



Influence of melatonin on physiological traits and yield potential of cassava under salt stress

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Abstract

Cassava (*Manihot esculenta* Crantz) is considered as an important staple crop among the tropics and subtropics region and is the third largest source of carbohydrates. The varying climatic conditions like heat stress and drought causes depletion of ground water and increase the concentration of salt in irrigation water is getting increased. It is sensitive to moderate concentration of salt, particularly at early stages of development. During irrigation salt deposition takes place in the soil and causes drying of leaves, shedding of leaves, reduced tuber yield and quality. It was efficient that the use of melatonin, an anti-stress compound plays an important role in plant stress defence mechanism mainly related to drought and salt stress. The present investigation aimed at studying the impact of melatonin on physiological characters in response to yield potential and in improving salt tolerance of cassava under salt stress condition. The present study was carried out in cassava variety Sree Athulya with nine treatments under 120mM NaCl salt stress condition. Different treatments viz., sett treatment and foliar application of 100 ppm melatonin was done at 30 DAP and 60 DAP of the crop growth. Control (salt stress + no melatonin) and absolute control (no stress and no melatonin) also maintained for comparison purpose. The salt stress of 120 mM NaCl was imposed from day one to 120 days. The results showed that, under salt stress condition among the treatments, sett treatment of melatonin 100 ppm plus foliar application at 30 DAP and 60 DAP followed by foliar spray of melatonin only at 30 DAP maximum photosynthetic rate, stomatal conductance and transpiration rate along with osmotic adjustment. The melatonin treated plants are physiologically efficient and provides higher yield than untreated one under salt stress condition.

Keywords: cassava, melatonin, salt stress, gas exchange parameters, osmotic adjustment

Introduction

Salinity is one of the major abiotic factors limiting the crop yield and threatening food security worldwide (Sah *et al.* 2016)^[38]. The soil deterioration in agricultural lands is due to the rise in the global climate changes. Salt stress is faced as the major by plants and leads to reduced plant growth and productivity. The unsustainable irrigation practices and deforestation causes increase in area of salt stress (Munns and Gilliam, 2015)^[34]. Worldwide about 800 million hectares of land are affected by salt and this accounts about 6 per cent of total land area (FAO, 2008). During irrigation, salt deposition takes place in the soil resulting in unproductive condition. Salinization of groundwater is becoming an increasing problem in many parts of the cassava growing areas. The semiarid and arid zones have the natural cause of accumulation of salts, the soluble salts such as chlorides, sulfates, carbonates are released due to the weathering of parental rocks. The abundant cause is the release of sodium chloride and accumulation of oceanic salt by wind and rain. It was reported that 6-50 mg/kg of sodium chloride are present in rainwater. When the soils contain NaCl more than 40 mM (EC 4 ds/m or more) is said to be salt affected soil (Munns and Tester, 2008)^[33]. The pH of saline soils is lower than the sodic soils (less than 8.5) and have exchangeable sodium potassium ratio of lesser than 15 (IRRI, 2011). Under salt stress, plants are prone to the production of excessive reactive oxygen species (ROS), membrane lipids or proteins peroxidation occurs which destroys the cell membranes leading to cell death of a normal plant. Salt at high concentration causes osmotic stress with reduced water potential in plant roots,

also the uptake of water and nutrients gets affected that inhibits the growth and development of plant that results in wilting and death of plants (Julkowska and Testerink., 2015; Liu *et al.*, 2018)^[17, 50]. Due to rise in sea level and the groundwater getting contaminated, there is a need for the development of salt tolerant crops.

Cassava (*Manihot esculenta* Crantz) is the most widely cultivated tuber crop in the tropics as a food crop due to high starch content of the roots. It has its own inherent tolerance to stressful environment, it is therefore considered to abiotic stress-tolerant (Bull *et al.*, 2011)^[4]. According to FAO, cassava is said to be moderately sensitive to salt stress (Gleadow *et al.*, 2016)^[37]. Cassava cultivation is likely expanded in salt affected soil zones in order to promote agriculture in unproductive lands (Carretero *et al.*, 2007)^[6] due to increased demand in cassava production (Shabala *et al.*, 2013)^[39]. Cassava being the important staple crop among the tropics has its tolerance to drought and high temperatures but its response to salt tolerance is unknown. The successful development of crops to survive under salt stress has been a long time concern (Munns 2002)^[27]. Plant growth regulators are extensively used to regulate plant growth and to enhance plant stress tolerance. Melatonin is a pleiotropic molecule and has many cellular and physiological functions in varied kingdoms (Arnao and Hernandez-Ruiz., 2015)^[23] present in both plants and animals (Barratt *et al.* 1977; Dubbels *et al.* 1995; Reiter *et al.* 2011; Nawaz *et al.* 2016; Shi *et al.* 2016)^[3, 10, 35, 28, 41]. Melatonin was found to be involved in regulation of

plant growth and development which protects plants against abiotic and biotic stresses such as salt, drought, cold, heat and heavy metal stresses (Reiter *et al.*, 2015) [36]. It was reported that application of exogenous melatonin effectively improves salt tolerance in certain plants. In *Malus hupehensis*, the pretreatment with melatonin reduced the inhibitory effects of salt stress such as degradation and loss of chlorophyll (Li *et al.*, 2012) [20]. Plants show the sign of declination in net photosynthetic rate and chlorophyll content in drought and high salt stress condition whereas, the treatment with melatonin reverts this inhibition of growth (Zhang *et al.*, 2014) [52]. Under salt stress, application of 50–150 μM melatonin increased the total chlorophyll content and enhanced photosynthetic capacity in cucumber (Wang *et al.*, 2016) [48]. In apple and tomato the melatonin pretreatment increased photosynthetic efficiency (Li *et al.*, 2012; Yin *et al.*, 2019) [20, 47]. According to Ren *et al.* (2020) [16] maize seedlings treated with melatonin showed higher leaf area and photosynthetic activity in salt stress. Similarly the maize seedlings showed higher transpiration rate and photosynthetic rate under drought (Qiao *et al.*, 2020; Huang *et al.*, 2019) [49, 15]. Hwang *et al.* (2020) [30] proved that the antioxidants present in melatonin enhanced photosynthesis. Thus exogenous melatonin exhibits a major role in ROS reduction and increases antioxidants and secondary metabolites (Shakhawat *et al.*, 2020; Shakeel *et al.*, 2020) [24, 40]. Altaf *et al.* (2020) [22] in tomato seedlings under salt stress discovered the increase in gas exchange parameters when treated with melatonin. Ren *et al.* (2020) [16] suggested that melatonin treated plants had higher osmolyte contents so the osmotic potential was lower compared to control. Organic osmolytes such as soluble sugars maintain osmotic adjustment and further sucrose and fructose levels enhance melatonin treated plants under salt stress. The present study was proposed to evaluate melatonin on physiological mechanisms with relation to tuber yield under salt stress in cassava.

Materials and methods

Plant materials

The cassava variety Sree Athulya, a central variety released by Central Tuber Crop Research Institute, Trivendrum was used for the study.

Treatments

The study was carried out during 2019 at the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore. Salt stress was imposed with 120mM NaCl once in three days, from day one of planting to 120 days after planting for all the treatments except absolute control as per the method of Kalarani *et al.* (2018) [26]. The deposited salt were flushed out once in a week with normal water. The plants of absolute control were maintained with normal irrigation and without melatonin. Various treatments *viz.*, absolute Control (without salt and melatonin), control (salt + no melatonin), sett treatment with 100 ppm melatonin, foliar spray of 100 ppm melatonin at 30 DAP, foliar spray of 100 ppm melatonin at 60 DAP, foliar spray of 100 ppm melatonin at 30 and 60 DAP, sett treatment of 100 ppm melatonin + foliar spray of 100 ppm melatonin at 30 DAP, sett treatment of 100 ppm melatonin + foliar spray of 100 ppm melatonin at 60 DAP, sett treatment of 100 ppm melatonin + foliar spray of 100 ppm melatonin at 30 and 60 DAP. The following measurements were taken.

Growth measurements

Observations on physiological parameters were taken on 45 days, 75 days and 135 days after planting. Three replicates were taken for each treatment and the mean calculation was done for each measurement. The physiological parameters *viz.*, gas exchange parameters (photosynthetic rate, transpiration rate and stomatal conductance) osmotic potential and osmotic adjustment were taken. Tuber yield was taken during harvest.

Leaf gas exchange parameters

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 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ 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 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, stomatal conductance expressed as $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ and transpiration rate expressed as $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$.

Osmotic potential and osmotic adjustment

The penultimate fully expanded leaf on the main stem was cut, wrapped in a plastic bag and soaked in water in the refrigerator for 24 hours to rehydrate the tissue. The rehydrated leaf placed in aluminium foil, frozen with liquid nitrogen for 30 seconds to stop the physiological function of its cells and stored in a -80°C freezer. The sap was collected by squeezing the leaf sample with the help of a sterile syringe and the osmolality (mmol kg^{-1}) of the expressed sap was determined using a vapour pressure osmometer (Vapro Model 5520 Wescor Inc., Logan, UT, USA). Osmotic potential (π) was calculated as $\pi = -cRT$, where c is concentration, R is the universal gas constant (0.0832) and T is the temperature in degrees Kelvin (310o K). The following conversion equation was used to compute osmotic potential (inMPa).

$$\text{Osmotic potential} = \frac{[(\text{Osmolality mmol kg}^{-1}) (0.0832) (310)]}{10000}$$

Osmotic adjustment was calculated as the difference between the turgid potential in normal watered treatment and stress treatment (Babu *et al.*, 1999) [1].

Tuber yield per plant

The weight of all marketable tubers per plant was recorded in each replication was added and an average yield per plant was worked out and expressed in kg per plant.

Statistical analysis

The data collected from this experiment on various parameters were statistically analyzed in Completely Randomized Design (CRD) as suggested by Gomez and Gomez (1992) [12]. The

critical difference (CD) was computed at five per cent probability and were furnished.

Result and Discussion

Physiological characters

Gas exchange parameters

Irrespective of the treatments, photosynthetic rate was increased from 45 DAP to 135 DAP. The photosynthetic rate decreased under stress condition. The rate of photosynthesis shows a significant difference between control and other treatments. The results showed that the salt treated plants has a declined net photosynthetic rate in all the three stages (13.59, 15.19 and 16.13 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$). Among the treatments, sett treatment plus foliar spray of melatonin at 30 DAP and 60 DAP was recorded highest photosynthetic rate under salt stress condition 21.81, 19.08 and 18.76 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ at 45, 75 and 135 DAP followed by foliar application of melatonin only at 30 DAP. Photosynthesis, physico-chemical process which by utilizing light energy forms organic compounds for plant growth, development and production (Barnawal *et al.*, 2017) [2]. Melatonin preserves chlorophyll and improves the efficiency of photosynthesis under stress condition (Jiang *et al.*, 2016; Li *et al.*, 2018) [7, 21].

According to Zhang *et al.* (2014) [52] melatonin protects chlorophyll and delays leaf senescence further maintains photosynthetic rates. These findings support the present investigation.

Similar to photosynthetic efficiency, the transpiration rate and stomatal conductance shows a significant difference. Initially under salt stress condition there was a high transpiration rate in control than all other treatments. Later transpiration rate increased in melatonin treated plants. Under salt stress condition, maximum rate of transpiration and stomatal conductance was observed in foliar spray of 100 ppm melatonin at 30 DAP in all the three stages which is on par with sett treatment plus foliar application of 100 ppm melatonin at 30 and 60 DAP. Salt plus melatonin treatment showed higher stomatal conductance than plants in salt alone but it was less compared to absolute control plants which is not affected by salt stress with melatonin. It was concluded that stomatal conductance hold larger stomatal opening (Zhang *et al.*, 2020) [29].

Chen *et al.* (2018) [9] reported that the exogenous melatonin mitigates from salt stress and decrease the reduction in stomatal conductance in maize. Zhang *et al.* (2019) [51] observed that water deficit stress on photosynthesis can be reduced by melatonin in soybean leaves which enhances stomatal conductance, transpiration rate and maintains normal photosynthetic rate for normal growth and development.

Wang *et al.* (2013) [43] and Weeda *et al.* (2014) [45] reported that melatonin activates CAB gene, which is associated in chlorophyll biosynthesis and reduced PAO gene which degrades chlorophyll. This might be the reason that melatonin increases the photosynthetic rate. The mechanism for increase in transpiration and assimilation rate might be the down regulation of ABA

Synthetic gene and up regulation of ABA catabolic genes by melatonin. As a result ABA level gets reduced in stress induced plant and the stomata remains open Hasan *et al.* (2015) [14]. The earlier findings collaborated well with present study.

Osmotic potential (-Mpa) and osmotic adjustment (Mpa)

The osmotic potential and osmotic adjustment were observed in control plants and stressed plants. The relationship between osmotic potential and osmotic adjustment is inverse under stress condition. It was observed that data on osmotic potential and osmotic adjustment shows the effect of melatonin compared to control (Fig. 1). Sett treatment plus foliar application of 100 ppm melatonin at 30 DAP and 60 DAP indicates more osmotic adjustment and less osmotic potential in all the three stages 0.85 (Mpa) and -2.06 (-Mpa) at 45 DAP, 0.94 (Mpa) and -1.75 (-Mpa) at 75 DAP, 0.88 (Mpa) and -1.47 (-Mpa) at 135 DAP respectively followed by melatonin spray only at 30 DAP. Absolute control shows the maximum reduced potential -1.57 (-Mpa), -0.81 (Mpa) and -0.59 (-Mpa) at 35 DAP, 75 DAP and 135 DAP respectively. The effective strategy for plants to resist salt stimulated osmotic stress is osmotic adjustment (Yin *et al.*, 2013) [46]. Chen *et al.* (2014) [8] suggested the decrease in osmotic potential and an increase in osmotic adjustment has been observed in melatonin treated plants under salt stress condition. Under salt and drought stress condition, the effect of melatonin in maintaining water status was reported by Chen *et al.* (2018), Su *et al.* (2019) and Ye *et al.* (2016) [8, 42, 48]. These earlier findings confirm the present study.

Yield trait

Tuber yield (kg/plant)

The data on yield potential is presented in (Table. 2). The application of melatonin has an improved effect on tuber yield of plants compared to the control plants under salt stress condition. Apart from absolute control (5.21 kg plant⁻¹), foliar spray of 100 ppm melatonin at 30 DAP (4.01 kg plant⁻¹) recorded maximum yield (3.95 kg plant⁻¹) which is on par with sett treatment plus foliar spray of 100 ppm melatonin at 30 DAP and 60 DAP. Among the various treatments stressed plant (control) recorded the least yield (2.13 kg plant⁻¹). Beyon and Back (2014) [5] explained that melatonin may alter plant characters such as seedling growth, senescence and yield. The increase in yield and nutritional quality of crops has been observed in plants which are introduced with melatonin biosynthetic genes (Nawaz *et al.*, 2016) [28].

Similarly, foliar application 100 mM melatonin in moringa under drought was known to increase growth rate, yield and yield components (Sadak *et al.*, 2020) [25]. Pre- soaking treatment of wheat seed in 100 μM improved grain yield and other yield components (Ye *et al.*, 2020 higher grain yield *viz.*, number of pods, number of grains per pod and total pod weight was observed (Zhang *et al.*, 2019) [51]. Earlier studies of moringa, wheat and soybean confirmed the present investigation.

Table 1: Effect of melatonin on Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$), transpiration rate ($\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$) of cassava under salt stress condition

Treatments		Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$)				Transpiration rate ($\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$)			
		45 DAP	75 DAP	135 DAP	Mean	45 DAP	75 DAP	135 DAP	Mean
T ₁	Absolute Control (without salt and melatonin)	23.08	25.14	27.03	25.08	8.10	8.74	9.06	8.63
T ₂	Control (with salt)	13.59	15.19	16.13	14.27	9.14	6.74	6.03	7.30
T ₃	Sett treatment with 100 ppm melatonin	14.76	16.83	17.97	16.52	7.30	7.40	7.88	7.52
T ₄	Foliar spray of 100 ppm melatonin at 30 days after planting	19.20	21.74	23.51	21.48	8.03	8.35	8.97	8.45
T ₅	Foliar spray of 100 ppm melatonin at 60 days after planting	14.18	14.87	14.35	14.80	7.52	7.63	7.91	7.68
T ₆	Foliar spray of 100 ppm melatonin at 30 and at 60 days after planting	16.81	14.01	13.83	14.88	7.72	7.97	8.46	8.05
T ₇	Sett treatment+ Foliar spray of 100 ppm melatonin at 30 days after planting	17.94	15.28	15.02	16.08	7.68	7.93	8.42	8.01
T ₈	Sett treatment+ Foliar spray of 100 ppm melatonin at 60 days after planting	16.33	16.17	15.81	16.10	7.37	7.52	7.97	7.62
T ₉	Sett treatment+ Foliar spray of 100 ppm melatonin at 30 and 60 days after planting	21.81	19.08	18.76	19.88	7.94	8.22	8.71	8.29
	Mean	17.52	17.59	18.27	17.75	7.87	7.83	8.16	7.95
	SEd	0.46	0.47	0.49		0.20	0.20	0.21	
	CD (0.05)	0.96**	0.98**	1.03**		0.43**	0.44**	0.45**	

NS- Non significant *- Significant **- Highly significant

45 DAP: 15 days after 1st spray, 75 DAP: 15 days after 2nd spray, 135 DAP: Recovery stage

Table 2: Effect of melatonin on stomatal conductance ($\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$) of cassava under salt Stress condition

Treatments		Stomatal conductance ($\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$)			
		45 DAP	75 DAP	135 DAP	Mean
T ₁	Absolute Control (without salt and melatonin)	0.48	0.82	0.94	0.74
T ₂	Control (with salt)	0.32	0.36	0.45	0.37
T ₃	Sett treatment with 100 ppm melatonin	0.31	0.34	0.50	0.38
T ₄	Foliar spray of 100 ppm melatonin at 30 days after planting	0.42	0.53	0.84	0.59
T ₅	Foliar spray of 100 ppm melatonin at 60 days after planting	0.31	0.32	0.52	0.38
T ₆	Foliar spray of 100 ppm melatonin at 30 and at 60 days after planting	0.35	0.40	0.64	0.46
T ₇	Sett treatment+ Foliar spray of 100 ppm melatonin at 30 days after planting	0.36	0.41	0.62	0.46
T ₈	Sett treatment+ Foliar spray of 100 ppm melatonin at 60 days after planting	0.33	0.38	0.56	0.42
T ₉	Sett treatment+ Foliar spray of 100 ppm melatonin at 30 and 60 days after planting	0.38	0.44	0.73	0.51
	Mean	0.36	0.44	0.64	0.48
	SEd	0.18	0.19	0.20	
	CD (0.05)	0.38**	0.40**	0.43**	

NS- Non significant *- Significant **- Highly significant

45 DAP: 15 days after 1st spray, 75 DAP: 15 days after 2nd spray, 135 DAP: Recovery stage

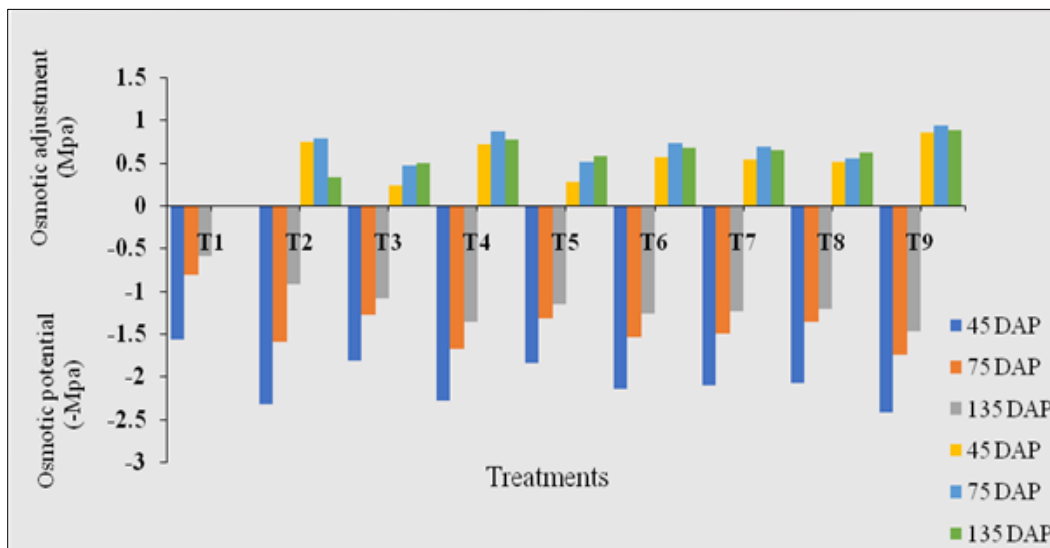


Fig 1: Effect of melatonin on osmotic potential (-MPa) and osmotic adjustment (MPa) of cassava under salt stress condition

Table 3: Effect of melatonin on Tuber yield plant⁻¹ of cassava under salt stress condition

Treatments		Tuber yield (kg plant ⁻¹)
T ₁	Absolute Control (without salt and melatonin)	5.21
T ₂	Control (with salt)	2.13
T ₃	Sett treatment with 100 ppm melatonin	2.56
T ₄	Foliar spray of 100 ppm melatonin at 30 days after planting	4.01
T ₅	Foliar spray of 100 ppm melatonin at 60 days after planting	2.48
T ₆	Foliar spray of 100 ppm melatonin at 30 and at 60 days after planting	3.05
T ₇	Sett treatment+ Foliar spray of 100 ppm melatonin at 30 days after planting	3.12
T ₈	Sett treatment+ Foliar spray of 100 ppm melatonin at 60 days after planting	2.73
T ₉	Sett treatment+ Foliar spray of 100 ppm melatonin at 30 and 60 days after planting	3.95
Mean		3.25
SE d		0.08
CD (0.05)		0.17**
NS- Non significant *- Significant **- Highly significant		

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