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# Melatonin alters photosynthesis related traits in finger millet (*Eleusine coracana* L.) under drought condition

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**Abstract**
Finger millet is an important grain crop provides food and nutritional security to the marginal farmers in the rainfed dry lands. The yield levels are far below its actual potential because it is predominantly grown under dry, semi-arid to sub-humid drought-prone agro ecosystems. Drought is an important environmental constraint that limits the productivity and affects both quality and quantity of yield. To mitigate the effect of drought on photosynthesis related traits of finger millet, an experiment was conducted with melatonin applied as seed treatment and foliar spray @ 40 and 60 µM. The finger millet was imposed with drought during vegetative and reproductive stages by withholding water for a period of 10 days or until moisture level reaches below 20% in pots. The experiment was laid out in completely randomized block design with three replications. From the results, it is observed that melatonin treatment has positive effect on photosynthesis related traits such as photosynthetic rate, transpiration rate, stomatal conductance, chlorophyll content, soluble protein and chlorophyll fluorescence in finger millet under drought. Melatonin @ 60 µM given as seed treatment plus foliar spray or foliar spray alone during drought at vegetative and reproductive stages significantly increased the photosynthetic rate and related traits which would help in better biomass buildup and yield production in finger millet under drought.

**Keywords:** Finger millet, drought, melatonin, photosynthesis, chlorophyll fluorescence

## Introduction
Finger millet (*Eleusine coracana* (L.) Gaertn), is an important nutri-cereal belongs to Poaceae family, subfamily Chloridoidae (Dida *et al*., 2008) [4]. As a subsistence food crop, it is cultivated in diverse eco-geographical areas worldwide and displays high genetic and morphological variability and diversity (Hilu and de Wet, 1976; Liu *et al*., 2011) [9, 15]. Its wide adaptability may be attributed to its C4 nature (Holt, 2000) [10] and can be cultivated in a wide range of soils and climates. Because of its short growing season, it is of specific importance in arid and semi arid regions (Mbithi-Mwikya *et al*. 2000) [17]. Limitation of water source, irregular annual rainfall during growth season and lack of resource management cause severe decrease in crops yield at these regions (Eack, 1996) [6]. Finger millet has high nutritional value (National Research Council, 1996) [21] and excellent storage qualities (Duke, 1978) [5]; hence it fits well in farmers' risk avoidance strategies in drought-prone areas (Holt, 2000) [10]. The crop is grown as food grain both in Africa and south East Asia (mainly India and Nepal) (Upadhyaya *et al*., 2007) [25]. It constitutes about 81% of the minor millets produced in India.

Factors contributing to the decline in finger millet production include unfavorable environmental conditions including frequent droughts, pests and diseases, low soil fertility, use of unimproved cultivars and poor management practices (Oduori, 1993) [22]. Drought is an important environmental constraint that limits the productivity of many crops and affects both quality and quantity of yield. Furthermore, it causes changes in a number of physiological and biochemical processes governing plant growth and productivity, limiting photosynthesis and consequently the yield of plants (Alexieva *et al*., 2001) [1]. The physiological efficiency of a plant can be improved by improving photosynthetic efficiency, reducing photorespiration, better partitioning of photoassimilates and stimulating nitrogen metabolism. All these processes are inter-linked through several interactions and influence growth and productivity. Natural and synthetic chemical substances acting as plant growth regulators (PGRs) have been found to influence these processes in one way or the other.

For increasing the production of finger millet, either more area has to be brought under cultivation or the productivity per unit area has to be augmented under unfavorable environmental conditions. In the existing situation, it is very difficult to bring more area under millets by replacing other staple crops.

Hence, only option available is alleviation of harmful effects due to drought that affect the yield potential of finger millet growing extensively under rainfed areas. To mitigate the effects of water deficit, management options such as external application of essential nutrients and growth regulating substances at critical stages have been tried over a period of time apart from water conservation methods. Plant growth regulators (PGRs) are employed increasingly in the recent years to overcome physiological constraints leading to enhanced production in several crops. Application of PGRs proved better in manipulating physiological mechanisms to withstand under stress during vegetative and reproductive stages and resulted in lesser yield reduction due to water deficit and positive effects of such practices have been widely reported in a number of agricultural and horticultural crops (Harris *et al*., 2001; Musa *et al*., 2001) [8, 20]. Hence, it is highly imperative that the use of plant growth regulating substances as one of the management options to realize better yield of this wonder millet under drought condition. One such growth regulating natural compound is "Melatonin" which has several growth regulating activities in plants including alleviation of the effect of biotic and abiotic stresses in plants.

Melatonin (N-acetyl-5-methoxytryptamine), a well-known animal hormone, is present in different parts of all the plant species (Arnao and Ruiz, 2006) [2] and shares a common biosynthetic pathway with IAA. In plants, it acts as a growth regulator similar to IAA in function (Zhang *et al*., 2015) [30]. Murch and Saxena (2002) [19] reported the antioxidant activity of melatonin against biological free radicals. Thus, it has role in alleviation of oxidative stress due to various abiotic stresses in plants. In this study, the role of melatonin in altering gas exchange parameters and other traits related to photosynthetic efficiency of finger millet subjected to water deficit was investigated.

## Materials and Methods

The study was conducted in rain out shelter, Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore during *Rabi* 2018. For pot culture experiment, pot mixture was prepared by using red soil, sand and vermicompost in the ratio of 3:1:1 and the pots (D26×H30 cm size) were filled with 14 kg of soil. The seeds of finger millet (var. CO15) were directly sown in the pot and after the establishment of seedlings, thinning was done to maintain three seedlings per pot uniformly across the replications. Fertilizer dosage for pot culture was calculated using per hectare recommendations of finger millet and other cultivation operations including plant protection measures were carried out as per recommended package of practices of Tamil Nadu Agricultural University, Coimbatore. Plants were maintained weed free by hand weeding. The experiment was laid out with eight treatments in Factorial Completely Randomized Block Design (FCRD) with three replications.

All plants, in both the control and treatment groups, were watered regularly up to the flowering stage and then the irrigation was withheld to create drought at reproductive stage. The plants regularly irrigated till maturity were considered as absolute control. Best concentrations of melatonin, 40 and 60 µM obtained from the lab study, were used for seed treatment and foliar application in the pot study. Foliar spray of melatonin was given after three days of withholding of water. Soil moisture content was measured using moisture meter (Delta-T Soil moisture kit - Model: SM150, Delta-T Devices, Cambridge) periodically and rewatering was done, when the soil moisture reached below 20 per cent and 1/3rd of leaves started drying.

Gas exchange parameters *viz*., photosynthetic rate, transpiration rate and stomatal conductance were recorded using an advanced portable photosynthesis system (LI-6400 XT, LicorInc, Nebraska, USA). The readings were recorded from 10.00 am to 12.00 noon on a clear sunny day when the photo synthetically active radiation was more than 1000 µmol photons $m^{-2}$ $s^{-1}$ also which avoid effects of photo-inhibition. Fully expanded leaf from the top was clamped inside the leaf chamber and held perpendicular to incident light and computed values were recorded. The instrument maintained a constant $CO_2$ flux to leaf chamber, which was maintained at ambient concentration. Relative humidity was maintained at a steady level equal to the ambient relative humidity to simulate a condition similar to that of ambient air. The photosynthetic rate expressed as µmol $CO_2$ $m^{-2}$ $s^{-1}$, stomatal conductance expressed as mmol $H_2O$ $m^{-2}$ $s^{-1}$ and transpiration rate expressed as mmol $H_2O$ $m^{-2}$ $s^{-1}$.

Chlorophyll index (SPAD value) were recorded using chlorophyll meter (SPAD 502) designed by the Soil Plant Analytical Development (SPAD) section, Minolta, Japan. The Minolta SPAD-502 measures chlorophyll content as ratio of transmittance of light at wavelength of 650 nm and 940 nm. Three readings were taken from each replication in each treatment and the average value computed as described by Minolta (1989) and Monje and Bughree (1992) [18]. The chlorophyll fluorescence was measured using the fluorescence meter (Plant PAM-210 (Teaching PAM), Heinz Walz, Germany). The key fluorescence parameters *viz*., Fo, (initial fluorescence), Fv (variable fluorescence), Fm (maximal fluorescence) and the ratio of Fv/Fm were measured. Fv/Fm is a useful ratio that depicts the proportion of quantum yield relation to a high degree of photosynthesis. Soluble protein content of the leaf was estimated at 660 nm by using Folin-Ciocalteau reagent by following the procedure described by Lowry *et al*. (1951) and expressed as mg $g^{-1}$ of fresh weight. The data collected on these traits were statistically analyzed in a FCRD as suggested by Gomez and Gomez (1992). The critical difference (CD) was computed at five per cent probability.

## Results and Discussion

Photosynthesis is the physicochemical process by which plants use light energy to drive the synthesis of organic compounds and it is the basis of plant production (Xu *et al.*, 2014) [27]. Drought is a serious environmental stress, inhibiting photosynthesis. The limitation of ambient CO2 diffusion to the site of carboxylation which induced by stomatal closure, is usually considered the main reason for the decline in photosynthetic rate under water stress (Liu *et al.*, 2013) [13]. The data on gas exchange parameters such as photosynthetic rate, transpiration rate and stomatal conductance show not only significant difference between irrigated (absolute control) and control (drought) treatments but also between melatonin treatments (Table 1). The photosynthetic rate was a greatly reduced under both vegetative (14.20 µmol $CO^2$ $m^{-2}$ $s^{-1}$) and reproductive stages (13.96 µmol $CO^2$ $m^{-2}$ $s^{-1}$) as against irrigated condition (38.60 and 35.28 µmol $CO^2$ $m^{-2}$ $s^{-1}$). Among the two concentrations of melatonin, application of 60 µM melatonin as foliar spray recorded the highest photosynthetic rate of 27.98 and 30.24 µmol $CO^2$ $m^{-2}$ $s^{-1}$ under drought at vegetative and reproductive stages, respectively, which was on par with seed treatment plus foliar spray of 60 µM melatonin (27.03 and 29.23 µmol $CO^2$ $m^{-2}$ $s^{-1}$)

(Table 1). Sarropoulou *et al*. (2012) [23] observed that exogenous melatonin at low concentrations enhanced the content of photosynthetic pigments, total biomass and total carbohydrates indicating a role for melatonin in the plant stress metabolism. Transcriptome analysis revealed that melatonin exerts its functions mainly through regulation of photosynthesis, and starch or sucrose metabolism. Maize seedlings under drought, when compared with untreated plants, the melatonin treated plants maintained high photosynthetic rates under drought stress, enabling a much greater supply of assimilates to growing tissue (Ye *et al.*, 2016) [28]. Treatment with melatonin significantly reverses the inhibition on net photosynthetic rates and chlorophyll content of plants under drought conditions (Zhang *et al.*, 2014) [29]. It maintains the photosystem II (PSII) function under stress and delayed the typical reduction in chlorophyll content.

In this study, melatonin applied as seed treatment plus foliar spray @ 60 µM melatonin (5.67 and 3.62 mmol $H_2O$ $m^{-2}$ $s^{-1}$) recorded maximum transpiration rate, which was on par with foliar spray of 60 µM (5.37 and 3.37 mmol $H_2O$ $m^{-2}$ $s^{-1}$), followed by seed treatment plus foliar spray of 40 µM melatonin (4.62 and 3.84 mmol $H_2O$ $m^{-2}$ $s^{-1}$) which was on par with foliar spray of 40 µM melatonin (4.57 and 3.63 mmol $H_2O$ $m^{-2}$ $s^{-1}$) under both the stages of drought (Table 1). Li *et al.* (2015) [12] also reported that apple seedlings treated with melatonin significantly have higher CO2 assimilation rates, transpiration rate and stomatal conductance under drought conditions. In maize, the enhanced stomatal conductance was associated with foliar sprayed melatonin that might contribute to high photosynthetic rate during drought stress (Ye *et al.*, 2016) [28].

Stomatal conductance also showed similar trend as that of photosynthetic and transpiration rates in finger millet subjected to drought at vegetative and reproductive stages (Table 1). The finger millet plants treated with melatonin @ 60 µM given as seed treatment and foliar spray recorded the highest stomatal conductance (0.26 and 0.29 mmol $H_2O$ $m^{-2}$ $s^{-1}$) followed by melatonin @ 60 µM as foliar application alone (0.26 and 0.25 mmol $H_2O$ $m^{-2}$ $s^{-1}$) at both the stages of drought. In plants, drought is known to up regulate the production of abscisic acid (ABA) which leads to the closure of the stomata and the expression of drought stress related genes (Sperry *et al.*, 2002) [24]. Melatonin down regulated ABA synthetic gene (*NCED3*) and up regulated its catabolic genes (*CYP707A1* and *CYP707A2*) and as a result, reduced ABA levels in drought stimulated plants. This reduction caused the stomata to remain open. This mechanism might be the reason for significant increase in transpiration rate and stomatal conductance to considerable extent in the present study. Li *et al.* (2015) [12] reported that apple seedlings treated with melatonin significantly had higher CO2 assimilation rates and stomatal conductance under drought conditions.

**Table 1:** Effect of melatonin on gas exchange parameters of finger millet under water deficit condition

| Treatments | Photosynthetic rate (µmol $CO^2$ $m^{-2}$ $s^{-1}$) | | Transpiration rate (mmol $H_2O$ $m^{-2}$ $s^{-1}$) | | Stomatal conductance (mmol $H_2O$ $m^{-2}$ $s^{-1}$) | |
|---|---|---|---|---|---|---|
| | D@VS | D@RS | D@VS | D@RS | D@VS | D@RS |
| T1: Absolute control | 38.60 | 35.28 | 8.18 | 7.91 | 0.28 | 0.30 |
| T2: Control | 14.20 | 13.96 | 2.06 | 1.85 | 0.07 | 0.06 |
| $T_3$ : Seed treatment with 40 µM melatonin | 16.34 | 16.90 | 2.86 | 2.04 | 0.15 | 0.14 |
| $T_4$ : Foliar spray of 40 µM melatonin | 24.08 | 21.08 | 4.57 | 3.63 | 0.23 | 0.23 |
| $T_5$ : Seed treatment + foliar spray of 40 µM melatonin | 26.54 | 25.53 | 4.62 | 3.84 | 0.24 | 0.22 |
| $T_6$ : Seed treatment with 60 µM melatonin | 16.18 | 13.68 | 2.72 | 2.60 | 0.16 | 0.08 |
| $T_7$ : Foliar spray of 60 µM melatonin | 27.98 | 30.24 | 5.37 | 3.37 | 0.26 | 0.25 |
| $T_8$ : Seed treatment + foliar spray of 60 µM melatonin | 27.03 | 29.23 | 5.67 | 3.62 | 0.26 | 0.29 |
| Mean | 23.87 | 23.24 | 4.51 | 3.61 | 0.21 | 0.19 |
| SEd | 1.31 | 1.02 | 0.25 | 0.31 | 0.04 | 0.02 |
| CD (p = 0.05) | 2.74 | 2.18 | 0.54 | 0.66 | 0.08 | 0.04 |

D@VS: Drought during vegetative stage; D@RS: Drought during reproductive stage

Melatonin seed treatment plus foliar spray @ 60 µM showed better photosynthetic efficiency (Chlorophyll fluorescence) in the finger millet plants under water deficit during vegetative and reproductive stages, in the present study (Table 2). Stress often induces damage of PSII in a leaf (Maxwell and Johnson, 2000) [16], which could be a measure of photosynthetic efficiency. PSII photosynthetic efficiency, represented by the expression of Fv/Fm, was kept at higher values in melatonin treated plants than untreated ones and melatonin given as seed treatment. Melatonin treated cucumber plants maintained higher PSII, ETR and lower NPQ (Wang *et al.*, 2013) [26]. Liu *et al*. (2015) [14] reported that after 20 days of drought, the Fv/Fm was five per cent higher for plants exposed to melatonin than control seedlings of tomato. These results suggested that melatonin could protect the drought induced damage in photosynthetic system.

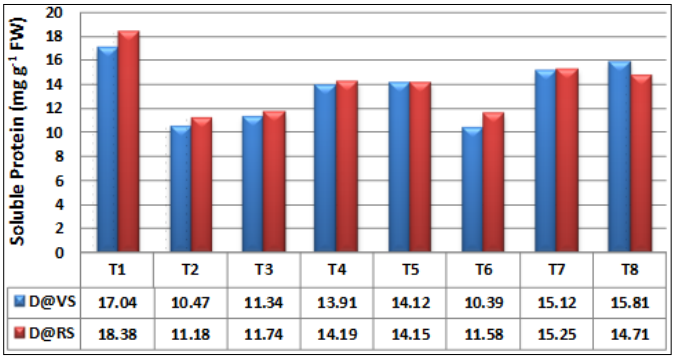
**Table 2:** Effect of melatonin on chlorophyll index and chlorophyll fluorescence of finger millet under water deficit condition

| Treatments | Chlorophyll Index (SPAD value) | | Chlorophyll fluorescence (Fv/Fm) | |
|---|---|---|---|---|
| | D@VS | D@RS | D@VS | D@RS |
| T1: Absolute control | 49.2 | 64.50 | 0.711 | 0.726 |
| T2: Control | 20.6 | 35.59 | 0.512 | 0.501 |
| $T_3$ : Seed treatment with 40 µM melatonin | 24.9 | 36.93 | 0.518 | 0.514 |
| $T_4$ : Foliar spray of 40 µM melatonin | 34.8 | 42.97 | 0.613 | 0.629 |
| $T_5$ : Seed treatment + foliar spray of 40 µM melatonin | 35.6 | 41.73 | 0.613 | 0.636 |
| $T_6$ : Seed treatment with 60 µM melatonin | 24.7 | 34.87 | 0.517 | 0.520 |
| $T_7$ : Foliar spray of 60 µM melatonin | 38.8 | 45.17 | 0.622 | 0.621 |
| $T_8$ : Seed treatment + foliar spray of 60 µM melatonin | 38.4 | 44.83 | 0.615 | 0.635 |
| Mean | 33.3 | 43.32 | 0.59 | 0.60 |
| SEd | 0.74 | 0.62 | 0.036 | 0.032 |
| CD (p = 0.05) | 1.51 | 1.26 | 0.073 | 0.060 |

D@VS: Drought during vegetative stage; D@RS: Drought during reproductive stage

Chlorophyll is an extremely important biomolecule critical in photosynthesis. Melatonin improves chlorophyll index in plants which were subjected to drought at vegetative and reproductive stages. Melatonin @ 60 µM given as seed treatment plus foliar spray recorded higher chlorophyll index, which was statistically on par with 60 µM given as foliar spray, compared to melatonin given as melatonin given as foliar spray or seed treatment @ 40 µM (Table 2). Arnao and Hernandez-Ruiz (2009) [3] suggested that melatonin may have an inhibiting action on chlorophyll degrading enzyme, such as chlorophyllase and pheophorbide a-oxygenase which enhances chlorophyll content and delays drought induced senescence. This might be the one of the reasons behind the maximum chlorophyll index of melatonin treated plants. Under drought, contents of photosynthetic pigments were reduced significantly when compared with unstressed plants and finally end up in senescence. However, those pigments were largely preserved in the melatonin treated plants by down regulating senescence associated genes and that resulted in increased chlorophyll content and photosynthetic rates and compared to untreated plants (Wang *et al.*, 2013) [26].

The soluble protein content in finger millet was significantly reduced under drought irrespective of treatments compared to irrigated condition. At the same time, melatonin has positive influence on soluble protein content under drought given at both vegetative and reproductive stags. Seed treatment plus foliar spray of 60 µM melatonin recorded the highest soluble protein content among the melatonin treatments followed by melatonin @ 60 µM as foliar spray after three days of drought at vegetative and reproductive stages (Fig.1). During drought, by accumulation of reactive oxygen species, chloroplast degeneration occurs through the progressive loss of proteins, such as RuBisCO and chlorophyll binding proteins (Hortensteiner, 2006) [11]. It is possible that, low concentration levels of melatonin act as an antioxidant, preventing the accumulation of free radicals in chloroplast, preventing chloroplast degeneration which ultimately protects chlorophyll binding proteins and soluble protein like RuBisCO (Arnao and Hernandez-Ruiz, 2009) [3].




| | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 |
|---|---|---|---|---|---|---|---|---|
| D@VS | 17.04 | 10.47 | 11.34 | 13.91 | 14.12 | 10.39 | 15.12 | 15.81 |
| D@RS | 18.38 | 11.18 | 11.74 | 14.19 | 14.15 | 11.58 | 15.25 | 14.71 |

**Fig 1:** Efficet of melationin on soluble protein of finger millet under water de ficit conditioin

From this study, it is inferred that melatonin treatment has positive effect on photosynthesis related traits such as photosynthetic rate, transpiration rate, stomatal conductance, chlorophyll content, soluble protein and chlorophyll fluorescence in finger millet under drought. Melatonin @ 60 µM given as seed treatment plus foliar spray or foliar spray alone during drought at vegetative and reproductive stages significantly increased the photosynthetic rate and related traits in finger millet which would help in biomass buildup and yield production.

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