Asian Journal of Biochemistry, Genetics and Molecular Biology

6(4): 39-45, 2020; Article no.AJBGMB.59675
ISSN: 2582-3698

Effect of Salinity Stress on Enzymatic Antioxidants Defense System of Two Maize (*Zea mays* L.). Varieties

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Authors' contributions

This work was carried out in collaboration among all authors. Author ZS performed data mining and all experimental work. Author ALA helped author ZS in experiments and manuscript writing. Authors MM, AML, ZHB and SS managed the analyses of the study and literature searches. All authors read and approved the final version of the manuscript.

Article Information

DOI: 10.9734/AJBGMB/2020/v6i430161
Editor(s):
(1) Dr. Stephen M. Ghogomu, University of Buea, Cameroon.
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Reviewers:
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(3) Naveed Kamal, Maize Breeding Sub Station, Pakistan.
Complete Peer review History: http://www.sdiarticle4.com/review-history/59675

Original Research Article

Received 20 July 2020
Accepted 23 September 2020
Published 21 January 2021

ABSTRACT

Maize is an economically important cereal crop cultivated worldwide depending on suitable climate condition. maize production is hindered by biotic and abiotic factors. Salinity is one of the major factors that affect maize yield. Plant respond to salinity by changes in their antioxidant enzymes activities which include Catalase (CAT), Superoxide dismutase (SOD), Peroxidase (POX), ascorbate peroxidase (APX) and glutathione reductase (GR). In the present study, maize seedlings were subjected to salinity stress at a different concentrations of 50 mM, 100 mM and 150 mM NaCl and were watered regularly with normal pure water. Root, mature leaves and young leaves were collected after 21 days of sowing and antioxidant enzyme activities in the collected samples were

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assayed using enzymatic method. A significant (P<0.05) increase in MDA and H_2O_2 concentration was observed in the roots and young leaves for NaCl-treated samples as compared to the control. Activities of CAT increased significantly (P<0.05) in all organs (root, mature leaves and young leaves) of salt treated maize seedling, while SOD and POX increased specifically in mature leaves. This indicates a possible role of reactive oxygen species (ROS) in the systemic signalling from roots to leaves, allowing leaves to activate their defence mechanism for better protection against salt stress.

Keywords: Salinity stress; maize; enzyme; antioxidant; ROS.

1. INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereals grown worldwide in a wider range of environments because of its greater adaptability Kogbe and Adediran, [1]. Maize is a good source of carbohydrate, vitamins, minerals and dietary fiber Allen and Ort, [2]. It is mainly used as a food source and has become the most important raw material for animal feed Pooja and Rajesh, [3]. Maize are glycophyte species and generally show limited growth and development due to soil salinity Ashraf and Harris, [4]. It is an economically-important crop and its production is affected by soil salinity in various parts of the world (Konopka et al., 2009).

Salinity is defined as the presence of an excessive concentration of salt in the soil which suppresses plant growth and productivity Zaki, [5]. Salinity stress induces a multitude of responses in plants including morphological, physiological, biochemical and molecular changes Ambede et al. [6]. It causes ionic imbalance, which result in ionic toxicity, osmotic stress, and generation of reactive oxygen species (ROS) Chaparzadeh et al. [7]. One of the biochemical changes occurring in plant subjected to environmental stress condition is the production of ROS Munne-Bosch, [8]. ROS attack protein, nucleic acids and lipid, and the degree of damage depends on the balance between formations of ROS (Vranova et al., 2002) [9].

The ability of plants to cope with salinity stress is an important determinant of crop distribution and productivity in many areas, so it is important to understand the mechanisms that confer tolerance to saline environment Gilbert et al. [10]. High salinity induces the formation of ROS within plant cells and to scavenge high ROS levels, an efficient system of non-enzymatic and enzymatic antioxidant is required Apel and Hirt, [11]. Non-enzymatic antioxidants include phenolics, flavonoids, tocopherols, ascobate and glutathione Munne-Bosch, [8]; (Rai et al. 2013), SOD, POX, CAT and APX (GR) that detoxify ROS Gill and Tuteja, [12]. Antioxidants could be used as potential growth regulator to improve salinity stress resistance in several plant species Gunes et al. [13]. These Antioxidant enzymes are very good biochemical makers of stress and their increased activity my attest the potential for remediation Vangronsveld and Clijstrs, [14].

Different organs and/or leaf tissues of different developmental stages may respond differently to salinity stress and trigger specific defences mechanisms. Roots are the first organs to encounter salinity stress and show greater reduction in growth than shoots (Lazof and Bernstein, 1999). Moreover, for some developmental stages, including germinating seeds, young leaves, and matured leaves are more sensitive to salt stress, and therefore the effects of high salinity depends on the developmental stages Houle et al. [15]. However, differences in oxidative stress and antioxidant defences, in different organs and developmental stages of plant species are much less studied, therefore the aim of this study was to evaluate the effect of salinity stress on the antioxidant defence responses of roots, mature leaves, and young leaves of maize cultivars (Oba super 2 16-11-kd-155-159 and Sammaz 37) cultivated at various NaCl concentrations.

2. MATERIALS AND METHODS

2.1 Plant Material and Stress Treatment

Two maize cultivars (Oba super 2 16-11-kd-155-159 and sammaz-37) used in this study were obtained from Sokoto Agricultural Development Project (SADP), Sokoto State, Nigeria. Seeds were directly sown into sandy soil (85.9 % sand, pH 6.34) initially containing 0.64 % carbon, 0.060 kg nitrate-nitrogen, 0.3 9 kg sodium, 0.82 kg potassium, 5.8 kg cation exchange capacity, 0.45 kg calcium and 0.50 kg magnesium. Germination

was carried out in the Botanical Garden of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto. Daily salinity irrigation treatments were applied as sodium chloride (NaCl) solution at concentrations of 0.00, 50, 100 and 150 mM NaCl. Each treatment was replicated three times daily. Roots, mature and and young leaves were harvested after 21 days of salt treatment and samples were immediately taken for analysis.

2.2 Enzyme Assay

0.5 g of each fresh tissue (young leaves, matured leaves and roots) were harvested from 21 days old salt-treated and non-treated maize seedling were washed and homogenized with a motar and pestle in a 3 ml ice-cold 100 mM potassium phosphate buffer pH 7.6 containing 0.1 mM EDTA for 5 min. After filtration, the homogenate was centrifuged at 3000 rpm for 15 min and the supernatant was used for the determination of antioxidant enzyme activities.

2.3 Determination of Enzymatic Antioxidant Activity

2.3.1 Superoxide dismutase (SOD)

SOD was assayed according to the method of Velikova et al. [16]. Briefly, to the supernatant 0.2 ml of the enzyme extract was added, followed by 1.0 ml of potassium phosphate buffer (pH 7.0) and 0.83 ml distilled water in a test tube. The mixture was incubated at 25^0C for 10 min. and 0.02 ml of pyrogallol was also added to the mixture which was then transferred into a cuvette and change in absorbance was read at 420 nm after 3 min.

2.3.2 Catalase (CAT)

Activity of catalase was determined according to the method of Aebi [17]. Crude enzyme preparation was added to 1.0 ml of potassium phosphate buffer (pH 7.0), 0.1 ml of H_2O_2 and 0.1 M EDTA. This mixture was incubated at standard temperature for 3 min, mixed by inversion and absorbance was measured at 240 nm.

2.3.3 Peroxidase (POX)

Activity of peroxidase was determined according to the method of Kar and Mishra, [18]. Peroxidase activity of roots, young leaves and mature leave tissues of maize was determined by adding 1.0 ml of the enzyme extract to 2.40 ml of 0.1 M potassium phosphate buffer pH 6.3, 0.30 ml of pyrogallol and 0.2 ml H_2O_2 in a test tube. The mixture was incubated for 5 min of 25^0C. and the amount of purpurogallin formed was determined by measuring the absorbance at 420 nm.

2.4 Determination of Lipid Peroxidation Marker

2.4.1 Malondialdehyde (MDA)

MDA was determined using the method of Hodges et al. [19]. Fresh roots, young leaves and mature leaves (0.5 g) each were homogenized in 1.0% metaphosphoric acid, incubated at room temperature for 30 min. and 1.0 ml of 10 % trichloroacetic acid (TCA) added and centrifuged at 2000 rpm for 15 min. The supernatant was used for the assay.

1.0 ml of the supernatant was mixed with 1.0 ml of 5 % thiobarbituric acid (TBA) in the test tubes, followed by addition of equal volume of 40% trichloroacetic acid. The mixture was placed in boiling water bath for 30 min at 95 ^{0}C. The samples were allowed to cool at room temperature and then the absorbance was measured at 532 nm.

2.5 Hydrogen Peroxide (H_2O_2)

Hydrogen peroxide content was estimated according to the method of Velikova et al. [16]. Fresh tissues of roots (0.5 g), mature and young leaves were homogenized in 5.0 ml of 0.1% (w/v) trichloroacetic acid (TCA) in an ice bath. The homogenate was centrifuged at 3000 rpm for 15 min. Then 0.5 ml of the supernatant was made up to 1 ml with 10 mM potassium Phosphate buffer (pH 7.0) to which 1 ml of 1.0M KI was added. The absorbance was measured at 390 nm

2.6 Procedure

2.6.1 Statistical analysis

Statistical analysis was carried out using the Instat software. Parameters were analysed statistically by one-way analysis of variance (ANOVA). Results were presented as Means±SEM and significant difference between

means ($p<0.05$) was established using the Duncan multiple range test.

3. RESULTS AND DISCUSSION

Adverse effects of Salinity in plants include reduction in overall growth and productivity due to perturbation of various physiological and biochemical parameters, conveniently, a number of such parameters have saved as markers of stress response, as well as, indicators of the severity of stress. To contribute to our understanding of the mechanisms underlying salinity stress responses, maize seedlings were exposed to three different salinity levels (50, 100 and 150 mM), and investigate responses in roots, mature leaves (M.L) and young leaves (Y.L). Salinity tolerance is an important trait for plants such as maize that grows in arid and semi-arid areas where water has high concentrations of salts Yaish and Kumar, [20]. Plant species and cultivars vary in their ability to tolerate salinity, due to changes in their genetic and epigenetic makeup which took a long time to evolve Yaish, [21]. Tolerance may involve a single mechanism or several mechanisms, such as the ability to avoid salts in the soil, the ability to compartmentalize Na^+ ions among different tissues and cells, or the ability to deal with the consequences of excessive amounts of salt in cells by producing additional quantities of antioxidants Munns and Tester, [22]. This study focused on the role of antioxidants in salt tolerance in two varieties of maize. The salt tolerance in (Oba super 2 16-11-kd-155-159 and sammaz-37) may also involve a high activity of SOD, CAT and POX when the seedlings are exposed to salinity.

Malondialdehyde and Hydrogen peroxide: Salinity has been reported to induce oxidative stress in different plants and tissues (Ashraf and Harris, 2004; Chawla et al. [23]. Under salinity stress, the levels of ROS increase in the plant tissues as a result of irregularities in the electron transport chain and accumulation of photo reducing power. One of the early changes in plants physiology and metabolism during abiotic stresse such as salinity is the production of reactive oxygen species (ROS) which include O_2^- , H_2O_2, and $OH.^-$ Among these, H_2O_2 appears to be one of the earliest stress signalling factor, and a stable ROS intermediate Gill and Tujeta, [12]. This study showed increase in MDA and hydrogen peroxide (H_2O_2) in root, mature leaves and young leaves of both the two varieties. Tatar and Gevrek [24] have also reported high MDA content with increase in the degree of salt stress in wheat. However, another study reported by Weisany et al. (2012) showed an increase in MDA and H_2O_2 levels in soybeans.

Superoxide dismutase SOD plays central role in defence against oxidative stress; it is the most effective intracellular enzymatic antioxidant because it catalyses the dismutation of superoxide to molecular oxygen and hydrogen peroxide Racchi et al. [25]. SOD activity has been reported to increase in plants exposed to various environmental stresses, including salinity Sharma and Dubey, [26]. Increased SOD activity indicates tolerance of the plant against environmental stresses Noctor and Foyer, [27]. In this study, salt stress resulted in an increase in SOD activity in mature leaves of both the two varieties (Oba super and sammaz 37) which is similar with the findings of Chawla et al. [23] where they reported increased SOD levels in leaves of tolerant rice cultivar under salinity. The result of the present study also showed decrease in SOD activity in root and young leaves of Oba super and sammaz 37 variety with respect to the control which is similar to the result of Chorianopoulou et al. (2012) that had also reported a decrease in SOD activity in root and leaf sheaths of maize under salinity stress.

Catalase: catalase catalyzes the dismutation of H_2O_2 into water and oxygen Corpas et al. [28]. It has been reported that environmental stresses cause enhancement of CAT activity. In this study, an increase in CAT activity under salinity stress was observed in the roots, young and mature leave of both the two varieties. CAT has been reported by Gao et al. [29] to be a major enzymatic antioxidant in radicles of *Jatropha curcas* L. challenged with salinity, especially under moderate salinity levels of 50 to 100 mM NaCl, compared to *hypocotyls and cotyledons*. Similarly, Chaparzadeh et al. (2004) that showed increased CAT activity in leaves of *Calendula offıcinalis* under NaCl concentrations.

Peroxidase: POX are a large group of enzymatic antioxidants which play a role in various biological processes. They are named after the fact that they commonly break up peroxide (Atamna, 2006). Results of the present study reveal an overall decrease in POX activity in response to salinity stress which is similar to the report of Chaparzadeh et al. (2004) in their where POX activity did not increase in either leaves or roots of *Calendula offıcinalis* under salinity stress.

Table 1. MDA and H_2O_2 (µg/g) levels of two varieties of maize exposed to different concentrations of NaCl for 21 days

Stress markers	NaCl (mM)	OBA SUPER 2			SAMMAZ 37		
		Root	Young Leaf	Mature Leaf	Root	Young Leaf	Mature Leaf
	0	0.153 ± 0.015	0.138 ± 0.002	0.228 ± 0.002	0.016 ± 0.002	0.104 ± 0.002	0.153 ± 0.015
MDA	50	0.569 ± 0.213^b	0.523 ± 0.015^b	0.523 ± 0.015^b	0.092 ± 0.025	0.511 ± 0.007^b	0.456 ± 0.005^b
(µg/g)	100	0.736 ± 0.002^b	1.195 ± 0.003^b	0.740 ± 0.005^b	0.37 ± 0.015^b	1.155 ± 0.002^b	0.642 ± 0.004^b
	150	1.156 ± 0.001^b	2.072 ± 0.006^b	1.809 ± 0.004^b	1.349 ± 0.010^b	1.525 ± 0.001^b	0.974 ± 0.002^b
	0	0.039 ± 0.000	0.042 ± 0.001	0.037 ± 0.001	0.037 ± 0.000	0.042 ± 0.001	0.038 ± 0.005
H_2O_2	50	0.038 ± 0.001	0.048 ± 0.002	0.037 ± 0.000	0.038 ± 0.003	0.048 ± 0.000	0.044 ± 0.003^b
(µg/g)	100	0.038 ± 0.001	0.051 ± 0.002^b	0.044 ± 0.001^b	0.039 ± 0.000	0.052 ± 0.001^b	0.051 ± 0.002^b
	150	0.042 ± 0.001^b	0.054 ± 0.002^b	0.051 ± 0.001^b	0.045 ± 0.001^b	0.053 ± 0.000^b	0.056 ± 0.001^b

Values are expressed as mean ± SEM of three replicates. And significant difference between the means, as determined by Duncan test ($p < 0.05$), are indicated by different superscript letters compared to control

Table 2. SOD, CAT and POX activity (unit/ml) of two varieties of maize exposed to different concentrations of NaCl for 21 days

Stress makers	NaCl (mM)	OBA SUPER 2			SAMMAZ 37		
		Root	Young Leaf	Mature Leaf	Root	Young Leaf	Mature Leaf
	0	22.447 ± 0.023^a	44.473 ± 0.014^a	4.823 ± 0.047^a	89.232 ± 0.667^a	89.232 ± 0.667^a	14.640 ± 0.021^a
SOD	50	4.267 ± 0.033^b	2.053 ± 0.023^b	22.447 ± 0.023^c	89.232 ± 0.667^a	22.447 ± 0.023^b	22.447 ± 0.023^c
(unit/ml)	100	2.453 ± 0.098^b	17.760 ± 0.030^b	44.413 ± 0.018^c	44.430 ± 0.012^b	22.447 ± 0.023^b	44.430 ± 0.012^c
	150	44.430 ± 0.012^c	89.232 ± 0.667^a	89.232 ± 0.667^c	89.232 ± 0.667^a	89.233 ± 0.667^a	89.232 ± 0.667^c
	0	2204.1 ± 47.933^a	2204.1 ± 47.933^a	4800.0 ± 50.000^a	3049.2 ± 87.100^a	5716.7 ± 91.667^a	4850.0 ± 50.000^a
CAT	50	3993.0 ± 159.730^c	3993.0 ± 159.730^c	5202.4 ± 273.800^c	4723.2 ± 205.370^c	5753.3 ± 3.333^a	5800 ± 50.000^c
(unit/ml)	100	4723.2 ± 205.370^c	3993.0 ± 159.730^c	5202.4 ± 273.800^c	4928.6 ± 0.000^c	5753.3 ± 3.333^a	5800.0± 50.000^c
	150	5756.0 ± 6.000^c	4928.6 ± 0.000^c	6516.7 ± 383.333^c	8625.0 ± 0.000^c	6900.7 ± 0.667^c	6900.0 ± 0.667^c
	0	7.557 ± 0.007	6.363 ± 0.007	5.875 ± 0.007	4.827 ± 0.007	10.180 ± 0.010	5.063 ± 0.007
POX	50	7.110 ± 0.005	6.070 ± 0.006	5.010 ± 0.005	4.193 ± 0.012	15.267 ± 0.033^b	7.733 ± 0.012^b
(unit/ml)	100	5.420 ± 0.005^a	6.273 ± 0.003	4.320 ± 0.006	4.307 ± 0.007	8.290 ± 0.010^a	4.333 ± 0.009
	150	4.507 ± 0.007^a	5.680 ± 0.005	7.390 ± 0.010^b	3.920 ± 0.006	4.267 ± 0.016^a	4.207 ± 0.007

Values are expressed as mean ± SEM of three replicates. And significant difference between the means, as determined by Duncan test ($p < 0.05$), are indicated by different superscript letters

4. CONCLUSION

This study revealed that the antioxidants system invoked by root, mature leaves and young leaves of Oba super 2 and sammaz 37 maize seedling under salt stress comprise of enzymatic (CAT, SOD and POX). These biochemical events thus make the maize seedling tolerant to salinity stress up to 150 mM.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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