

Research Article

## Standardization of optimum melatonin concentration for drought tolerance at germination and early development stage in rice (CO-54)

### R. Megala

Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore - 641003 (Tamil Nadu), India

### M.K. Kalarani\*

Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore - 641003 (Tamil Nadu), India

### P. Jeyakumar

Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore - 641003 (Tamil Nadu), India

### N. Senthil

Center for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore – 641003 (Tamil Nadu), India

### R. Pushpam

Department of Rice, Tamil Nadu Agricultural University, Coimbatore - 641003 (Tamil Nadu), India

### M. Umapathi

Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore - 641003 (Tamil Nadu), India

\*Corresponding author. E mailkalarani.mk@tnau.ac.in

### Article Info

<https://doi.org/10.31018/jans.v14i3.3766>

Received: July 9, 2022

Revised: August 28, 2022

Accepted: August 2, 2022

### How to Cite

Megala, R. *et al.* (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. <https://doi.org/10.31018/jans.v14i3.3766>

### Abstract

Drought stress poses a serious threat to production and nutritional security. In recent years, foliar application of plant growth regulators (PGRs) and nutrients are increasingly employed to overcome physiological constraints resulting in enhanced crop production. Melatonin is a new biomolecule recently found to ameliorate the effect of biotic and abiotic stresses in crop plants. Hence, the present experiment was conducted to assess the optimum concentration of melatonin to mitigate the adverse effect of drought stress on germination and growth components in rice variety CO-54. In this experiment, PEG-mediated drought stress (-0.5 MPa) was imposed with different concentrations of melatonin (at doses of 50, 100, 150, 200, and 250  $\mu$ M) seed treatments. Together, these results indicated that 200  $\mu$ M melatonin-treated seeds showed a greater germination percentage (60%), root length (12.23cm), shoot length (8.23cm), fresh and dry weight (0.126g and 0.095g), high vigor index (1910.22), promptness index (64.83), and germination stress index (100) respectively. The result of this experiment provides a shred of strong evidence suggesting that seed treatment of 200  $\mu$ M melatonin could be considered an effective technique for mitigating the detrimental effects of drought by promoting seed germination and thereby increasing the growth components of seedlings in rice. The study demonstrates that melatonin can shield rice seedlings from the effects of drought stress.

**Keywords:** Drought tolerance, Germination, Melatonin, Polyethylene glycol, Rice

### INTRODUCTION

Drought is a major problem worldwide because of changing climate and constrained water resources. Constant global climate changes worsen the scenario and limit crop growth and yield. More than 23 million hectares of rainfed rice cultivation in Asia is affected by

drought (Kumbhar *et al.*, 2015). Hence, the need to develop various drought tolerance, escape and adaptation strategies to cope with the diminishing water supplies is becoming increasingly important (Pandey and Sukla, 2015). Rice, being widely grown under flooded conditions, exhibits various morphological changes in response to drought (Henry *et al.*, 2016). The sensitivity

ty of rice to water stress alters with duration, the severity of drought stress, timing, rice growth stage, and variety (Sokoto and Muhammad, 2014).

Seed germination is a complex plant life cycle process involving breaking radicles through the seed coat. It is a crucial developmental stage referring to the uptake of water by the seed and resulting in protrusion of the radicle (Bewley *et al.*, 1997). It is a multifaceted process regulated and coordinated by various cellular, metabolic and molecular events (Rajjou *et al.*, 2004). Drought stress influences the plant's morphological, physiological, and biochemical processes. Seed germination is very susceptible to drought stress. It can be classified as a critical period to estimate the survival rate of plants under adverse environmental conditions. Seed germination is the initial point of a plant's life cycle where the plant gets exposed to the external environment. Improper seed germination it directly affects growth and yield. Hence, germination has its own ecological and economic significance (Weitbrecht *et al.*, 2011). Plant hormones are important signaling molecules that respond to environmental changes in seed germination. Phytohormones, mainly Gibberellins (GA) and abscisic acid (ABA), play a vital role in seed germination and early seed establishment. Hence, the imbalance of GA synthesis and ABA catabolism greatly affect seed germination and seedling growth. Therefore, it is crucial to improve seed germination in rice under drought.

Melatonin, an indoleamine compound, plays an important role in plant stress defense. In plants, the tryptophan-derived compound was first identified in some edible plants like cucumber and tomato in 1995. Zhang *et al.* (2014) reported that melatonin plays an important role in plant stress defense mechanisms mainly related to abiotic stresses such as drought, radiation, extreme temperature, and heavy metal stress. It is also involved in plant growth and development mainly in stress-affected developmental transitions including seed germination, flowering, fruiting, and senescence. Moreover, the effect of melatonin on seed germination is dose-dependent, where lower concentrations promote seed germination, while higher concentrations of melatonin inhibit or do not affect seed germination (Hernandez-Ruiz *et al.*, 2004).

Melatonin was hypothesized as a plant growth hormone by Hernandez-Ruiz *et al.*, 2004. A study on cucumber proved that melatonin application could refuse the PEG stress and showed a positive influence on the promotion of seed germination (Zhang *et al.*, 2013). The studies of Umapathi *et al.* (2018) illustrated that exogenous melatonin at optimum concentration could counteract the cadmium toxic effect and enhance tomato seed germination and seedling characteristics. Seed germination involves the dynamic balance of synthesis and transport of Abscisic acid (ABA) and gibberellins (GAs)

(Steven *et al.*, 2011). GAs can promote seed germination, break dormancy and initiate subsequent seedling growth. Under water deficit conditions and during seed germination, ABA levels increase. Studies have proved that exogenous melatonin application regulates seed germination by elevating the level of GAs and reducing ABA content during the early development stage (Xiao *et al.*, 2019). Despite several prior theories suggesting melatonin exogenously might encourage seed germination under drought conditions (Meng *et al.*, 2014; Zhang *et al.*, 2012). There is not much information available on the morphological and physiological processes that melatonin infers to reduce drought stress. Additionally, little is understood about how melatonin affects the physiology and microstructure of the epidermis during drought stress, as well as how rice seeds germinate. Henceforth, in this study, the optimum concentration of melatonin that is potential enough to alleviate the adverse impacts of drought stress in rice at germination and early development stage is evaluated.

## MATERIALS AND METHODS

### Plant material

A laboratory experiment was conducted at the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, in April 2021. Newly released rice variety CO 54 seed material was used in this experiment.

### Standardization of drought stress using polyethylene glycol

Healthy seeds were surface sterilized with 0.1% Mercuric chloride ( $\text{HgCl}_2$ ) for 2-3 min and then washed thoroughly with distilled water. Germination assays were performed on sterilized 10 cm diameter plastic Petri dishes with one layer of blotting paper. Each plate containing an optimum number of seeds and polyethylene glycol 6000 (PEG 6000) was used to impose artificial drought stress on the seeds of rice CO 54. The blotting papers were moistened with 10 mL of different concentrations of PEG 6000 solution (-0.2, -0.3, -0.4, -0.5, and -0.6 MPa) and only with distilled water (Control) and incubated at room temperature (25-30 °C). The experiment was laid out in a completely randomized block design with six levels of drought stress and three replications. From each treatment as well as the replication, 10 seeds were used to assess the germination percentage. The seed germination rate was monitored regularly and, after 14 days, identified the optimal PEG-6000 concentration.

### Standardization of optimum melatonin concentration for drought tolerance

In CO 54 rice variety, the drought stress was created

by using polyethylene glycol (PEG 6000). Based on the trial mentioned above, a concentration of 0.5 MPa PEG-6000 was chosen. Seeds were soaked overnight with different concentrations of about 0 (control), 50, 100, 150, 200, and 250  $\mu\text{M}$  of melatonin. Petri dishes were sterilized using 0.01 percent  $\text{HgCl}_2$  and 70 percent ethanol and finally washed with distilled water. Pre-soaked seeds (10 seeds) of each melatonin concentration were placed on blotting papers which were moistened with PEG6000 (-0.5 MPa) in each petri dish separately. A control lacking melatonin treatment and an absolute control without PEG-6000 was also maintained. The petri dishes were kept in the laboratory at room temperature (25-30  $^{\circ}\text{C}$ ). The seeds were allowed to germinate in petri dishes and moistened periodically with -0.5 MPa of PEG solution at regular intervals. The experiment was performed with three replications.

### Germination percentage

The number of germinated seeds was recorded daily (24 h intervals) for up to 14 days. When the radical and plumule reached a minimum of 2 mm in length, those seeds were counted as germinated seeds and it was calculated by using the formula and expressed as a percentage.

Germination percentage = Number of germinated seeds / Number of seeds kept for germination x 100 Eq. 1

### Shoot length

Seedlings from each replication were randomly taken and shoot length was measured on the 14<sup>th</sup> day from the collar region to the longest leaf tip and expressed in cm.

### Root length

Root length of rice seedlings was measured on the 14<sup>th</sup> day from the stem base to the longest root tip of the seedlings and expressed in cm.

### Vigor index

The vigor index of the seedlings was calculated using the following formula proposed by Abdul-Baki and Anderson (1973).

vigor index = (Shoot length + Root length) x Germination percentage Eq. 2

### Promptness index and Germination stress index

Promptness index (PI) and germination stress index (GSI) was calculated using the method developed by Sapra *et al.* (1991) and Bouslama and Schapaugh (1984) respectively.

$\text{PI} = ((\text{nd}2(1.4) + \text{nd}4(1.2) + \text{nd}6(1) + \text{nd}8(0.8) + \text{nd}10(0.6) + \text{nd}12(0.4) + \text{nd}14(0.2))$  Eq. 3

Where, nd2, nd4, nd6, nd8, nd10, nd12 and nd14 denote the percentage of germinated seeds after 2, 4, 6,

8, 10, 12 and 14 days after sowing, respectively.

Germination stress index =  $\text{PIS}/\text{PINS} \times 100$  Eq. 4

Where PIS is PI under drought stress situations and PINS is PI under normal condition.

### Fresh weight and dry weight

Seedlings were randomly picked from each replication and the fresh and dry weights were recorded. The fresh weight of the seedling was measured and those seedlings were kept in a hot air oven at 70 $^{\circ}\text{C}$  for 48 hours. Later their dry weight was recorded and expressed as  $\text{mg seedling}^{-1}$ .

### Plant height stress index and root length stress index

Plant height stress index (PHSI) and root length stress index (RLSI) were estimated on 14<sup>th</sup> day by using the formula stated by Ellis and Roberts (1981) and expressed as a percentage.

$\text{PHSI} (\%) = \text{Plant height stress plant} / \text{Plant height of controlled plant} \times 100$  Eq. 5

$\text{RHSI} (\%) = \text{Root height stress plant} / \text{Root height of controlled plant} \times 100$  Eq. 6

### Statistical analysis

Data analysis was done as suggested by Gomez and Gomez (1984). The collected data on various germination traits, presented with means and standard errors by ANOVA were analyzed using SPSS 13.0 (Version 133, LEAD Technologies Inc.) software. The germination percentage, shoot and root length, fresh and dry weight were graphed using Origin Pro 9 software. Principal component analysis (PCA), influenced by different melatonin treatments, was carried out using R software.

## RESULTS AND DISCUSSION

### Germination percentage

Drought stress during germination leads to poor crop establishment in soybean (Liu *et al.*, 2020). In present study, drought stress significantly ( $p \leq 0.05$ ) reduced the germination of rice CO 54 in control (when compared to absolute control). Melatonin is identified as a promising agent for enhancing seed germination. Irrespective of the concentration, melatonin treatment had a significant progressive effect on germination under drought. The germination percent was found to be maximum in absolute control (93%) and least in the control treatment (34%) (Fig. 1). Among the treatments with different concentration of melatonin, 200  $\mu\text{M}$  treated seeds showed the maximal effect on germination percentage of 74 %, whereas, when the melatonin concentration was increased to 250  $\mu\text{M}$ , the germination percentage began to decrease (53 %) over the control treatment.

These results were relevant to the cucumber research outcomes of Zhang *et al.* (2013). The germination rate improved upon exogenous melatonin application in cucumber seeds by 9.5 per cent compared to the PEG-mediated drought-induced seeds. The mechanism includes a decrease in ABA levels and an increase in active GAs such as GA<sub>3</sub> and GA<sub>4</sub> by melatonin treatment, thereby promoting seed germination under stress conditions (Hai-Jun *et al.*, 2015). Another possible mechanism is that melatonin pre-treatment may increase the water uptake and enhance the energy supply to the seed. Posmyk *et al.* (2008) studies revealed that a higher concentration of melatonin was found to inhibit seed germination in red cabbage while a lower dose increased the germination rate by 17%. Likewise, a 100 µM concentration of melatonin in oat plants positively impacted germination rate, survival rate, potential, and index (Gao *et al.*, 2018).

### Shoot length

The physiological mechanisms of melatonin are similar to auxin, which aids in seedling growth and development under drought stress (Huang *et al.*, 2019). The shoot length of rice seedlings was substantially reduced with respect to drought stress. Whereas the shoot length varied significantly among the treatments ranging between 4.27 to 8.23 cm (Fig. 2 & 3). The reduced shoot length in control may be due to inhibition of cell division, leading to stunted plant growth under drought-stress conditions (Hossain *et al.*, 2020). However, the melatonin pre-seed treatment, with 200 µM and 250 µM of melatonin had a higher shoot length of 8.23 cm and 7.60 cm, respectively. Similarly, Melatonin application increased the seedling length of kidney beans by 1.6 times more than the control (Chen *et al.*, 2009). This positive role of melatonin has also been established in transgenic rice plants (Kang *et al.*, 2010).

### Root length

Melatonin is known to promote lateral root growth under water deficit condition. Aguilera *et al.* (2015) demonstrated that melatonin was able to enhance root growth in lentils and kidney beans. In the present study, based on the concentration of melatonin-pre treatment, the seedling root length varied significantly. In contradiction with shoot length, the control plants had a comparatively higher root length of 8.40 cm than the absolute control (6.33 cm) (Fig. 2 and 3). Among the melatonin pre-treatment, a maximum root length was observed at 200 µM melatonin pre-treatment by 12.23 cm and 250 µM melatonin had a negative effect on root length of 10.27 cm, respectively. Melatonin can modify plant growth in a dose-dependent manner. It improves the lateral and taproot growth by enhancing the catalase activity at the root tips (Dai *et al.*, 2020). In sweet cherry grown under in vitro conditions, a low concentration of 1 µM melatonin was found to enhance the number of roots, root length, and rooting percentage. In contrast, a higher melatonin concentration of 10 µM inhibited proper root growth and development (Sarropoulou *et al.*, 2012). This inhibition of rooting at higher concentrations is attributed to Ca<sup>2+</sup> calmodulin system being linked to melatonin (Benitez-King *et al.*, 1996). The root architecture and its physiology are greatly affected under water stress, ultimately impairing nutrient and water uptake (Kuromorie *et al.*, 2018). Exogenous melatonin application was found to improve the seminal root growth without altering the number of adventitious roots in transgenic rice (Back and Park, 2012).

### Vigor index

The vigor index is a product of germination percentage and seedling length. The positive effects of melatonin as seed treatment are attributed to seed development, quality, crop growth, and yield (Zhang *et al.*, 2014).

**Table 1.** Effect of melatonin treatments on stress indices

Treatments	Vigor index	Promptness index	Germination Stress Index	Root length stress index	Plant height stress index
T1 : Absolute control	1135.56 <sup>cd</sup>	79.33 <sup>a</sup>	-	-	-
T2 : Control	760.00 <sup>e</sup>	39.43 <sup>f</sup>	64.29 <sup>e</sup>	132.63 <sup>d</sup>	73.14 <sup>f</sup>
T3 : Melatonin of 50 µM	1021.78 <sup>d</sup>	46.09 <sup>e</sup>	78.57 <sup>d</sup>	143.68 <sup>cd</sup>	82.86 <sup>e</sup>
T4 : Melatonin of 100 µM	1184.00 <sup>c</sup>	50.87 <sup>d</sup>	85.71 <sup>c</sup>	145.26 <sup>cd</sup>	96.00 <sup>d</sup>
T5 : Melatonin of 150 µM	1166.00 <sup>c</sup>	51.28 <sup>d</sup>	78.57 <sup>d</sup>	156.32 <sup>bc</sup>	102.86 <sup>c</sup>
T6 : Melatonin of 200 µM	1910.22 <sup>a</sup>	64.83 <sup>b</sup>	100 <sup>a</sup>	193.16 <sup>a</sup>	141.14 <sup>a</sup>
T7 : Melatonin of 250 µM	1548.44 <sup>b</sup>	59.92 <sup>c</sup>	92.86 <sup>b</sup>	162.11 <sup>b</sup>	130.29 <sup>b</sup>
Mean	1246.57	55.97	83.33	155.53	104.38
SE(d)	131.268 <sup>*</sup>	1.890 <sup>*</sup>	3.512 <sup>*</sup>	16.096 <sup>*</sup>	6.686 <sup>*</sup>
C.D.	5.955	4.092	7.736	5.754	3.561

Data on the same row with different superscript (a, b, c,) are significantly different at  $p < 0.05$

Drought stress eventually leads to a reduction in the vigor and productivity of the crop. Melatonin pre-treatment showed a discernible effect on root and shoot length and it was reflected in the vigor index. Accordingly, the maximum vigor index of 1910.22 was recorded in 200  $\mu\text{M}$  melatonin-treated seeds while 250  $\mu\text{M}$  melatonin-treated seeds had a comparatively lower vigor index of 1548.44 (Table 1). Exogenous melatonin application can mitigate the adverse effect of drought stress by improving the physiological and growth traits of plants (Debnath *et al.*, 2019). The results obtained were in corroboration with the findings of Aguilera *et al.* (2015), who reported that priming cucumber seeds with melatonin, elevated the endogenous melatonin content and contributed to the mobility of nutrients to the metabolic site of germinating seeds thereby improving the seedling quality. Likewise, the vigor index and germination potential of cotton seeds were enhanced upon treatment of melatonin in lower concentrations thereby promoting seed germination (Xiao *et al.*, 2019).

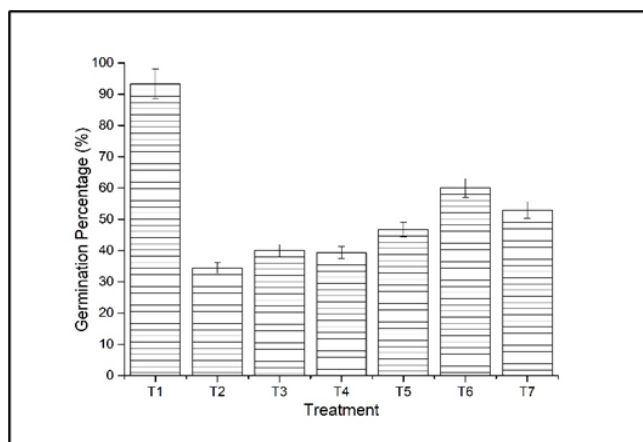
#### Promptness index and Germination stress tolerance index

The promptness index is a summary assessment of the impact of stress on crop growth and development. The promptness index showed a significant ( $p \leq 0.05$ ) difference between control and melatonin treatments in rice (Table 1). Apart from absolute control, a significant result was observed in melatonin pre-treatment. 200  $\mu\text{M}$  melatonin-treated seeds had a moderately higher promptness index of 64.83, followed by 250  $\mu\text{M}$  melatonin-treated seeds (59.92). Similar to the conclusions of the present study, melatonin application enhanced seedling emergence performance in pepper seeds alleviating the negative impacts of chilling stress (Korkmaz *et al.*, 2017). This positive effect may be due to the melatonin-induced elevation in antioxidants.

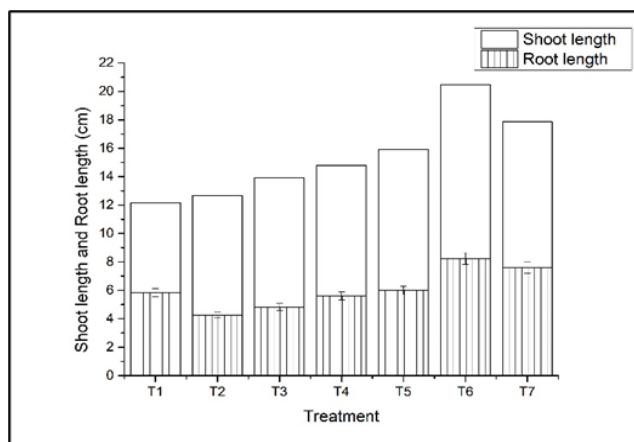
**Table 2.** Eigen value, variability % and cumulative % of germination traits among the melatonin treatments

	Principal component 1	Principal component 2
Germination Percentage	-0.454	-0.170
Shoot length	-0.289	0.496
Root length	0.060	0.622
Vigor index	-0.261	0.527
Promptness index	-0.474	-0.027
Fresh weight	-0.463	-0.133
Dry weight	-0.447	-0.206
Eigen values	4.411	2.489
Variability (%)	63.010	35.550
Cumulative (%)	63.010	98.570

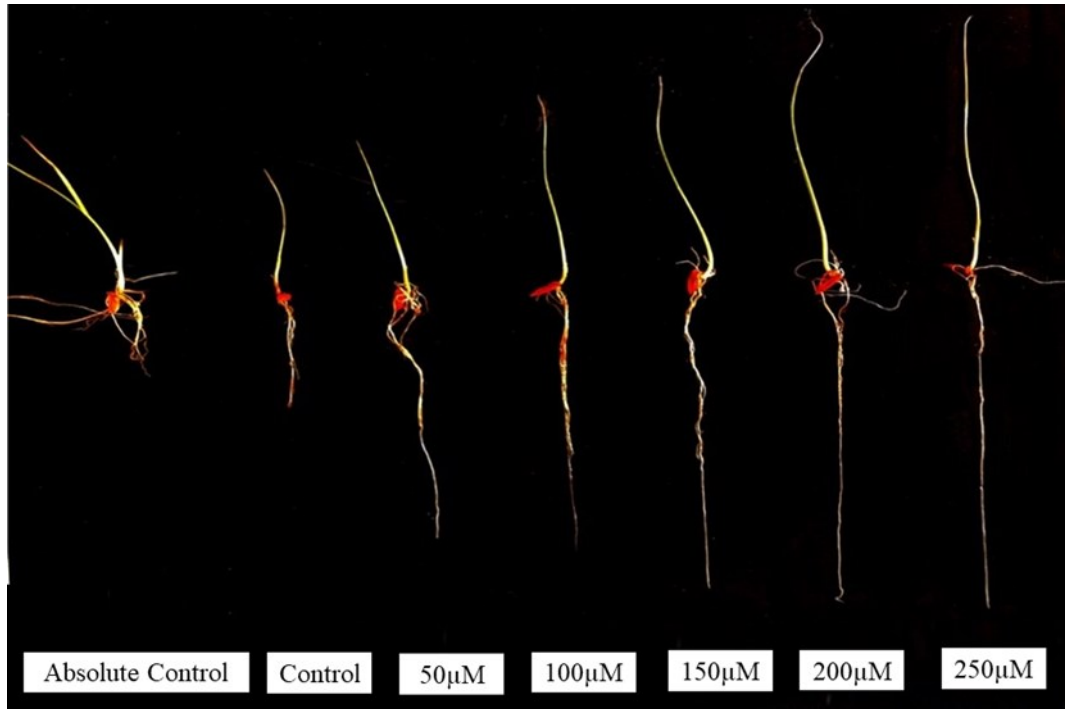
The germination stress tolerance index is an integrated calculation that indicates a higher level of tolerance to drought by using the promptness index and their data is presented in Table 1. A significant ( $p \leq 0.05$ ) variation was observed in the GSI between control and melatonin treatments. The least GSI value was observed in control (64.29) and the highest GSI value was noticed in 200  $\mu\text{M}$  melatonin-treated seeds (100.00). Nevertheless, an increase in melatonin concentration did not produce more effect on the GSI of 250  $\mu\text{M}$  melatonin-treated seeds (92.86). From this, it is concluded that the optimum level of 200  $\mu\text{M}$  melatonin concentration could enhance the tolerance of rice seedlings to drought stress. The key mechanism of stress tolerance involves crosstalk between melatonin and other phytohormones like gibberellin, auxin, cytokinin and ABA (Tiwari *et al.*, 2020). Chen *et al.* (2009) reported that a lower dose of melatonin stimulated seed germination and root growth faster than a higher concentration. Mel-



**Fig. 1.** Effect of melatonin treatments on germination percentage in rice under drought stress condition (T1 : Absolute control; T2 : Control; T3 : Melatonin of 50  $\mu\text{M}$ ; T4 : Melatonin of 100  $\mu\text{M}$ ; T5 : Melatonin of 150  $\mu\text{M}$ ; T6 : Melatonin of 200  $\mu\text{M}$ ; T7 : Melatonin of 250  $\mu\text{M}$ )



**Fig. 2.** Shoot and root length of rice seedlings influenced by melatonin treatments under water deficit condition (T1 : Absolute control; T2 : Control; T3 : Melatonin of 50  $\mu\text{M}$ ; T4 : Melatonin of 100  $\mu\text{M}$ ; T5 : Melatonin of 150  $\mu\text{M}$ ; T6 : Melatonin of 200  $\mu\text{M}$ ; T7 : Melatonin of 250  $\mu\text{M}$ )



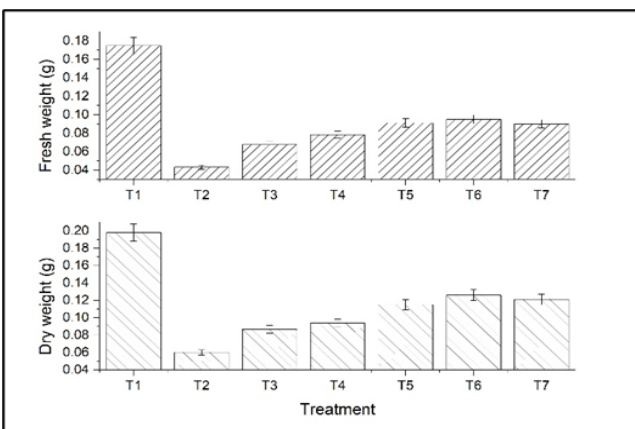
**Fig. 3.** Effect of melatonin seed treatment on shoot and root length of rice seedlings under drought stress condition

atonin pre-treatment as foliar application, seedling and root dipping treatments regulated germination, osmoregulation and photosynthesis in plants under drought stress conditions (Li *et al.*, 2018., Liang *et al.*, 2019). These changes may be attributed to the upregulation of ABA catabolism genes and GA biosynthetic genes resulting in a rapid decrease in ABA and an increase in GA respectively (Zhang *et al.*, 2014). Zhang *et al.* (2019) and Khan *et al.* (2020) stated that under abiotic stress conditions, melatonin elevates gibberellic acid synthesis and content.

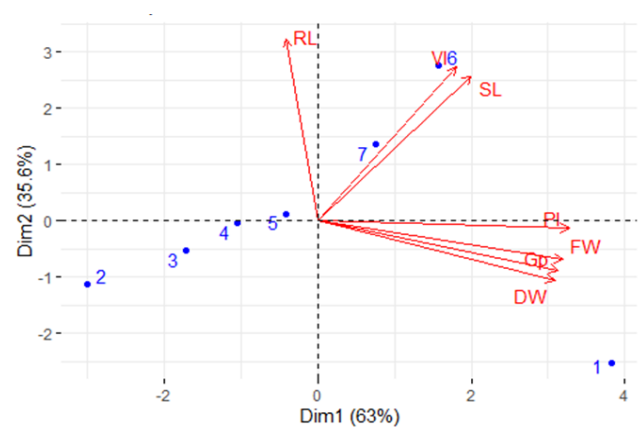
**Fresh weight and dry weight**

Melatonin acts as a regulatory factor in seed germination

and energy metabolism (Zhang *et al.*, 2017). Fresh and dry weight was measured, and the data is presented in Fig. 4. The results revealed a significant ( $p \leq 0.05$ ) difference between the control and melatonin treatments. Melatonin application progressively reduces the inhibitory effect of PEG-induced drought stress on germination and it was imitated in the fresh weight of rice seedlings. Among the treatments, the control seedlings had the least fresh and dry weight of 0.06 g and 0.04 g, respectively. Maximum fresh and dry weight was recorded in absolute control seedlings (0.198 g and 0.175 g). Seedling fresh and dry weight was gradually increased in a dose-dependent manner (50-200  $\mu\text{M}$ ) up to 0.095 g and 0.126 g respectively but the higher concentration of mela-



**Fig. 4.** Impact of melatonin treatments on fresh and dry weight of rice seedlings under drought (T1 : Absolute control; T2 : Control; T3 : Melatonin of 50  $\mu\text{M}$ ; T4 : Melatonin of 100  $\mu\text{M}$ ; T5 : Melatonin of 150  $\mu\text{M}$ ; T6 : Melatonin of 200  $\mu\text{M}$ ; T7 : Melatonin of 250  $\mu\text{M}$ )



**Fig. 5.** Principal component analysis (PCA) of rice as influenced by different melatonin treatments (Variables are GP, Germination percentage; VI, vigour index; SL, Shoot length; RL, Root length; FW, Fresh weight; DW, Dry weight; PI, Promptness index)

tonin (250  $\mu\text{M}$ ) significantly reduced the fresh and dry weight of seedlings (0.121 g and 0.090 g). The overall results give proof that the exogenous application of melatonin played a vital role in biomatter production. These results are in consistence with a previous study by Bai *et al.* (2020), who articulated that melatonin seed treatment improves the fresh weight of cotton in a dose-dependent manner. This enhancement effect of melatonin may be attributed to the cell division and up-regulation of genes responsible for DNA replication (Hernandez-Ruiz *et al.*, 2004). The results were in corroboration with the findings of Wei *et al.*, 2014 where the seed treatment of melatonin in soybean resulted in higher plant growth, development and yield. Gao *et al.*, 2018 reported that, melatonin treated oat seedling showed improved fresh weight, dry weight, stem thickness and phenotype under drought stress. In *Moringa olifera*, melatonin had a beneficial effect by improving the foliage yield, fresh and dry weight of shoots and leaves under water limited conditions (Sadak and Ahmed 2020).

#### **Plant height, stress index and Root length stress index**

The role of melatonin as a growth promoter was illustrated in etiolated *Lupinus albus* (Hernandez-Ruiz *et al.*, 2004). Similar to IAA, exogenous application of melatonin at micromolar concentrations, induced active growth of hypocotyls. In contrast, they exhibit an inhibitory effect at higher doses. The results of our experiment revealed that a significant ( $p \leq 0.05$ ) difference exists between the melatonin treatments (Table 1). The values of PHSI and RLSI ranged between a maximum of 141.14-73.14 in PHSI and 193.16-132.63 in RLSI at a dose of 50-250  $\mu\text{M}$  melatonin treatment and the control treatment. The PHSI and RLSI of 200  $\mu\text{M}$  melatonin pre-treatment has significantly ( $p \leq 0.05$ ) higher than those of all other treatments. This indicates that the ideal level of melatonin concentration can promote the PHSI and RLSI than the high concentration of melatonin treatment. Root length is a vital trait against any abiotic stress and in general, varieties with longer roots can cope with abiotic stress (Leishman and Westoby, 1994). Melatonin positively regulates root growth (Sharif *et al.*, 2018). These positive effects in regards to shoot, root and total dry matter production are due to the reduction in intercellular pH and cell wall loosening induced by melatonin, which is responsible for the cell wall elongation and expansion in lupin (Arnao and Hernandez, 2007).

#### **Principal component analysis (PCA) of seed germination and growth traits in rice as influenced by melatonin treatments**

Principal component analysis was used to determine variability among each germination trait. The first two

components in the PCA with eigen values greater than 1 contributed 98.57 percent to the variability (Table 2). Principal component 1 with an eigen value of 4.41 and Principal Component 2 with eigen value of 2.48 contributed 63.01 percent and 35.55 percent to total variability respectively. For the first principal component root length (0.060) contributed positively to 63.01 percent of the total variability. The second principal component accounted for 35.55 percent of the total variation and had high contributing characteristics observed in shoot length (0.496), root length (0.622), and vigor index (0.527).

Since the first two components contributed to nearly 98.57 percent of total variation the rest of the components can be neglected. Hence, the biplot was constructed with principal components 1 and 2. X and Y axis show principal component 1 and principal component 2, which explains 63% and 35.6% of the total variance, respectively. It is thus possible to notice that among the melatonin treatments, T6 (200  $\mu\text{M}$  melatonin) and T7 (250  $\mu\text{M}$  melatonin) performed the best (Fig. 5). When considering the contribution of each measured variable, it is possible to distinguish that root length, shoot length, and vigor index had the most significant impact on the imposed treatments.

#### **Conclusion**

In conclusion, rice variety CO 54 was very sensitive to drought stress. The study has substantiated that, with the varying levels of water stress, seed germination and early seedling development were adversely affected. Irrespective of the concentration, melatonin was able to ameliorate the negative impacts of drought stress on rice seedlings. However, prime germination percentage, shoot, and root length, fresh and dry weight, vigor index, promptness index, germination stress index, shoot and root length stress index were recorded when the seeds were treated with 200  $\mu\text{M}$  melatonin concentration. This was the optimum melatonin concentration for drought stress tolerance at germination and early development stage in rice. Additionally, the study also revealed that melatonin at higher concentrations (250  $\mu\text{M}$ ) had an inhibitory effect on seed germination and seedling growth and development of rice.

#### **Conflict of interest**

The authors declare that they have no conflict of interest.

#### **REFERENCES**

1. Abdul-Baki, A. A. & Anderson, J. D. (1973). Vigor determination in soybean seed by multiple criteria. *Crop Science*, 13 (6), 630-633. <https://doi.org/10.2135/cropsci1973.0011>

- 183X001300060013x
2. Aguilera, Y., Herrera, T., Liébana, R., Rebollo-Hernanz, M., Sanchez-Puelles, C. & Martín- Cabrejas, M. A. (2015). Impact of melatonin enrichment during germination of legumes on bioactive compounds and antioxidant activity. *Journal of Agricultural and Food Chemistry*, 63(36), 7967–7974. <https://doi.org/10.1021/acs.jafc.5b03128>
  3. Arnao, M. B. & 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. <https://doi.org/10.1111/j.1600079X.2006.00396.x>
  4. Back, K. & Park, S. (2012). Melatonin promotes seminal root elongation and root growth in transgenic rice after germination. *J Pineal Res.*, 53: 385–389. <https://doi.org/10.1111/j.1600-079X.2012.01008.x>
  5. Bai, Y., Xiao, S., Zhang, Z., Zhang, Y., Sun, H., Zhang, K. ... & Liu, L. (2020). Melatonin improves the germination rate of cotton seeds under drought stress by opening pores in the seed coat. *Peer.J.*, 8, e9450. <https://doi.org/10.7717/peerj.9450>
  6. Benitez-King, G. A., Rios, A. Martinez. & Anton-Tay, F. (1996). In vitro inhibition of Ca<sup>2+</sup>/ calmoduline dependent kinase II activity by melatonin. *Biochim. Biophys. Acta.*, 1290, 191-196. [https://doi.org/10.1016/0304-4165\(96\)00025-6](https://doi.org/10.1016/0304-4165(96)00025-6)
  7. Bewley, J.D. (1997). Seed germination and dormancy. *Plant Cell*, 9(7), 1055–1066. doi: 10.1105/tpc.9.7.1055
  8. Bouslama, M. & W. Schapaugh. (1984). Stress tolerance in soybeans. I. Evaluation of three screening techniques for heat and drought tolerance. *Crop Science*, 24 (5), 933-937. <https://doi.org/10.2135/cropsci1984.0011183X002400050026x>
  9. Chen, Q., Qi, W. B., Reiter, R. J., Wei, W. & Wang, B. M. (2009). Exogenously applied melatonin stimulates root growth and raises endogenous indoleacetic acid in roots of etiolated seedlings of *Brassica juncea*. *Journal of Plant Physiology*, 166(3), 324–328. <https://doi.org/10.1016/j.jplph.2008.06.002>
  10. Dai, L., Li, J., Harmens, H., Zheng, X. & Zhang, C. (2020). Melatonin enhances drought resistance by regulating leaf stomatal behaviour, root growth and catalase activity in two contrasting rapeseed (*Brassica napus* L.). *Plant Physiol Biochem*, 149, 86–95. DOI: 10.1016/j.plaphy.2020.01.039
  11. Debnath, B., Islam, W., Li, M., Sun, Y., Lu, X., Mitra, S., Hussain, M., Liu, S. & Qiu, D. (2019). Melatonin mediates enhancement of stress tolerance in plants. *Int J Mol Sci.*, 20, 1040. <https://doi.org/10.3390/ijms20051040>
  12. Ellis, R. & Roberts, E. (1981). The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology (Netherlands)*, 9 (2), 373-409.
  13. Gao, W., Zhang, Y., Feng, Z., Bai, Q., He, J. & Wang, Y. (2018). Effects of Melatonin on Antioxidant Capacity in Naked Oat Seedlings under Drought Stress. *Molecules.*, 23, 1580. <https://doi.org/10.3390/molecules23071580>
  14. Gomez, K.A. & Gomez, A. A. (1984). *Statistical procedures for agricultural research*, John Wiley & Sons.
  15. Hai-Jun, Z., Na, Z., Rong-Chao, Y., Li, W., Qian-Qian, S., Dian-Bo, L., et al. (2015). Melatonin promotes seed germination under high salinity by regulating antioxidant systems, ABA and GA? Interaction in cucumber (*Cucumis sativus* L.). *Journal of Pineal Research*, 57(3), 269–279. <https://doi.org/10.1111/jpi.12167>
  16. Henry, A., Wehler, R., Grondin, A., Franke, R. & Quintana, M. (2016). Environmental and physiological effects on grouping of drought tolerant and susceptible rice varieties related to rice (*Oryza sativa*) root hydraulics under drought. *Annals of Botany*, 118(4), 711–724. <https://doi.org/10.1093/aob/mcw068>
  17. Hernandez-Ruiz, J., Cano, A. & Arnao, M. B. (2004). Melatonin: A growth stimulating compound present in lupin tissues. *Planta*, 220(1), 140–144. <https://doi.org/10.1007/s00425-004-1317-3>
  18. Hossain, M.S., Li, J., Sikdar, A., Hasanuzzaman, M., Uzizerimana, F., Muhammad, I., Yuan, Y., Zhang, C., Wang, C. & Feng, B. (2020). Exogenous Melatonin Modulates the Physiological and Biochemical Mechanisms of Drought Tolerance in Tartary Buckwheat (*Fagopyrum tataricum* (L.) Gaertn). *Molecules*, 25, 2828.
  19. Huang, B., Chen, Y.E., Zhao, Y.Q., Ding, C.B., Liao, J.Q., Hu, C., Zhou, L.J., Zhang, Z.W., Yuan, S. & Yuan, M. (2019). Exogenous melatonin alleviates oxidative damages and protects photosystem ii in maize seedlings under drought stress. *Front Plant Sci.*, 10, 1–16. <https://doi.org/10.3389/fpls.2019.00677>
  20. Kang, K., Lee, K., Park, S., Kim, Y. S. & Back, K. (2010). Enhanced production of melatonin by ectopic overexpression of human serotonin *N*-acetyltransferase plays a role in cold resistance in transgenic rice seedlings. *Journal of Pineal Research*, 49(2), 176–182. <https://doi.org/10.1111/j.1600-079X.2010.00783.x>
  21. Khan, M.N., Khan, Z., Luo, T., Liu, J., Rizwan, M., Zhang, J., Xu, Z., Wu, H. & Hu, L. (2020). Seed priming with gibberellic acid and melatonin in rapeseed: Consequences for improving yield and seed quality under drought and non-stress conditions. *Ind Crops Prod*, 156, 112850. DOI: 10.1016/j.indcrop.2020.112850
  22. Korkmaz, A., Karaca, A., Kocacinar, F. & Cuci, Y. (2017). The effects of seed treatment with melatonin on germination and emergence performance of pepper seeds under chilling stress. *Tarim Bilimleri Dergisi – Journal of Agricultural Sciences*, 23, 167–176.
  23. Kumbhar, S.D., Kulwal, P.L., Patil, J.V., Sarawate, C.D., Gaikwad, A.P. & Jadhav, A.S. (2015). Genetic diversity and population structure in landraces and improved rice varieties from India. *Rice Science*, 22(3), 99–107. <https://doi.org/10.1016/j.rsci.2015.05.013>
  24. Kuromori, T., Seo, M. & Shinozaki, K. (2018). ABA Transport and Plant Water Stress Responses. *Trends Plant Sci*, 23 (6), 513-22. DOI: 10.1016/j.tplants.2018.04.001
  25. Leishman, M.R. & Westoby, M. (1994). The role of seed size in seedling establishment in dry soil conditions- experimental evidence from semi-arid species. *J. Ecology*, 82 (2), 249-258. <https://doi.org/10.2307/2261293>
  26. Li, J., Zeng, L., Cheng, Y., Lu, G., Fu, G., Ma, H., Liu, Q., Zhang, X., Zou, X. & Li, C. (2018). Exogenous melatonin alleviates damage from drought stress in *Brassica napus* L. (rapeseed) seedlings. *Acta Physiol Plant*, 40, 1–11. DOI: 10.7717/peerj.7793
  27. Liang, D., Ni, Z., Xia, H., Xie, Y., Lv, X., Wang, J., Lin, L., Deng, Q. & Luo X. (2019). Exogenous melatonin promotes biomass accumulation and photosynthesis of ki-



- wifruit seedlings under drought stress. *Sci Hortic (Amsterdam)*, 246, 34–43. DOI: 10.3390/ijms21030852
28. Liu, J., Shabala, S., Zhang, J., Ma, G., Chen, D., Shabala, L., Zeng, F., Chen, Z., Zhou, M., Venkataraman, G. & Zhao, Q. (2020). Melatonin improves rice salinity stress tolerance by NADPH oxidase-dependent control of the plasma membrane K<sup>+</sup> transporters and K<sup>+</sup> homeostasis. *Plant Cell Environ pce.*, 13759. <https://doi.org/10.1111/pce.13759>
  29. Meng, J. F., Xu, T. F., Wang, Z. Z., Fang, Y. L., Xi, Z. M. & Zhang, X. W. (2014). The ameliorative effects of exogenous melatonin on grape cuttings under water-deficient stress: antioxidant metabolites, leaf anatomy, and chloroplast morphology. *Journal of Pineal Research*, 57(2), 200–212. <https://doi.org/10.1111/jpi.12159>
  30. Pandey, V. & Shukla, A. (2015). Acclimation and tolerance strategies of rice under drought stress. *Rice Science*, 22(4), 147–161. <https://doi.org/10.1016/j.rsci.2015.04.001>
  31. Posmyk, M.M., Kuran, H., Marciniak, K. & Janas, K. M. (2008). Presowing seed treatment with melatonin protects red cabbage seedlings against toxic copper ion concentrations. *J Pineal Res.*, 45, 24–31. <https://doi.org/10.1111/j.1600-079X.2007.00552.x>
  32. Rajjou, L., Gallardo, K., Debeaujon, I., Vandekerckhove, J., Job, C. & Job, D. (2004). The effect of alpha-amanitin on the Arabidopsis seed proteome highlights the distinct roles of stored and neosynthesized mRNAs during germination. *Plant Physiology*, 134(4), 1598–1613. <https://doi.org/10.1104/pp.103.036293>
  33. Sadak, M.S. & Ahmed Bakry, B. (2020). Alleviation of drought stress by melatonin foliar treatment on two flax varieties under sandy soil. *Physiol Mol Biol Plants*, 1, 1–3. <https://doi.org/10.1007/s12298-020-00789-z>
  34. Sapra, V., E. Savage, A. A. & Beyl, C. (1991). Varietal differences of wheat and triticale to water stress. *Journal of Agronomy and Crop Science*, 167(1), 23–28. <https://doi.org/10.1111/j.1439-037X.1991.tb00929.x>
  35. Sarropoulou, V., Dimassi-Theriu, K., Therios, I. & Koukourikou-Petridou, M. (2012). Melatonin enhances root regeneration, photosynthetic pigments, biomass, total carbohydrates and proline content in the cherry rootstock PHL-C (*Prunus avium* × *Prunus cerasus*). *Plant Physiology and Biochemistry*, 61, 162–168. <https://doi.org/10.1016/j.plaphy.2012.10.001>
  36. Sharif, R., Xie, C., Zhang, H., Arnao, M.B., Ali, M., Ali, Q., Muhammad, I., Shalmani, A., Nawaz, M.A., Chen, P. & Li, Y. (2018). Melatonin and its effects on plant systems. *Molecules*, 23(9), 2352. doi: 10.3390/molecules23092352
  37. Sokoto, M.B. & Muhammad, A. (2014). Response of rice varieties to water stress in Sokoto, Sudan Savannah, Nigeria. *Journal of Bioscience Med.*, 2, 68–74. DOI: 10.4236/jbm.2014.21008
  38. Steven, F., Isabel, D.S., Heather, C. & Finch-Savage, W.E. (2011). Dormancy cycling in Arabidopsis seeds is controlled by seasonally distinct hormone-signaling pathways. *Proceedings of the National Academy of Sciences of the United States of America*, 108(50), 20236–20241. <https://doi.org/10.1073/pnas.1116325108>
  39. Tiwari, R.K., Lal, M.K., Naga, K.C., Kumar, R., Chourasia, K.N., S, S., Kumar, D. & Sharma, S. (2020). Emerging roles of melatonin in mitigating abiotic and biotic stresses of horticultural crops. *Sci Hortic*, 272, 109592. <https://doi.org/10.1016/j.scienta.2020.109592>
  40. Umapathi, M., Kalarani, M. K. & Srinivasan, S. (2018). Optimization of Melatonin to Mitigate Cadmium Stress at Seedling Level in Tomato. *Madras Agricultural Journal*, 105. 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. (2014). Melatonin enhances plant growth and abiotic stress tolerance in soybean plants. *Journal of Experimental Botany*, 66(3), 695–707. <https://doi.org/10.1111/jpi.12367>
  41. Weitbrecht, K., Muller, K. & Leubnermetzger, G. (2011). First off the mark: early seed germination. *Journal of Experimental Botany*, 62(10), 3289–3309. <https://doi.org/10.1093/jxb/err030>
  42. Xiao, S., Liu, L., Wang, H., Li, D., Bai, Z., Zhang, Y., Sun, H., Zhang, K. & Li, C. (2019). Exogenous melatonin accelerates seed germination in cotton (*Gossypium hirsutum* L.). *PLoS One*, 14(6), 0216575. <https://doi.org/10.1371/journal.pone.0216575>
  43. Zhang, L., Jia, J., Xu, Y., Wang, Y., Hao, J. & Li, T. (2012). Production of transgenic *Nicotiana glauca* plants expressing melatonin synthetase genes and their effect on UV-B-induced DNA damage. *In Vitro Cellular & Developmental Biology- Plant*, 48(3), 275–282. <https://doi.org/10.1007/s11627-011-9413-0> Zhang, N., Zhao, B., Zhang, H.J., Weeda, S., Yang, C., Yang, Z.C., Ren, S. & Guo, Y.D. (2013). Melatonin promotes water-stress tolerance, lateral root formation, and seed germination in cucumber (*Cucumis sativus* L.). *J Pineal Res.*, 54, 15–23. <https://doi.org/10.1111/j.1600-079X.2012.01015.x>
  44. Zhang, H.J., Zhang, N., Yang, R.C., Wang, L., Sun, Q.Q., Li, D.B., Cao, Y.Y., Weeda, S., Zhao, B., Ren, S., et al. (2014). Melatonin promotes seed germination under high salinity by regulating antioxidant systems, ABA and GA4 interaction in cucumber (*Cucumis sativus* L.). *J Pineal Res.*, 57, 269–279. <https://doi.org/10.1111/jpi.12167>
  45. Zhang, J., Shi, Y., Zhang, X., Du, H., Xu, B. & Huang, B. (2017). Melatonin suppression of heat-induced leaf senescence involves changes in abscisic acid and cytokinin biosynthesis and signaling pathways in perennial ryegrass (*Lolium perenne* L.). *Environmental and Experimental Botany*, 138, 36–45.
  46. Zhan, H., Nie, X., Zhang, T., Li, S., Wang, X., Du, X., Tong, W. & Song, W. (2019). Melatonin: A small molecule but important for salt stress tolerance in plants. *Int J Mol Sci*, 20(3), 709. <https://doi.org/10.3390/ijms20030709>