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Assessment of physiological efficiency of cassava genotypes under salt stress

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Abstract
The present investigation was carried out during 2019-2020 at Tapioca and Castor Research Station, Tamil Nadu Agricultural University, Yethapur to assess the physiological efficiency of cassava genotypes under salt stress. The experiment was initiated with cassava YTP2 and TCMS 7 (salt tolerant), H226 and Kunguma rose (moderately salt tolerant) and Sree Athulya (salt susceptible) with two level of salt treatments *viz*., control and 120mM NaCl. Cement structures were established with the height of 20cm and width of 30cm for adding salt water. Only one plant was maintained in each structure. Salt water treatments were imposed from day one of planting to 120 days after planting once in three days. Experiment was replicated five times and followed Completely Randomized Factorial Design. The results revealed that, cassava genotype YTP2 recorded maximum super oxide dismutase, catalase and peroxidase activity. Higher osmotic adjustment of 1.9 Mpa and 1.7 Mpa was seen in YTP2 and H226 leaves respectively. With respect to osmotic potential, more reduced potential was observed in TCMS7 (-3.8 Mpa) followed by YTP2 (-3.6 Mpa) and also minimum reduction on tuber yield was observed in YTP2 (17.3 %) under salt stress.

Keywords: cassava, salt stress, superoxide dismutase, catalase, peroxidase, osmotic potential

1. Introduction
Soil salinity is a major stress that limits production of food crops. Salt tolerance has been widely and intensively studied on a wide array of other plants (Ibrahim, 2016) [15]. It is partially associated with osmo protectants such as sugars, proline and endogenous phytohormone (You and Chan, 2015) [22]. Cassava is one of the most important tropical food crops due to its high starch content of the roots (Alves, 2002) [1]. This crop can grow in marginal areas where other crops fail, where the soil is infertile and climatic conditions are very harsh with low rainfall (Bull *et al.*, 2011) [6] and has its own inherent tolerance to stressful environments (ElSharkawy, 2004) [10] and considered as abiotic stresses-tolerant crop (Ceballos *et al*., 2004) [7]. In Tamil Nadu, cassava (*Manihot esculenta* Crantz.) is mainly cultivated for sago and starch. It is called as a "famine reserve crop" since it can cultivate on poor soil where other crops not grow well.In India, Kerala and Tamil Nadu account for about 80% of the total acreage of the crop in India. Cassava is the most widely grown root crop in the world and the tuberous roots are the principle source of calories for many of the world's poorest people (Nweke *et al.*, 2002; FAO, 2014) [11,18]. This crop is being cultivated in large scale in Salem, Namakkal, Erode, Cuddalore, Villupuram, Dharmapuri and Kanyakumari districts of Tamil Nadu.
During irrigation, salt deposition takes place in the soil resulting in unproductive condition (Church *et al*., 2013; Nunn, 2013) [8, 17]. Salinization of groundwater is becoming an increasing problem in many parts of the cassava growing area in Tamil Nadu. The released varieties and farmers collections are low tuber and starch yielding and highly susceptible to salt stress. Evaluation of suitable salt-tolerant accession is necessary if cassava is continued to expand its role as a staple in a future, more saline world. The salt tolerant cassava accessions will be highly useful for cultivation in parts of Salem, Namakkal and Erode districts of Tamil Nadu in the changing scenario. Based on this background, this work has been carried out to identify suitable cassava genotypes for salt tolerance with high tuber yield and starch content.

2. Materials and Methods
Experiment was initiated with YTP2 and TCMS 7 (salt tolerant), H226 and Kunguma rose (moderately salt tolerant) and Sree Athulya (salt susceptible) during April '2019 with two level of salt treatments *viz*., control and 120mM NaCl. Cement structures were established with the height of 20cm and width of 30cm for adding salt water. Only one plant was maintained in each structure. Salt water treatments were imposed once in three days from day one of planting to 120 days after planting as per the method followed by Ros Gleadow *et al.* (2016) [20]. Structures were flushed weekly with water to prevent accumulation of salt in the soil. Recommended package of practices for cassava was followed. After 120 days of planting, biochemical parameters were assessed.
SOD activity was determined by using nitrobluetetrazolium (NBT) salt as described by Beau-champ and Fridovich (1971) [4] and expressed in enzyme unit mg protein^{-1}min^{-1}. Peroxidase activity was determined as per Nakano and Asada method (1981) [16] and expressed in enzyme unit min^{-1} g^{-1} of fresh weight. Catalase activity was determined as per Gopalachari (1963) [14] method and expressed in enzyme unit µg of H_2O_2 g^{-1} min$^{-1.}$ Osmotic potential was estimated by adopting method of Babu *et al*. (1999) [2].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 -80ºc 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 (ψ_s) was calculated as, ψ_π = -c RT, 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 (in Mpa).

Osmotic potential = [(Osmolality mmol kg^{-1}) (0.0832) (310)] / 10000

Osmotic adjustment was calculated as the difference between the turgid potential in the well watered treatment and stress treatment (Babu *et al*. 1999) [2]. The data collected on the different characters from field experiments were statistically analyzed in a FCRD (Factorial Completely Randomized Design) as suggested by Gomez and Gomez (1992) [13]. The critical difference (CD) was computed at five per cent probability.

3. Results and Discussion

The result of superoxide dismutase, catalase and peroxidase activities of cassava leaves under control and salt stressed situations were presented in Table 1. When compared to unstressed leaves, salt stressed leaves recorded less antioxidant enzyme activity with irrespective of genotypes. Superoxide dismutase activity was increased under saline condition in all the genotypes but YTP2 variety was recorded maximum activity of 14.52 units mg^{-1} protein. Catalase activity has inverse relation with hydrogen peroxide, so it is expressed as remaining unbroken H_2O_2. Significant difference was observed among the treatments. Salt stress increased the catalase and peroxidase activity in all the genotypes. Remaining H_2O_2 was minimum in YTP2 (83.88 µg H_2O_2 g^{-1} min^{-1}) followed by TCMS 7 (102 µg H_2O_2 g^{-1} min^{-1}) under salt stress which denotes maximum catalase activity. Among the genotypes, YTP2 recorded highest peroxidase activity ((1.82 change in OD at 430 nm g^{-1} min^{-1}) in salt stressed leaves followed by TCMS7 (1.33 change in OD at 430 nm g^{-1} min^{-1}). Irrespective of the genotypes, low catalase and peroxidase activity was noticed in control plants. Catalase is an important antioxidant enzyme that plays a significant role in salinity stress condition in omitting H2O2 and other harming factors (Bassuany *et al.*, 2014) [3]. The gradual increase of catalase rate due to the increase of salinity stress has been reported in three flax genotypes (El-(Bassiouny, *et al.*, 2015) [9]. This increase was higher in the tolerant genotype of comparing to the other two genotypes. Studies have shown that to deal with the toxic effects of oxidative stress, the plants used a series of mechanisms and using peroxidase and catalase enzyme system are two main systems in order to prevent cellular damages due to the stress (Sridevi and Venkatesan, 2009) [21]. In accordance with the results in this study, other studies also show that this enzyme functions as a defense mechanism in plants under stress condition (Pourakbar and Holasso, 2015) [19]. The earlier findings corroborated well with the present study

Osmotic potential and osmotic adjustment were observed in the leaves of control and salt stressed plants and presented in Table 2. Osmotic potential and osmotic adjustment have inverse relationship under stress. More osmotic adjustment of 1.9 Mpa and 1.7 Mpa was seen in 120 mM NaCl treated cassava YTP2 and H226 leaves respectively. With respect to osmotic potential, more reduced potential was observed in TCMS7 (-3.8 Mpa) followed by YTP2 (-3.6 Mpa) under salt stress. Unstressed control plants recorded osmotic potential ranging from -1.6 to -1.9 MPa. Effect of salt on number of tubers, tuber yield and starch content was recorded for cassava genotypes and presented in Table 3. All the genotypes recorded high number of tubers, tuber yield and starch content in control which was reduced under salt stress condition. Minimum reduction on tuber yield was observed in YTP2 (17.3 %) with starch content of 25.5 per cent respectively. Among the genotypes, TCMS 7 recorded minimum tuber yield of 2.19 kg $plant^{-1}$ and minimum starch content in Kungumarose (19 %) followed by Sree Athulya (2.84 kg $plant^{-1}$). The major contributors for maintaining the cellular osmotic potential are Na^+ and Cl^- (Flowers *et al*., 2015) [12]. The tolerant variety accommodates high concentrations of Na^+ and Cl^- in tissues by intracellular compartmentation and the synthesis of compatible solutes. The bulk of the ions are compartmentalized within vacuoles and organic solutes such as sucrose, sugar alcohols, proline, and glycinebetaine are accumulated by halophytes and most probably contribute to osmotic adjustment in the cytoplasmic compartments of vacuolated cells rather than the whole cell (Flowers *et al.*, 2015) [12].This might be the reason for YTP2 adjusted osmotically and recorded less reduction in tuber yield and starch content. The results obtained in the present investigation coincides the findings of previous studies.

Table 1: Effect of salt stress on physiological parameters

Genotypes / Treatments	Superoxide dismutase Units mg^{-1} protein			Catalase (µg of H_2O_2 g^{-1} min^{-1})			Peroxidase (change in OD at 430 nm g^{-1} min^{-1})		
	Control		120mM NaCl	Control		120mM NaCl	Control		120mM NaCl
TCMS 7				93.50		102.02	1.23		1.33
Me 681	14.52		17.44	81.08		83.88	1.42		1.82
H226	12.06		15.42	95.60		114.83	1.21		1.24
Kungama Rose	13.12		15.89	72.30		132.5	1.56		1.03
Sree Athulya	13.5		13.6	87.40		159.2	1.65		0.96
	T	V	T X V	T	V	T X V	T	V	T X V
SEd	0.33	0.23	0.46	0.759	1.200	1.697	0.009	0.015	0.022
CD(P =0.05)	0.66	0.46	0.94	2.05	3.24	4.591	0.02	0.03	0.044

Table 2: Effect of salt stress on osmotic potential and osmotic adjustment

Genotypes / Treatments	Osmotic potential (-Mpa)			Osmotic adjustment (Mpa) 120mMNaCl		
	Control	120mM NaCl				
TCMS 7	-1.8	-3.8		2		
Me 681	-1.9	-3.6		1.7		
H226	-1.5	-2.9		1.4		
Kungama Rose	-1.6	-2.7		1.1		
Sree Athulya	-1.8	-2.1		0.3		
	T	V	T X V	T	V	T X V
SEd	0.01	0.02	0.03	0.003	0.007	0.010
CD(P =0.05)	0.02	0.04	0.05	0.007	0.015	0.022

Table 3: Effect of salt stress on tuber yield and starch content

Genotypes / Treatments	No of tubers plant^{-1}			Tuber yield (kg Plant $^{-1}$)			Starch (%)		
	Control		120mM NaCl	Control		120mM NaCl	Control		120mM NaCl
TCMS 7	7.0		4.5	5.85		2.19	25.0		19.0
Me 681	6.5		5.5	6.30		5.21	29.0		25.5
H226	5.5		4.5	5.42		4.20	27.0		18.0
Kungama Rose	6.0		5.0	5.50		4.15	28.0		17.0
Sree Athulya	6.0		4.0	5.10		2.84	27.0		15.0
	T	V	T X V	T	V	T X V	T	V	T X V
SEd	0.03	0.06	0.08	0.03	0.05	0.07	0.16	0.26	0.38
CD (P =0.05)	0.08	0.12	0.18	0.07	0.11	0.15	0.34	0.54	0.768

4. Conclusion

The data presented in this work cassava YTP2 tolerate extreme saline conditions by reduce ROS production. Our results clearly indicate that high salinity induced production of O^{2-} is scavenged by constitutively higher enzyme activity of superoxide dismutase. Increased activity of superoxide dismutase induces an overproduction H_2O_2 which is counter balanced by increased activity of catalase and high level of peroxidase activity thereby maintaining appropriate levels of H_2O_2. The coordinated changes in the activity of various antioxidative enzymes efficiently scavenge salinity induced ROS production thereby protecting the membrane integrity. In general, considering the results of this study, it could be concluded that the salinity stress leads to component change of biochemical factors in different cassava genotypes and this change benefits the plant in tolerant genotypes while it leads to cellular damage in sensitive genotypes. Our data revealed that sustainable utilization of YTP2 as a genetic resource can lead to develop salt tolerant crops by genetic engineering or breeding strategies.

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