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EFFECTS OF PRIMING ON GERMINATION AND BIOCHEMICAL ATTRIBUTES OF THREE MAIZE LINES UNDER NaCl STRESS CONDITION

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ABSTRACT

The adverse effect of salinity has been established to vary between different varieties of the same plant species. This study was therefore conducted to determine the tolerability of three newly released lines of maize to saline conditions when primed with ethylene diamine tetraacetic acid (EDTA) and salicylic acid (SA) each at 1.0 M concentration; seeds primed with distilled water were used as control. Concentrations of 0, 50, 100, 150 and 300 mM NaCl were tested. The experiment followed a 3x3x5 factorial arrangement of treatments with three replications. The results revealed significant interaction between maize lines and salinity where the germination percentage of line SWAN-LSR-Y was much more affected than the other lines at the salinity level of 50 mM, and its speed of germination was more affected than the others when passing from 150 to 300 mM NaCl. The seedling lengths (radicle and plumule) and seed vigor index were influenced by significant interaction between the primer and salinity, where EDTA enhanced better seedling growth than the other primers when the salinity did not exceed 150 mM. OMR-LSR-SY maize line did not show tolerability at this concentration. Similarly, EDTA and SA treated maize lines showed lower accumulation of reactive oxygen species such as superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), as well as a decrease in the malondialdehyde (MDA) contents, most importantly in SWAN-LSR-Y and BR9928-OMR-SR-Y maize lines. Catalase (CAT) and superoxide dismutase (SOD) activities were enhanced in SWAN-LSR- Y and BR9928-OMR-SR-Y upon application of EDTA.

Additional keywords: EDTA, salicylic acid, salinity, *Zea mays*

RESUMEN

Efecto de impregnar la semilla con EDTA en la germinación y atributos bioquímicos en tres líneas de maíz bajo estrés de NaCl

Se ha demostrado que los efectos adversos de la salinidad pueden variar entre distintas variedades de una misma especie vegetal. Este estudio se realizó para determinar la tolerabilidad de tres líneas de maíz recién liberadas a condiciones salinas cuando se impregnaron con ácido etilendiaminotetraacético (EDTA) y ácido salicílico (SA), cada uno a una concentración de 1,0 M; como control se usaron semillas humedecidas con agua destilada. Se probaron concentraciones salinas de 0, 50, 100, 150 y 300 mM de NaCl. El experimento tuvo un arreglo factorial de tratamientos de 3x3x5 con tres repeticiones. Los resultados revelaron una interacción significativa entre las líneas de maíz y la salinidad donde el porcentaje de germinación de la línea SWAN-LSR-Y fue

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mucho más afectado que las otras líneas al nivel de salinidad de 50 mM, y su velocidad de germinación fue más afectada que las otras al pasar de 150 a 300 mM de NaCl. Plántulas (radícula y plúmula) y el índice de vigor de la semilla fueron influenciados por una interacción significativa entre el agente impregnante y la salinidad, donde EDTA incrementó el crecimiento de las plántulas más que los otros impregnantes cuando la salinidad no sobrepasó l50 mM. La línea de maíz OMR-LSR-SY no mostró tolerabilidad a esta concentración. De manera similar, las líneas de maíz tratadas con EDTA y SA mostraron una menor acumulación de especies reactivas de oxígeno, como el radical anión superóxido (O_2^-) y el peróxido de hidrógeno (H_2O_2), así como una disminución en los contenidos de malondialdehído (MDA), principalmente en las líneas SWAN-LSR-Y y BR9928-OMR-SR-Y. Las actividades de la catalasa (CAT) y la superóxido dismutasa (SOD) se incrementaron en SWAN-LSR-Y y BR9928-OMR-SR-Y luego de la aplicación del EDTA.

Palabras-clave adicionales: Ácido salicílico, salinidad, *Zea mays*

INTRODUCTION

Maize or corn (*Zea mays* L.) is a very important food cereal crop cultivated globally. The crop ranks the third most important cereal following wheat and rice in terms of area of production (Tian et al., 2014). In nature, plants are continuously subjected to multiple environmental stresses during their different phases of growth (Zhu et al., 2021). Salinity is one of the deleterious abiotic stresses which inhibits crop growth and development (Raja et al., 2021). Salt stress has great effects on plant functioning and metabolism and significantly impedes productivity (Valenzuela et al., 2022).

Under elevated salt concentration, the capacity of crops to absorb water decreases resulting in rapid decrease in growth rates. Thus, salinity is a crucial limitation to food production as it decreases crop yields and reduces land use. In the semi-arid and arid areas where there is usually high soil salt and precipitation may be insufficient, salinity ranks high as an inhibitor to crop development (El Sabagh et al., 2020). Salinity happens through natural processes (such as geological, hydrological and pedological) or human-induced procedures such as improper irrigation, drainage and overgrazing resulting in the soil water accumulation of dissolved salts to an extent that impedes plants development (Sahab et al., 2021).

Maize is regarded to be one of the cereals that is most salt-sensitive (Maas and Hoffman, 1977; Sabagh et al., 2021). Salinity is especially harmful to maize during germination and at the seedling growth stage (Akter et al., 2018). Like other abiotic stresses, salt stress in maize also leads to oxidative stress by the enhanced production of reactive oxygen species (ROS) (Shah et al., 2021). ROS triggers cellular harm through protein degradation, enzyme inactivation, gene modifications, and interferes in multiple metabolic pathways. This adversely leads to poor productivity (Nabavi et al., 2022).

Seed priming or seed reinforcement is one pragmatic approach to increasing crops output and productivity (Chimwemwe et al., 2021; Esper Neto et al., 2021). It also provides a means of increasing the efficiency of seed in many plant species in terms of synchronizing germination, reduction of emergence time and improving crop establishment most especially under stressful conditions (Chiu et al., 2002; Ibrahim, 2016). Different type of substances can be used in seed priming to promote tolerance to various abiotic stresses (Abdulbaki et al., 2019). Salicylic acid (SA) is considered as a hormone-like substance that has been used to increase germination and seedling emergence in various crops (Galviz et al., 2020). The mechanisms of how SA improves crop performance under saline condition is traceable to the protection of cell membrane, increase in carbon metabolisms, antioxidant system, osmo-protectant, photosynthetic pigments, regulation of stress defense proteins such as glutathione S-transferase APX and 2-cysteine peroxiredoxin (Kang et al., 2012; Sharma, 2017; Ghani et al., 2021). Ethylene diamine tetraacetic acid (EDTA), on the other hand, is a powerful chelating agent of metals and it has a wide range of applications due to its antecedent in forming stable complexes with most metals over a wide range of environmental hazards. In crops such as pepper and tomato, addition of EDTA had been reported to reduce the adverse effect of salinity during the germination of the aforementioned plants (Mgbeze et al., 2011; Olayinka et al., 2016). In *Brassica napus*, leaves and roots, EDTA amendment increased the activity of anti-oxidant enzymes by decreasing the concentrations of MDA and H_2O_2 (Habiba et al., 2015).

The severity of salinity stress on plant depends on both the degree of tolerance of plants and the specific mechanism underlying salinity stress

(Farooq et al., 2015). Maintaining high levels of antioxidant enzyme activities may contribute to salinity tolerance by affording the plant better protection mechanisms against oxidative damage. Therefore, comparing antioxidant defense systems, lipid peroxidation levels, and primers (EDTA and salicylic acid) contents in maize lines might allow a better understanding of the plants tolerance mechanisms to salinity stress (Ahmad et al., 2021; Iqbal et al., 2021). Hence, there is need to access the effect of different primers (EDTA and SA) on the salt stressed maize lines on the germination and enzyme activities.

MATERIALS AND METHODS

Germination experiment. Three maize lines (SWAN-LSR-Y, BR9928-OMR-SR-Y and OMRLSR-Y) used for the study were collected from the seed bank of Federal College of Agriculture, Moor Plantation, Ibadan, Oyo State, Nigeria. Seeds were surface-sterilized for 5 min using 5 % sodium hypochlorite (NaOCl) and were thereafter rinsed repeatedly with distilled water. They were then primed with distilled water (H_2O), EDTA and SA for 24 hours. The concentration of EDTA and SA used was 1.0 M. Twenty-five seeds were positioned in 9 cm each Petri dishes on two layers of Whatman filter papers. The primed seeds were subjected to varying concentrations (0, 50,100,150 and 300 mM) of sodium chloride (NaCl) for salt stress by irrigating the Petri-dishes (using a syringe) with 10 mL volume for each of the concentrations. The dishes containing the treated seeds were arranged in a growth chamber at 25^{o}C and with a 16-h light period following complete randomized design (CRD) in a 3x3x 5 factorial arrangement with three replications.

Germination assessment. Germination percentage was determined in lots of 25 seeds at the end of 10 days. The emergence of radicle was used as an indicator of germination.

Radicle and plumule measurements. This was done in all the seedlings at the 10 days after planting the seeds on Petri-dishes.

Speed of germination. The following formula of Maguire (1962) for lots with 100 seeds was used:

$$Speed\ of\ germination = n_1/d_1 + n_2/d_2 + n_3/d_3 \cdots,$$

where n: number of germinated seeds, d: number of days. However, since we used 25 seeds per lot, the formula was adjusted by a factor of 100/25 (Pire and Vargas, 2019).

Seedling vigor index (SVI) was measured as described by Maisuria and Patel (2009):

$$SVI = \text{Germination (\%)} \times \text{Seedling length (RL + PL)}$$

Where RL = radicle length, PL = plumule length, both measured in centimeters

Biochemical assessment.

Reactive oxygen species. Fresh plumule of randomly selected plants from each replicate of the different lines was homogenized and immediately frozen in liquid nitrogen prior to storage at -4 ^{o}C for usage later. The homogenate samples were used for the estimate of reactive oxygen species accumulation in the plant as follows:

Hydrogen peroxide (H_2O_2) concentration assay. This was estimated by the method described by Velikova et al. (2000). The H_2O_2 content was expressed as $\mu mol \cdot g^{-1}$ FW after using the extinction coefficient 0.28 $\mu M^{-1}cm^{-1}$ for its determination.

Superoxide anion radical (O_2^-) assay. The superoxide anion radical content was evaluated using the method stated by Ajiboye et al. (2016), where 100 µL of the homogenate were mixed with nitroblue tetrazolium (1 $mg \cdot mL^{-1}$) and incubated for 30 min at 37 ^{o}C, followed by addition 0.1 mL of HCl (0.1 mol/L) after incubation. The resulting homogenate was centrifuged at 1500 g for 10 min. The reduced nitroblue tetrazolium in the homogenate was extracted with dimethyl sulfoxide (DMSO), diluted with 80 µL phosphate-buffered saline (pH 7.5) and the absorbance was read using a microplate reader DR-200B at 575 nm. The superoxide anion radical concentration was estimated as follows

$$\text{Concentration} = \frac{\text{Absorbance}}{\text{Extinction coefficient}} \times \text{dilution factor}$$

Extinction coefficient = 17,000 $M^{-1} \cdot cm^{-1}$.

Determination of oxidative stress. Lipid peroxidation, an indicator of oxidative stress in cells and tissues was measured by estimating the MDA using the method described by Reilly and Aust (2001). The absorbance was measured and concentration determined with the same formula shown above.

Antioxidant enzyme activities. The plant sample tissue was mixed in extraction solution of 50 mM phosphate buffer (pH 7.0) at the ratio 1:5.

Homogenate derived was then centrifuge at 5000 rpm for 10 min at 4 °C. The supernatants were collected using a micro pipette to new sample tubes to be used for the subsequent analyses.

Superoxide dismutase (SOD) activity. The total superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed for by the method described by Afolabi et al. (2015).

$$\% \text{ Inhibition} = \frac{\text{increase in absorbance of sample}}{\text{increase in absorbance of blank}} \times 100$$

One unit of SOD activity was defined as the amount of SOD necessary to cause 50 % inhibition of the oxidation of adrenaline to adrenochrome during 1 minute.

Catalase (CAT) activity. Catalase (EC1.11.1.6) activity was assessed following the method of Hadwan and Abed (2016). The rate constant of a first-order reaction (k) equation is used to determine catalase activity:

$$CAT\ Activity\ of\ test\ kU = \frac{2.303}{t} \cdot \left[\frac{\log\ S^0}{S - M}\right] \cdot \frac{V_t}{V_S}$$

where t: time, S°: absorbance of standard tube, S: absorbance of test tube, M: absorbance of control test (correction factor), Vt: total volume of reagents in test tube, Vs: volume of serum

Ascorbate peroxidase (APX) activity. Ascorbate peroxidase (EC1.11.1.11) was assayed following the method of Nakano and Asada (1981).

Data analysis. Using Statistical Package for Social Sciences (SPSS 25.0) software, data were subjected to a three-way Analysis of Variance (ANOVA). The three factors considered were salinity levels, primers and maize lines. Interaction between the salt stress, primers and lines were analyzed. Means and standard error of data were obtained. The means were separated by Duncan Multiple Range Test ($P \leq 0.05$).

RESULTS

The results of germination percentage and speed of germination differed significantly ($P \leq 0.05$) due to maize line, salinity and their interaction, without effect of the priming (Table 1). The significant interactions between maize line and salinity showed that germination percentage and speed of germination of all the maize lines were adversely affected by salinity with increase in concentrations (Table 2), although SWAN-LSR-Y could be considered tolerant when compared to the other maize lines. Salinity as high as 300 mL recorded the lowest germination attributes in all the maize lines. However, BR9928-OMR-SR-Y was the most affected and showed the lowest germination percentage value of 38.7 % and speed of germination value of 13.2 (Table 2). EDTA recorded the highest germination percentage of 70.47 % and 19.92 for speed of germination over the other primers (Table 1). Irrespective of lines and primers, highest percentage germination and its speed were recorded in maize line treated with 0 mM of NaCl (control) and following in a decreasing order of magnitude in those maize lines irrigated with 50, 100, 150, and 300 mM of NaCl (Tables 1 and 2). The interaction effect between the maize line, salinity and primers was not significant indicating that the maize lines did not respond differentially to salinity and primer conditions (Table 1).

The radicle and plumule lengths and seedling vigor index were significantly influenced ($P \leq 0.05$) by the primer and salinity, not by the maize line. Maize line seeds primed with EDTA and SA showed significantly higher values of the foregoing aforementioned variables when compared to those primed with distilled water. The growth of the radicle and plumule was inhibited in maize lines receiving the highest concentration of salinity (300 mM). Seeds treated with 50 mM NaCl recorded a significant increase in radicle and plumule lengths, with a remarkable vigor compared to those treated with 100 and 150 mM NaCl (Table 3). The significant interaction effect between primer and salinity as shown in Table 4, indicated that the growth of radicle and plumule lengths and seed vigor index were not adversely impaired by different salinity level when primed with EDTA (Table 4). The seedling lengths in EDTA treated seeds showed no significant differences between 100 and 300 mL salinity levels when compared to those recorded for SA and H_2O where significant differences were observed (Table 4). Radicle and plumule lengths, and seedling vigor index were mainly affected in water treatment (control) when salinity level surpassed 50 mM, while priming treatments were less affected.

Table 1. Effects of primers on the germination parameters of three maize lines under salinity condition

	Germination (%)	Speed of germination (seed %·day^{-1})
Maize Line (L)		
SWAN-LSR-Y	77.14 a	21.96 a
BR9928-OMR-SR-Y	66.59 b	18.48 b
OMR-LSR-SY	66.67 b	18.28 b
Seed priming (P)		
H_2O	66.00 a	19.68 a
EDTA	70.47 a	19.92 a
SA	64.18 a	19.24 a
Salinity (mM) (S)		
0	83.08 a	25.20 a
50	77.60 ab	21.92 ab
100	67.20 bc	19.96 bc
150	61.11 c	19.48 cd
300	45.41 d	15.48 d
Interactions		
LxP	0.72	0.58
LxS	<0.001	<0.001
PxS	0.61	0.89
LxPxS	0.66	1.00

Means followed by the same literals are not significantly different according to Duncan's test ($P \leq 0.05$) SA: Salicylic acid

Table 2. Interaction effect of maize line x salinity on the germination percentage and speed of germination of the plant

	Salinity (mM)	Germination (%)	Speed of germination (seed %·day^{-1})
Maize Line			
	0	88.3 a	26.4 a
	50	78.5 b	25.2 ab
SWAN-LSR-Y	100	70.6 c	20.8 bc
	150	68.7 c	20.4 bc
	300	51.9 d	16.4 c
P-value		<0.001	<0.001
	0	81.1 a	24.4 a
	50	74.6 a	21.2 ab
BR9928-OMR-SR-Y	100	63.9 b	19.6 bc
	150	55.8 b	19.6 bc
	300	38.7 c	13.2 c
P-value		<0.001	0.007
	0	79.8 a	24.8 a
	50	79.7 a	22.0 ab
OMR-LSR-SY	100	67.1 ab	19.6 b
	150	58.9 b	18.4 b
	300	45.9 c	17.2 c
P-value		<0.001	<0.001

Means followed by distinct letters are significantly different according to Duncan's test ($P \leq 0.05$)

Table 3. Effects of primers on the radicle and plumule lengths, and seedling vigor index of three maize lines under salinity condition

	Radicle length(cm)	Plumule length (cm)	Seedling vigor index
Maize Line (L)			
SWAN-LSR-Y	6.86 a	5.81 a	515.15 a
BR9928-OMR-SR-Y	6.62 a	5.63 a	390.96 a
OMR-LSR-SY	6.51 a	5.70 a	396.73 a
Seed Priming (P)			
H_2O	4.64 b	2.72 b	340.33 b
EDTA	6.98 a	5.93 a	593.86 a
SA	6.05 a	4.12 a	553.75 a
Salinity (mM) (S)			
0	9.10 a	8.10 a	749.39 a
50	7.40 b	6.29 b	549.97 b
100	5.30 c	4.40 c	360.18 c
150	4.87 c	4.87 c	315.15 cd
300	3.27 d	3.27 d	182.02 d
Interactions			
LxP	0.94	0.91	0.36
LxS	0.75	0.49	0.51
PxS	<0.001	<0.01	<0.01
LxPxS	0.99	1.00	1.00

Means followed by distinct letters are significantly different according to Duncan's test ($P \leq 0.05$). SA: Salicylic acid

Table 4. Interaction effect of primer x salinity on the radicle and plumule length, and seedling vigor index of the maize lines

		Radicle length (cm)	Plumule length (cm)	Seedling vigor index
Primer	Salinity (mM)			
	0	9.1 a	8.3 a	751 a
	50	6.3 b	5.3 b	486 b
H_2O	100	3.3 c	3.1 c	251 c
	150	2.5 cd	4.2 cd	181 cd
	300	1.7 d	1.8 d	126 d
	P-value	<0.001	<0.001	<0.001
	0	9.3 a	8.1 a	756 a
	50	8.1 a	6.9 a	597 b
EDTA	100	6.5 b	5.2 b	432 c
	150	6.2 b	5.3 b	396 c
	300	5.2 b	4.1 b	215 d
	P-value	<0.001	<0.001	<0.001
	0	8.8 a	7.9 a	741 a
	50	7.8 a	6.6 b	486 b
SA	100	6.1 b	4.9 c	398 c
	150	5.9 b	5.1 c	368 c
	300	4.3 c	3.9 d	205 d
	P-value	<0.001	<0.001	<0.001

Means followed by distinct letters are significantly different according to Duncan's test ($P \leq 0.05$)

The MDA content was not affected by priming the maize lines. However, it varied according to the salinity level, and after an initial drop, it increased steadily from 100 to 300 mM NaCl. H_2O_2 was significantly influenced by the primer and salinity, with significant interaction between them (Table 5). Maize lines did not differ significantly for all the stress attributes. Seed primed with EDTA and SA showed significantly lower content of O_2^-, and H_2O_2 when compared to those primed with distilled water. In comparison with the control, the O_2^- values were generally higher in all the saline treatments. The H_2O_2 content consistently increased as salinity level was higher (Table 5). The significant interaction between primer and salinity with respect to production of H_2O_2 is indicated in Table 6. The production of O_2^- in each of the primer did not show statistical differences between control and the salinity levels. Statistical differences were observed for H_2O_2, where EDTA and SA show good efficiency in decreasing the accumulation of H_2O_2, most importantly at 50, 100, and 150 mM saline conditions. Production of H_2O_2 at 300 mM in all the primers was significantly higher than other salinity levels (Table 6).

Table 5. Effects of primers on the reactive oxygen species (O_2^- and H_2O_2) and MDA of three maize lines under salinity condition

	O_2^- µmol.mg^{-1}Fw	H_2O_2 µmol.mg^{-1}Fw	MDA µmol.g^{-1}Fw
Maize line (L)			
SWAN-LSR-Y	0.004 a	17.28 a	0.006 a
BR9928-OMR-SR-Y	0.006 a	21.06 a	0.005 a
OMR-LSR-SY	0.005 a	22.62 a	0.007 a
Seed priming			
H_2O	0.008 a	23.26 a	0.004 a
EDTA	0.004 b	11.20 b	0.006 a
SA	0.003 b	15.63 b	0.007 a
Salinity(mM) (S)			
0	0.0003 b	1.61 c	4.00 ab
50	0.007 a	4.91 c	3.00 ab
100	0.005 a	11.16 b	2.00 b
150	0.005 a	16.83 ab	3.00 ab
300	0.003 a	21.43 a	6.00 a
Interaction			
LxP	0.61	0.61	1.00
LxS	0.99	0.84	1.00
PxS	<0.001	<0.001	1.00
LxPxS	0.73	0.99	1.00

Means followed by distinct letters are significantly different according to Duncan's test ($P \leq 0.05$). SA: Salicylic acid

The antioxidant enzyme activities showed no statistical differences due to maize line except for SWAN-LSR-Y maize line which had significant increase in SOD activity over all other maize lines (Table 4). APX activity was not affected by any level of salinity or maize line (Table 7), but differed significantly with respect to priming agents with EDTA and SA enhancing the activities of reactive oxygen species scavenging enzymes respect to the control (primed with only distilled water). Significant interactions were recorded between the maize line and salinity only for CAT and SOD. In all the maize line, CAT and SOD activities were significantly lowest in the highest concentration (300 mM) and they increased with decrease in salinity levels. However, salinity levels of 50, 100 and 150 mM posed minimal damage to the activities of the enzymes in SWAN-LSR-Y and BR9928-OMR-SR-Y when compared to OMR-LSR-SY. It should be noted that salinity at the highest concentrations (300 mM) significantly reduced the production of these antioxidant enzymes in all the maize lines investigated (Table 8).

Table 6. Interaction effect of primer x salinity on the accumulation of singlet oxygen and hydrogen peroxide in the maize lines

	Salinity (mM)	O_2^- µmol·g^{-1}Fw	H_2O_2 µmol·mg^{-1}Fw
Primer			
	0	0.0006 a	1.6 e
	50	0.0145 a	5.2 d
H_20	100	0.0103 a	14.7 c
	150	0.0100 a	23.7 b
	300	0.0061 a	31.4 a
P-value		0.37	<0.0001
	0	0.0006 a	1.6 e
	50	0.0128 a	4.8 d
EDTA	100	0.0103 a	9.7 c
	150	0.0095 a	14.6 b
	300	0.0059 a	17.8 a
P-value		0.23	<0.0001
	0	0.0005 a	1.5 e
	50	0.0140 a	4.7 d
SA	100	0.0095 a	9.1 c
	150	0.0094 a	12.2 b
	300	0.0059 a	15.1 a
P-value		0.36	<0.0001

Means followed by distinct letters are significantly different according to Duncan's test ($P \leq 0.05$). SA: Salicylic acid FW: fresh weight

DISCUSSION

The establishment of any crop stands required that the process of germination was not hindered. In this study, it has been established that saline condition is detrimental to germination and the growth of embryonic parts in all the newly released maize lines investigated most importantly when the saline condition is above 150 mM. However, the interaction effect between maize line and salinity for germination and speed of germination had clearly shown that SWAN-LSR-Y performed better in the foregoing germination attributes when the salinity exceeded 150 mM.

The ability to withstand this stress could be attributed to reasons such as genetic make-up and primers most importantly the EDTA. Application of EDTA promoted faster uptake of water and trigger of enzymes needed for mobilization of food reserve that resulted in the improvement of germination and reduction of germination time. The foregoing explanation is further buttressed by significant interaction that existed between the primer and salinity for radicle and plumule lengths, where maize lines treated with EDTA was found to be most effective in ameliorating the adverse effect posed by salinity when compared to those primed with SA and H_2O. Generally, the use of EDTA and SA enhanced the aforementioned growth characters when compared to those primed with distilled water. The results agreed with earlier workers where EDTA and SA under salt condition increased seedling growth and development of tomato and wheat respectively (Liting et al., 2015; Olayinka et al., 2016; Ghafoor et al., 2020). Similarly, Aloui et al. (2014) had reported longer radicle and plumule for all the salinity levels in pepper plants whose seeds were primed.

Table 7. Effects of primers on catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities (µmol·min^{-1}·mg^{-1} protein) of three maize lines under salinity condition

	CAT activity	SOD activity	APX activity
Maize Line (L)			
SWAN-LSR-Y	112.04 a	66.59 a	2.05 a
BR9928-OMR-SR-Y	119.69 a	12.48 b	1.62 a
OMR-LSR-SY	118.55 a	1.25 c	1.75 a
Seed priming (P)			
H_2O	91.69 a	24.56 a	1.86 b
EDTA	94.67 a	30.51 a	3.46 a
SA	93.94 a	25.76 a	2.89 a
Salinity (mM) (S)			
0	151.49 a	45.36 a	1.53 a
50	146.53 a	35.02 ab	1.70 a
100	94.14 b	26.02 bc	1.61 a
150	42.92 c	18.86 cd	1.55 a
300	31.96 c	9.49 d	1.47 a
Interactions			
LxP	0.72	0.31	0.14
LxS	<0.01	<0.01	0.64
PxS	0.61	0.94	0.99
LxPxS	0.48	0.99	0.99

Means followed by distinct letters are significantly different according to Duncan's test ($p \leq 0.05$). SA: Salicylic acid

Table 8. Interaction effect of maize line x salinity on the catalase (CAT) and superoxide dismutase (SOD) activities (µmol. min^{-1}.mg^{-1} protein) in the plant

Maize Lines	Salinity (mM)	CAT	SOD
	0	149.5 a	45.4 a
	50	142.8 a	40.7 ab
SWAN-LSR-Y	100	87.2 b	33.4 bc
	150	36.3 c	28.4 c
	300	17.8 d	12.5 d
P-value		<0.001	<0.001
	0	151.5 a	44.7 a
	50	149.1 a	32.4 b
BR9928-OMR-SR-Y	100	101.7 b	28.5 b
	150	48.1 c	17.6 c
	300	39.4 c	8.9 d
P-value		<0.001	<0.001
	0	153.3 a	39.9 a
	50	146.7 a	31.9 b
OMR-LSR-SY	100	93.6 b	16.2 c
	150	44.4 c	10.7 d
	300	38.7 c	7.1 d
P-value		<0.001	<0.001

Means followed by distinct letters are significantly different according to Duncan's test ($P \leq 0.05$).

Considering, the production of H_2O_2 which is one of the measure of oxidative stress in alongside O_2^- and MDA, it was observed that EDTA and SA was more effective in preventing the accumulation of H_2O_2 most importantly when the salinity level did not exceed 150 mM. This observation is due

to ability of EDTA or SA to induce greater SOD activity which in turn offered protection against antioxidants by allowing detoxification of H_2O_2 produced in all the maize lines during stress (Hussain, 2016).

In this study, application of EDTA or SA allowed the maize lines to have an increased CAT and SOD activities that were needed to reduce the damaging effect resulting from salinity. The reduction of deleterious effect of salinity was much more pronounced in SWAN-LSR-Y and BR9928-OMR-SR-Y than OMR-LSR-SY in salinity levels between 50, 100 and 150 mM. These results agreed with findings of Ahanger and Agarwal (2017) who reported that application of SA enhanced the activity of the antioxidant systems by decreasing the damaging effect posed by salinity in wheat. Similarly, Farzana et al. (2020) reported reduction of adverse effect of reactive oxygen species (ROS) and increase in antioxidant enzymes in salt-stressed genotypes upon application of SA. In *Brassica napus*, EDTA has been found to increase antioxidant enzymes in copper polluted soil (Habiba et al 2015). The elevated activities of antioxidants in the aforementioned maize lines upon application of EDTA or SA gave the germinating seeds the strength to eliminate ROS and limit the accumulation of MDA that has been affirmed as indicator of stress in plants (Younesi and Moradi, 2014).

CONCLUSION

The present investigation had revealed that the germinating maize lines were adversely affected by salinity as high as 300 mM and that saline condition as high as 150 mM could be tolerated by all the maize lines when primed with EDTA and SA. SWAN-LSR-Y and BR9928-OMR-SR-Y maize lines were more remarkable in all the growth attributes at 150 mM than OMR-LSR-SY. Also, the use of EDTA was more effective than salicylic acid as priming agent on account of its greater ability to increase the activities of free radical scavenging antioxidant enzymes, notably SOD in SWAN-LSR-Y and BR9928-OMR-SR-Y salt-stressed maize lines at the tolerated concentration.

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