Physiological insights of Melatonin Hormone for alleviation of drought stress in rice

Abstract

Drought stress significantly hampers crop growth and yield by negatively impacting various biochemical and physiological plant processes. This studyaimed to assessdrought tolerance in traditional rice varieties using Poly Ethylene Glycol 6000 (PEG 6000)and focused on the effectiveness of melatonin applied through both seed treatment and foliar spray in mitigating the effects of drought stress. The optimal drought screening conditions were determined at a PEG concentration of -4 bars. As the PEG concentration increased, key factors such asgermination percentage, vigor index, root length, and shoot length decreased. This indicates that PEG can be a useful tool for early selection of drought tolerant rice varieties. Melatonin (*N*-acetyl-5-methoxytryptamine), known foralleviating abiotic stress, showed positive effects on seed germination at 200 ppm concentration. The study observed that stomatal closure is a natural response to drought stress, but melatonin application induced partial stomatal opening. Notably, a foliar spray of 100 ppm melatonin demonstrated better recovery from drought stress compared to the 200ppmconcentration. In conclusion, the research suggests that seed treatment with 200 ppm melatonin and foliar spraying with 100 ppm melatonin are the most effective approachesfor reducing the adverse effects of drought stress in rice plants.

Keywords: Drought stress; Foliar spray; Melatonin; Rice; Seed treatment;

Introduction

Drought is characterized by a water deficiency severe enough to hinder plant growth, significantly reducing rice yield as an abiotic factor. Plants exhibit consistent water loss through transpiration and limited water uptake due to decreased soil moisture under drought stress (Koffler et al., (7).To counter water deficit, plants employ various mechanisms at morphological, physiological, biochemical, cellular and molecular levels (Fang et al., (6). Drought has become a major limiting factor threatening rice, one of the world's most important food crops in its critical seed germination phase (Liu et al., (10). In response to drought stress, stomatal closure is the primary reaction in most plants, preventing water loss through transpirational channels.Chemical cues such as abscisic acid synthesis in drying roots, regulate stomatal closure, closely tied to soil moisture content rather that leaf water status (Pirastech-Anosheh et al., (14).Drought induced excessive reactive oxygen species (ROS) can damage chloroplast, impeding photochemical reactions, ultimately diminishing photosynthesis and agricultural productivity (Li et al., (9).

Recently, plant growth regulators have been widely used to regulate plant growth and improve plant stress tolerance. Melatonin (*N*-acetyl-5-methoxytryptamine) is an indole hormone widely present in plants and animals. It was identified and quantified for the first time in plant (Dubbels et al., (4). Melatonin has demonstrated its ability to activate signalling processes during plant responses to various stress conditions (Ahammed et al., (1). Studies have shown that melatonin enhances plant resilience to stress, inducing tolerance under abiotic stress conditions like heavy metals, high temperature, salinity, and leaf senescence (Zhange et al., (22); Zhang et al., (21). Melatonin promotes adaptive responses to stress, influencing stomatal conductance, photosynthetic rate, transpiration rate, mineral uptake, exudation of organic acid anions,phenolic compounds, hormonal regulation, sugar metabolism, and ROS scavenging. Additionally, it regulates antioxidant enzyme activity, reducing oxidative damage to lipids, proteins and nucleic acids (Ahammed et al. (1). Melatonin also has crosstalk with other plant growth regulators such as gibberellin, jasmonic acid and abscisic acid to regulate various physiological processes in plants under drought stress. In addition, melatonin regulated the transcription of various essential genes involved in antioxidative defense mechanism (Sharma et al., (16)

Physiological role of melatonin under drought stress has been reported in many plants' species. But its mechanism and effective concentration is remained unclear. Therefore, the present experiment aimed to identify the ideal PEG concentration for screening drought-tolerant traditional rice varieties under laboratory conditions and to investigate the effectiveness of melatonin in mitigating drought stress through seed treatment and foliar applications.

Materials and Methods

The experiment was conducted in the laboratory, Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore during 2022. Totally five traditional varieties namely Thooyamalli, Maranel, Thanga samba, Navara and KarungKuruvai taken for germination study using PEG 6000. Thepolyethylene glycol (PEG 6000) solutions with different water potential of 0, -1, -2, -3, -4 and -5 bars were prepared by dissolving 91.8,135,168.5,196.9 and 221.6 g of PEG in 1000 ml of distilled water. Water potential (ψ) was calculated by an equation that relates PEG concentration to water potential according to Michel (1983). $\Psi = 1.29\ [PEG]\ [PEG]^2T - 140\ [PEG]^{2\ -\ 4}\ [PEG]$. Where Ψ is the water potential of each treatment (bars), [PEG] is the concentration of PEG solution [g PEG (g $H_2O)^{-1}$] and T is the temperature (ºC).

Sterilized petri plates were taken and blotter papers were cut to the required size and were placed in the Petri plates.25 rice seeds were taken per concentration per variety. The rice seeds were surfacesterilized using 0.01 percent $HgCl_2$ and 70 percent ethanol and finally washed with distilled water to avoid fungal infection.Then the seeds were arranged in the Petri plates (14+10+1). Then 10 mL of Polyethylene glycol of different concentrations were poured to the respective plates. Five replications were maintained per treatment.A control was also maintained with adding distilled water. Periodic checking and observation of the plates were done to monitor any fungal infections in the seeds as well drying out of PEG.After 14 days, the number of seeds germinated, root length and shoot length of the seedlings were recorded. The experiment was performed using a completely randomized block design with five replications and statistical analysis was done as suggested by Gomez and Gomez (12) using SPSS 16.0 package.

Standardization of optimum concentration of melatonin for seed treatment

Seeds were surface sterilized using 0.01 percent $HgCl_2$ and 70 percent ethanol and finally washed with distilled water and petri plates were cleaned thoroughly with a cotton swab. The seeds were then subjected in germination test in petri dishes containing moistened blotting paper with -4 bars of PEG to induce drought stress and seed treatment with melatonin 100 ppm and 200 ppm along with an absolute control (no melatonin seed treatment and -4 bar of PEG to induce drought stress). Five replications were maintained for each treatment. At the end of 14th day, total germination percentage, root length and shoot length were measured as suggested by Megala et al. (10).

Standardization of optimum concentration of melatonin for foliar spray to withstand drought stress in pot culture

Drought stress was induced in the pot culture plants by withholding water for 10 days at panicle initiation stage. Then foliar application of melatonin was given at the concentration of 100 and 200 ppm respectively and five replications were maintained. The stomatal characters and gas exchange parameters were recorded before initiating drought, after drought stress and after melatonin spray using portable photosynthesis system (LICOR 6400).

Results and Discussion

The study primarily focuseson investigating the effect of melatonin in drought mitigation through both seed treatment and foliar application.Initially, the screening of traditional rice varieties for drought tolerance under laboratory conditions using polyethylene glycol (PEG 6000) was conducted. This was followed by the standardization of the optimum melatonin concentration to alleviate PEG-induced drought stress in rice varieties. Finally, the study attempted to assess theeffect of foliar application of melatonin for alleviating drought stress under pot culture. The study data, including tables and results are provided detailed under relevant headings.

Drought screening using PEG under Laboratory Conditions

Polyethylene glycol (PEG), a non-permeable osmolyte, is utilized to induce water and osmotic stress, decreasing cell water potential and serving as a tool for plant selection against drought stress. PEG-6000 is usually used to induce water deficit due to its capacity of lowering water potential in medium. It helps to test the seeds with in laboratory instead of adjusting soil water potential (Prabhakar *et al*., (15).

Germination Percentage

The germination percentage of the screened traditional rice varieties was calculated (Table 1). It progressively decreased with the rising concentration of PEG, indicating increasing water stress. The highest germination percentage occurred in the control with no PEG (92 %). All varieties exhibited germination upto -3 bars. The lowest germination percentages were recorded at -5 bars (22%) and -4 bars (51 %). However, concentrations exceeding -4 bars proved lethal to seed germination. Therefore, the optimal PEG concentration for drought screening was determined to be -3 bars (Fig 1a). PEG concentrations above -4 bars were detrimental to both germination and the subsequent growth of seedlings, resulting in negative correlations with germination and rice emergence rates (Purbajanti et al., (16). The impact of PEG on rice germination serves as a valuable tool for the early identification of drought tolerant rice varieties.

Shoot and Root length

Drought stress inhibits rice seedling growth due to lower turgor pressure, reduction in photosynthesis and cellular damage caused by ROS. The seedlings shoot length was observed (Fig 1b). As the PEG concentration increased, the shoot length of all varieties decreased. The maximum shoot length was observed in the control with no PEG (8.24 cm). Notably, no shoots were produced at -5 bar PEG concentration.Root length observations were recorded for the seedling and presented in Fig 1c. Similar to shoot length, root length decreased progressively with increasing PEG concentration. The higher root was observed in the control with no PEG (10.24 cm), while the minimum occurred at -5 bar PEG concentration (0.23 cm).the root architecture and its physiology are greatly affected under water stress, ultimately impairing nutrient and water uptake (Kuromoriet al., (8). The shoot length of rice seedlings was substantially reduced with respect to drought stress. The highest vigour index was observed in the control, whereas the lowest values were recorded at -5 bars followed by -4 bars (Fig 1D). drought stress leads to a reduction in the vigor and productivity of the crop.

Determination of optimum concentration of Melatonin to alleviate PEG 6000 induced drought stress

PEG induced drought stress was applied at a concentration of -4 bars. In T_1, the maximum germination percentage (92), shoot length (7.2cm), root length (10.78 cm) and vigour index were observed. In T_3, the next best values were recorded with a germination percentage of 82%, shoot length of 5.9 cm, root length of 10.11 cm, and vigour index of 1312.82. Melatonin is a plant growth regulator and plays an important role in regulating plant growth and development (Arnao and Hernandez-Ruiz, (2). It is believed that melatonin and IAA can have a co-regulatory impact on plant growth by working in a combined or similar fashion to promote root morphogenesis (Murch et al., (13). The least outcomes were in T_2 with a germination percentage of 70%, shoot length of 1.12 cm, root length of 4.96 cm, and vigour index of 435.6 (Table 1). Among the treatments, seed treatment with 200 ppm of melatonin exhibited superior outcomes in terms of seedling characters compared to the treatment with 100 ppm of melatonin in rice. Melatonin induced root growth leads to a more efficient acquisition of soil water and nutrients, therefore enhancing the growth in the aerial part of the plant (Arnao and Hernandez-Ruiz, (3).Hence, the optimal melatonin concentration for seed treatment to mitigate drought stress was identified as 200 ppm. This was consistent the findings of Megala et al. (11) in rice and Zhang et al., (21).

Foliar application of melatonin to mitigate drought stress

Stomata, responsible for essential physiological processes like photosynthesis, respiration, and transpiration are intricately regulated by complex signal transduction pathways and water balance. In the face of drought stress, plants adapt by controlling stomatal closure and decreasing transpiration to regulate cellular moisture content. In this study, the external application of melatonin played a crucial role in preserving high turgor pressure, effectively sustaining stomatal openness even under drought conditions. Stomatal frequency in the Thanga Samba variety remained constant under control conditions, post-drought stress and after melatonin spray at concentrations of 100 ppm and 200 ppm (Table 2). However, variations in stomatal opening and closing were evident across different treatments. Stomata were remained open in the control plants and their closure was observed under drought stress, signifying stomatal closure as an early response to drought.

Stomatal closure is a crucial mechanism for preventing water loss through transpiration routes and is primarily regulated by chemical signals, including the formation of abscisic acid (ABA) in dehydrating roots (Pirastech-Anosheh et al.,(14). The foliar spray of melatonin, a partial reopening of stomata was observed, indicating enhanced drought tolerance and a return to normal physiological conditions.The recovery from drought stress, as reflected in photosynthetic and transpiration rates, was more pronounced under the foliar application of 100 ppm melatonin compared to 200 ppm. Therefore, the optimal concentration for foliar application of melatonin to mitigate drought in the rice was determined to be 100 ppm. This finding aligns with the suggestion by Silalert et al. (20) that a foliar application of 100 µM melatonin is highly effective in alleviating the adverse effects of drought stress in rice plants. Drought stress directly affects the structure and activity of photosynthetic organs in plant leaves.

Stomatal frequency varying not only among species but also among different rice verities was examined. Varieties displaying higher photosynthetic rate and moderate transpiration rates were identified as suitable candidates for drought tolerance.In this study observed stomatal closure as a natural response to drought stress and recorded partial stomatal opening with melatonin application. Recent research results have suggested that application of melatonin can improve plat photosynthetic rates through which effectively inhibitthe chlorophyll degradation and increase the chlorophyll content by enhancing RuBisco activity and promote better photosynthesis (Shi et al., (19). Melatonin treatment may help to prevent chlorophyll degradation by down regulating the gene encoding the chlorophyllase enzyme (Sharma et al., (18).Notably foliar spray of melatonin at 100 ppm demonstrated better recovery from drought stress compared to the 200-ppm centration. These findings contribute valuable insights into the selection and mitigation strategies for drought tolerance.

Figure 1. Influence of PEG on Germination parameters in traditional rice varieties

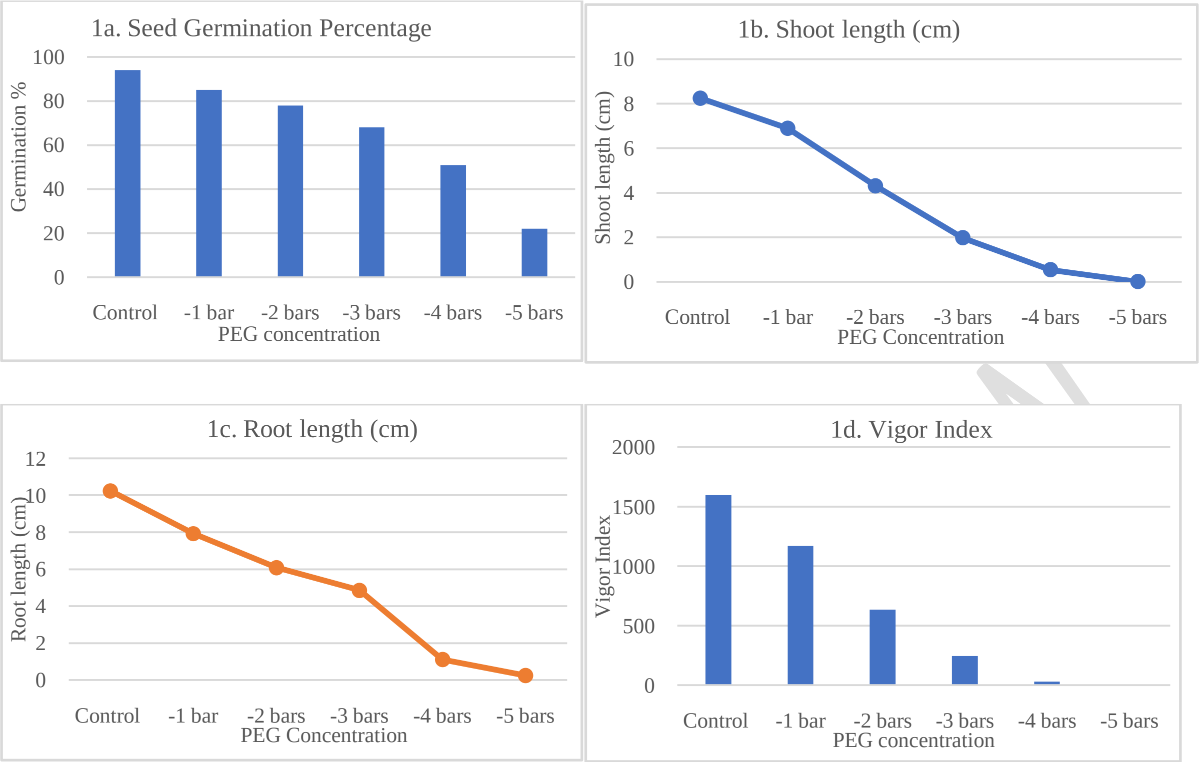


Table 1. Standardization of optimum concentration of melatonin to withstand PEG-6000 induced drought stress in rice

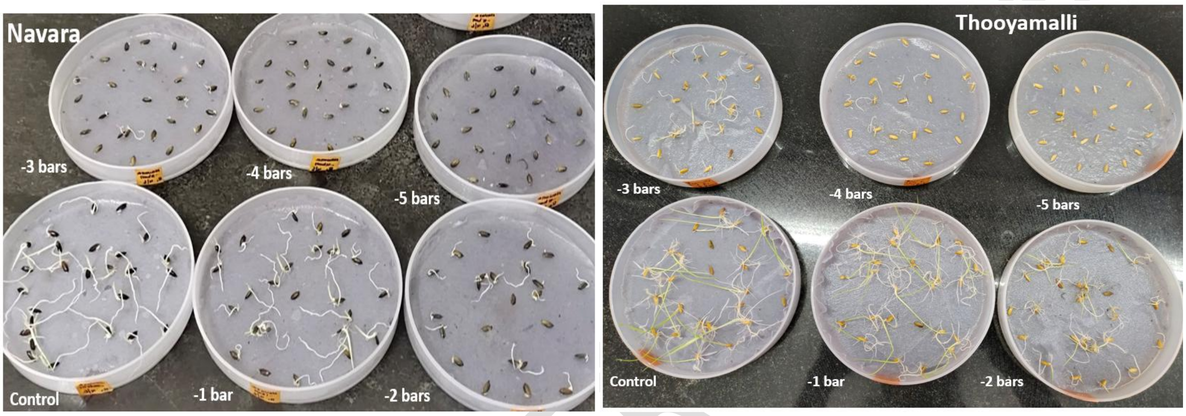
Treatments	Germination Percentage	Shoot length (cm)	Root length (cm)	Vigor Index
T_1: Control	86.8	7.20	10.78	1546.28
T_2: Drought stress (PEG@-4 bars)	70.2	1.12	4.96	435.62
T_3: Seed treatment with 100 ppm melatonin + PEG @-4 bars	79.6	4.50	8.74	1045.96
T_4:Seed treatment with 200 ppm melatonin + PEG @-4 bars	82.4	5.90	10.11	1312.82
Mean	**79.75**	**4.68**	**8.65**	**1085.17**
CD (0.05)	**4.765****	**2.016****	**2.004****	**369.07****

Table 2. Foliar spray of melatonin on gas exchange parameters in rice mitigating drought stress

Treatment	**Stomatal**	**Photo synthetic**	**Stomatal conductance**	**Sub stomatal CO_2concentra**	**Transpiration rate(mmol**	**Water use efficiency**

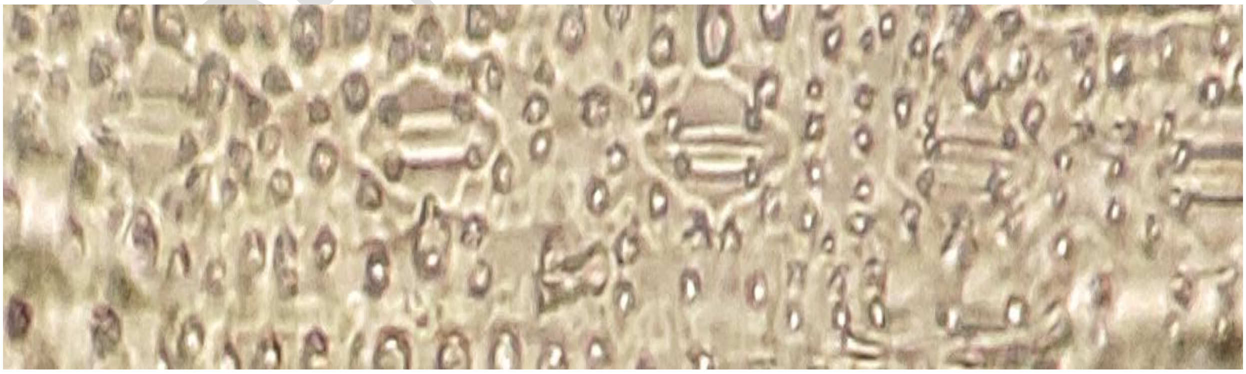
	frequency	**rate(µmol CO_2 m^{-2} S^{-1})**	**(mol H_2O m^{-2} S^{-1})**	**tion(µL L^{-1})**	**H_2O m^{-2} s^{-1})**	
T_1: Control	79	29.82	0.46	240.6	7.36	4.05
T_2: Drought (withheld of water for 10 days)	78	18.65	0.31	332.1	11.23	1.66
T_3: Drought + Foliar spray of 100 ppm melatonin	79	26.34	0.42	251.8	8.14	3.24
T_4: Drought + Foliar spray of 200 ppm melatonin	78	24.68	0.41	258.7	8.56	2.88
Mean	**79**	**24.87**	**0.40**	**270.8**	**8.82**	**2.96**
CD (0.05)	**0.251**	**3.40****	**0.049****	**32.19****	**1.294****	**1.440****

Picture 1. Seed germination with different concentration of PEG

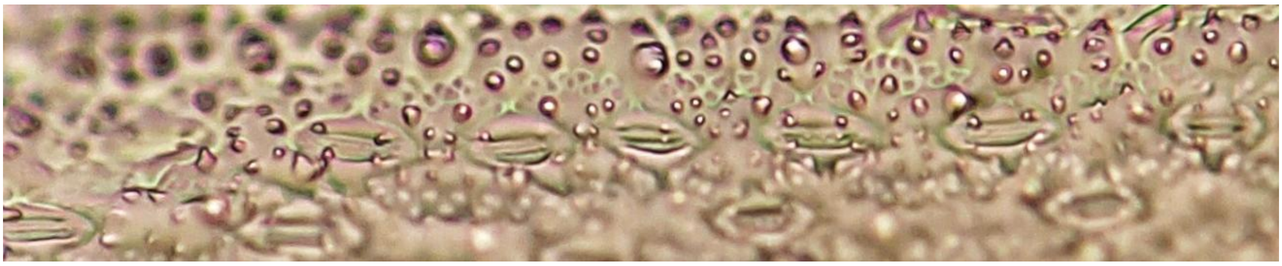


Picture 2. Stomatal characteristics of rice variety, Thanga samba

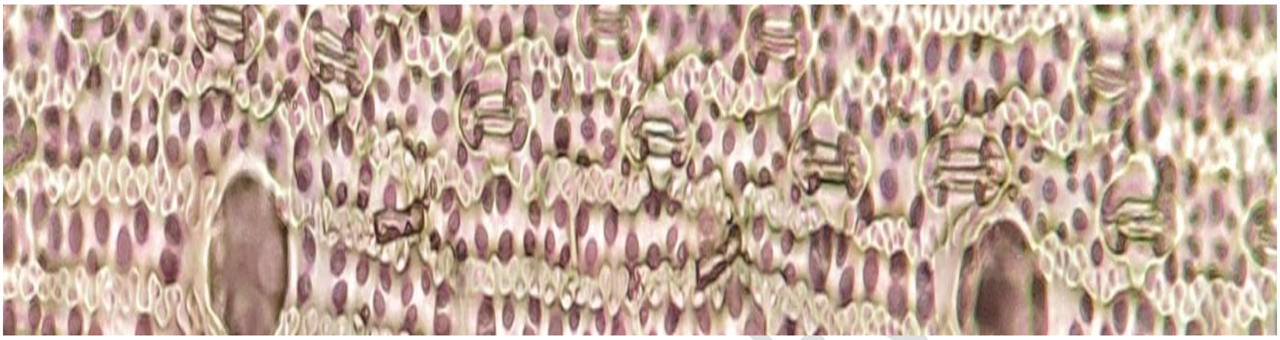
2a. Before drought: fully opened stomata



2b. After drought: closed stomata



2c. After melatonin application: partially opened stomata



Conclusion

To summarize, this study suggests that seed treatment with 200 ppm of melatonin improves seed germination in rice under PEG indued drought stress conditions. Further, foliar spray of 100 ppm melatonin could alleviate the adverse effects of drought stress in rice through partial opening of stomata, leading to continuous gas exchange for photosynthesis and transpiration. This process improves the water use efficiency of the rice plants. Hence, melatonin at 100 ppm was found to be the most effective concentration at alleviating the effects of drought stress in rice. However, further evaluation under field conditions is needed.

References

1. AhammedGJ, XuW,LiuA, ChenS. Endogenous melatonin deficiency aggravates high temperature-induced oxidative stress in *Solanum lycopersicum* L. *Environ. Exp. Bot.*, 2019;*161*: 303-311.
2. Arnao MB, Hernández-Ruiz J. Functions of melatonin in plants: a review. Journal of Pineal Research 2015; 59:133-150.
3. Arnao MB, Hernández-Ruiz J. Growth activity, rooting capacity, and tropism: three auxinic precepts fulfilled by melatonin. Acta Physiologiae Plantarum 2017; 39:127.

4. Dubbels R, Reiter RJ, Klenke E, Goebel A, Schnakenberg E, Ehlers C. Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography mass spectrometry. Journal of Pineal Research 1995; 18:28-31.
5. Gomez K A and Gomez AA. Statistical procedures for agricultural research. John Wiley & Sons.
6. Fang Y, Xiong L. General mechanisms of drought response and their application in drought resistance improvement in plants. Cell. Mol. Life Sci., 2015; 72(4): 673-689.
7. Koffler B E, Luschin-Ebengreuth N, Stabentheiner E, MüllerM, ZechmannB. Compartment specific response of antioxidants to drought stress in Arabidopsis. Plant Science, 2014; 227: 133-144.
8. KuromoriT, Seo M, Shinozaki K. ABA Transport and Plant Water Stress Responses. Trends Plant Sci, 2018; 23 (6), 513-22.
9. Li C, Tan D X, Liang D, ChangC, Jia D, Ma F. Melatonin mediates the regulation of ABA metabolism, free-radical scavenging, and stomatal behaviour in two Malus species under drought stress. J. Exp. Bot.2014; 66, 669–680.
10. Liu J, Wang W, Wang L, Sun Y. Exogenous melatonin improves seedling health index and drought tolerance in tomato. Plant Growth Reg., 2015; 77(3), 317-326.
11. MegalaR. et al. Standardization of optimum melatonin concentration for drought tolerance at germination and early development stage in rice (CO-54). Journal of Applied and Natural Science, 2022; 14(3), 1022 - 1030.
12. MitchellDJ, TiddyGJT, Waring L, BostockT, McDonaldMP. Phase-behavior of polyoxymethylene surfactants with water - mesophase structures and partial miscibility (cloud points). J. Chem. Soc. Faraday Trans. I, 79 1983, pp. 975-1000.
13. Murch SJ, Campbell SSB, Saxena PK. The role of serotonin and melatonin in plant morphogenesis: Regulation of auxin-induced root organogenesis in in vitro-cultured explants of St. John’s wort (*Hypericum perforatum* L.). In Vitro Cellular and Developmental Biology-Plant 2001; 37:786-793.
14. Pirasteh- Anosheh H, Saed- Moucheshi A, Pakniyat H, PessarakliM. Stomatal responses to drought stress. In: Parvaiz Ahmad (Ed). Water stress and crop plants: A sustainable approach, 2016; 1: 24-40.
15. Prabhakar S, HeshamMI,MarkusF,WilliamFS, ThorstenK.. Critical water potentials for germination of wheat cultivars in the dryland Northwest USA. Seed Sci. Res., 2013; 23: 189-198.

16. PurbajantiED, Kusmiyati F, FuskhahE, Rosyida R, AdinuraniP G,Vincēviča-GaileZ. Selection for drought-resistant rice (*Oryza sativa* L.) using polyethylene glycol. In: IOP Conference Series: Earth and Environ. Sci., 2019; 293(1): 012-014.
17. Sharma A, Wang J, Xu D, Tao S, Chong S, Yan D, Zheng B. Melatonin regulates the functional components of photosynthesis, antioxidant system, gene expression, and metabolic pathways to induce drought resistance in grafted Caryacathayensis plants. Science of the Total Environment. 2020; 713:136675.
18. SharmaA, ZhengB. Melatonin mediated regulation of drought stress: Physiological and molecular aspects. Plants, 2019; 8(7): 190.
19. Shi H, Reiter RJ, Tan DX, Chan Z. Indole3-Acetic acid Inducible 17 positively modulates natural leaf senescence through melatonin mediated pathway in Arabidopsis. Journal of Pineal Research. 2015;58: 26–33.
20. Silalert P,Pattanagul W. Foliar application of melatonin alleviates the effects of drought stress in rice (*Oryza sativa* L.) seedlings. Not. Bot. Horti. Agrobot. Cluj. Napoca., 2021; 49(3), 12417.
21. Zhang H J, Zhang N A, Yang R C, Wang L, Sun QQ, Li DB, Guo YD. Melatonin promotes seed germination under high salinity by regulating antioxidant systems, ABA and GA 4 interaction in cucumber (*Cucumis sativus* L.). *J. Pineal Res.*, 2014; *57*(3), 269-279.
22. ZhangN, Sun Q, Zhang H, Cao Y, Weeda S, Ren S, Guo Y D. Roles of melatonin in abiotic stress resistance in plants. *J. Exp. Bot.*, 2015; *66*(3), 647-656.