. and Nambara, E. (2014). The functions of the endosperm during seed germination. Plant and Cell Physiology. 55(9): 1521-1533.

Zhang, H.J., Zhang, N.A., Yang, R.C., Wang, L., Sun, Q.Q., Li, D.B. and 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.

Zhang, M., He, S., Zhan, Y., Qin, B., Jin, X., Wang, M. and Wu, Y. (2019). Exogenous melatonin reduces the inhibitory effect of osmotic stress on photosynthesis in soybean. PloS one. 14(12): e0226542.