



## Enhancing Germination and Seedling Growth in Salt Stressed Maize Lines through Chemical Priming

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**Abstract:** This study aimed to investigate the tolerance level and the use of primers (H<sub>2</sub>O, KNO<sub>3</sub>, ascorbic acid and salicylic acid), in mitigating stress in maize in the newly released cultivars (SWAN-LSR-Y, BR9928-OMR-SR-Y and OMR-LSR-SY). Activities of SOD, APX, CAT and GSH and lipid peroxidation were investigated, to measure the biochemical response of the primed maize seeds. Maize seeds primed with KNO<sub>3</sub> and ascorbic acid improved germination and anti-oxidative potential against ROS in ameliorating the salinity stress, while salicylic acid slowed germination. The same trend was followed in the seed vigour index and radicle length of seeds primed with ascorbic acid, which recorded the highest values. The control was observed to have the highest seed vigour index, while seeds primed with salicylic acid showed the least vigour index in the maize seeds. Increased salinity stress showed adverse effects on all growth parameters. Of the maize cultivars tested, SWAN-LSR-Y showed the most tolerance to salinity stress, in terms of germination. Significant high enzymatic activities and lipid peroxidation were recorded in seeds primed with ascorbic acid and KNO<sub>3</sub> show their importance in plant metabolic activities.

**Keywords:** Cultivar, Enzymatic activity, oxidative stress, Priming, Salinity.

## Introduction

Maize, not only a vital cereal crop but also a

cornerstone of global food security, plays a crucial role in ensuring a stable and sustainable food supply for populations worldwide (Farooq

*et al.*, 2015). Under salt stress, maize is moderately sensitive. It experiences reduced water uptake, leading to water scarcity in the plant tissues. To cope with salt stress, maize accumulates compatible solutes and activates antioxidant defense mechanisms to maintain cellular balance and protect against oxidative stress (Chinnusamy *et al.*, 2005). Soil minerals and nutrients are crucial for plant growth as they provide the required elements for metabolism and development. When these elements are not supplied adequately, plants may undergo stunted growth and nutrient deficiencies, leading to reduced productivity. However, the unnecessary presence of soluble salts causes severe ionic and osmotic stress in plants (Mosa *et al.*, 2017).

Salinity or salt stress is the accumulation of water-soluble salts in the soil column to a level that has a drastic impact on agricultural production and environmental health. Salt stress negatively impacts agricultural production as it disrupts seed germination, diminishes plant growth and crop yield, and affects the nutritional constituents of agricultural products. In addition, soil salinity can lead to environmental problems by contaminating water sources, altering soil pH, and affecting the microbial ecosystem (Stavi *et al.*, 2021). The accumulation of salts in soil over time eventually causes the loss of fertile land, making it unsuitable for plant growth and further worsen food security challenges. According to Food and Agriculture Organization's (FAO) reports on plant and land nutrition, the world is losing at least 3 hectares of fertile land every minute because of high soil salinity (Wang *et al.*, 2003). Water stress or elevated salinity contributes to hypertonic and hypotonic stress, which in turn impacts the plant's metabolic activities and could ultimately cause plant death (Zhu, 2002). High salinity

influences plants in a number of ways, which include water deficits, ion toxicity, nutritional disorders, oxidative stress, metabolic processes alteration, membrane disorganization, cell division, expansion reduction and genotoxicity (Munns, 2002).

On the onset and initiation of salt induced stress in a crop, significant biochemical and metabolic processes such as photosynthesis, protein production and energy and lipid metabolism are also influenced (Parida & Das, 2005). These effects are induced by either restricting the flow of water and nutrients into the plants or by direct injury to plant cells through the accumulation of toxic ions.

Seed priming of plants allows the metabolic processes of germination to occur without the emergence of radicle or plumule, leading to better seedling establishment. By priming seeds, they are provided with a protective layer that helps them withstand salt stress during germination and early growth stages (Lutts *et al.*, 2016). Different seed priming methods have been used over the years; by soaking seeds in distilled water (hydro-priming), soaking in solutions such as PEG, potassium salts (Osmo-priming), soaking in salt solutions (halo-priming) and soaking in plant growth hormones such as salicylic acid, indole acetic acid and thiamine (Khan *et al.*, 2016; Shehu *et al.*, 2019). There have been various reported evidences on the use of seed priming methods to improve seedling germination and early seedling growth under stress conditions (Lazim & Ramadhan, 2020; Olayinka *et al.*, 2022). This study intends to explore the tolerance level of three newly released maize cultivars and evaluate the effectiveness of primers, including H<sub>2</sub>O, KNO<sub>3</sub>, ascorbic acid, and salicylic acid, in mitigating

stress in maize.

## Materials & Methods

### Seeds collection

Three cultivars of maize namely SWAN-LSR-Y, BR9928-OMR-SR- Y and OMR-LSR-SY were collected from the seed bank of Federal College of Agriculture, Moor Plantation, Ibadan, Oyo State, Nigeria.

### Seeds treatment and experimental layout

Seeds were surface sterilized with 5% sodium hypochlorite for five min, thereafter rinsed with distilled water. The seeds were primed with related primers, [distilled water (H<sub>2</sub>O), Ethylene diamine tetra acetic acid (EDTA) and salicylic acid (SA)] for 24 hours (Sedghi *et al.*, 2010; Kim *et al.*, 2022), and then washed twice with distilled water. Twenty seeds were placed in nine cm each Petri dishes on two layers of Whatman filter papers. During the experiment, the primed seeds were subjected to varying concentrations (0, 50, 100, 150 and 300 mM) of sodium chloride (NaCl) for salt stress. Experimental layout followed the complete randomized design (CRD) in a factorial arrangement with three replications.

### Data collection

#### Determination of germination percentage

Germination count was done as indicated by clear emergence of radicle on daily basis for a period of 10 days. Plumule and radicle lengths were also measured using 10 cm rule at the termination of the studies. Germination percentage was calculated by the method adopted by Etejere & Olayinka (2014) based on the following formula.

$$\text{Germination Percentage (GP)} = \frac{Nf}{N}$$

### Speed of germination

Germination speed was calculated by the method provided by Zayneb *et al.* (2015) using the formula;

$$\text{Speed of germination} = \frac{n_1 + n_2 + n_3 \dots}{d}$$

n is the number of germinated seeds; d is the number of days.

### Seedling vigor index

The seedling vigor index measured and calculated as described by Abdul-Baki & Anderson (1973).

$$\text{Seed vigor index (SVI)} = \frac{SL \times GP}{100}$$

SL= Seedling length (Radicule + Plumule length) and GP=% germination.

### Biochemical studies

#### Collection of plant sample for biochemical assay

Fresh plumule of randomly selected plants from each replicate of the three varieties of maize cultivars were selected, homogenized and immediately frozen in liquid nitrogen, freeze-dried and stored at -4 °C for future usage.

#### Determination of Reactive Oxygen Species (ROS) concentration

Hydrogen Peroxide Concentration Assay: Hydrogen peroxide concentration was estimated by the method described by Velikova *et al.* (2000). The superoxide anion radical content was determined using the method described by Ajiboye *et al.* (2016).

#### Preparation of antioxidant enzymes extract

The plant sample tissue was mixed in extraction solution of 50mM phosphate buffer (pH 7.0) at ratio 1:5. Homogenate derived was then

centrifuge at 5000 g for 10 min at 4°C. The supernatants were collected using a micro pipette to new sample tubes to be used for further analyses.

### **Superoxide dismutase (SOD), catalase, and Ascorbate Peroxidase (APX) activities**

The SOD activity was assayed for by the method described by Afolabi *et al.* (2015). Catalase and APX activity were assessed by the method of Hadwan & Abed (2016) and Nakano & Asada (1981) respectively.

### **Reduced glutathione concentration (GSH)**

The procedure given by Ellman (1959) and Jollow *et al.* (1974) was used to assayed for the GSH.

### **Determination of oxidative stress**

Lipid peroxidation was measured by estimating the malondialdehyde content (MDA) using the method described by Reilly & Aust (1999)

### **Data analysis and presentation**

Data obtained from this study were subjected to a Three-way analysis of variance (ANOVA) using statistical package for social sciences (SPSS 25.0) software. The three factors considered were salinity levels, primers and cultivars. The means were separated by Duncan multiple Range Test (DMRT) and considered statistically significant at  $p \leq 0.05$ .

## **Results & Discussion**

### **Germination parameters**

#### **Speed of germination**

The influence of primer and different salinity levels on speed of germination is shown in (Table 1). There was no significant ( $p < 0.05$ ) interaction between the cultivars, the primers and the salinity stress on the germination speed

of the maize seedlings. However, there was a significant difference at  $p < 0.05$  between the interaction of just cultivars and primers, and the interaction between the primers and the various salinity stress levels. SWANLSR-Y germinated most rapidly, while the control (H<sub>2</sub>O) recorded the highest germination speed amongst the primers. Germination speed decreased with increasing salt stress, with the lowest magnitude achieved at 300 mM NaCl concentration. The commencement and rate of germination is delayed by salt stress during germination, which was the reason the control was found to record the highest germination speed compared to other primers (Farooq *et al.*, 2015). The speed of germination as affected by primer and salinity levels aligned with the work of (Akter *et al.*, 2018), who studied the effect of priming and salinity levels on maize using 5 seed priming and 4 salinity levels and observed that there was significant different in seed germination percentage due to seed priming treatments and various salinity stress levels. The effect of priming and salinity stress on germination was observed to be due to the prevention of water entry into the seed as a result of the decrease in the osmotic potential in the soil solution (El Sabagh *et al.*, 2021).

#### **Germination percentage**

There was no significant interaction between the salinity stress levels and the various primers used for the experiment on the maize cultivars (Table 1) on the germination percentage of the plants. SWAN-LSR-Y recorded the highest germination percentage at 52.33 %, followed by BR9928-OMR-SR-Y. Ascorbic acid had the greatest influence on the germination percentage (56.44 %) among the primers, followed by KNO<sub>3</sub> (55.33 %), the water (45.55 %) while

salinity stress (300 mM) negatively affected the germination percentage at 39.99%. There was no statistically significant difference on the effect of water and salicylic acid on the germination percentage, while significant different was observed on the effect of water and ascorbic acid on the germination percentage. This study disagreed with the study of Akter *et al.* (2018) who observed that hydro-priming for 48 hrs has the highest percentage of seed germination (95.7%), although, no statistical significant different was observed between hydro-priming and priming with salt as  $0.25 \text{ ds. m}^{-1}$ .

### Plumule length

There was significant difference the interaction of the cultivars and the primers across the different level of salinity stress at  $p < 0.05$  on the plumule length (Tables 1 and 2). Significant different at  $p < 0.05$  was observed for the salt stress, priming, and maize cultivars, priming and cultivars, priming and salt stress levels. The plumule length decreased with increasing concentration of salt from 8.98 cm to 1.04 cm at salt concentration 0 to 300 mM, respectively. In the priming treatments, plumule length was greater in the maize seeds primed with potassium nitrate. The plumule length of SWAN-LSR-Y was significantly lowest, while plumule lengths of OMR-LSR-SY and BR9928-OMR-LSR-SY were significantly similar. This aligned with the work of Khan *et al.* (2016) who studied the impact of fertilizer priming on seed germination behavior and vigor of maize. They observed that there were no significant interaction between priming treatments and maize varieties and as observed in this study, the highest plumule length was observed in seed treated with calcium ammonium nitrate (potassium nitrate was used in this study).

### Radicle length

There was no significant difference at  $p < 0.005$  in the interaction between the salinity stress, priming and the cultivars, priming and stress. However, significant different at  $p < 0.05$  was observed for the main effects of the primers and the stress on the radicle length of the plants (Table 1). Radicle length recorded for the three cultivars was statistically similar, which aligned with the work of Khan *et al.* (2016), who stated that there was a significant effect of priming and stress on root length, while no significant effect was observed on the varieties used. Salinity stress at 300 mM greatly reduced the radicle length. The seed treated with salicylic acid had the highest radicle length (6.17 cm), while seed treated with ascorbic acid had the lowest radicle length (5.65 cm). This deviate from the study of Khan *et al.* (2016), where non-primed seed had the highest radicle length (5.95 cm) compared with hydro-priming (4.47 cm) and osmo-priming (4.65 cm).

### Seedling vigour index

The primers and the levels of salinity stress influenced seedling vigour index as shown in table (1). No significant difference was observed at  $p < 0.05$ , in the interaction between the cultivars, primers and salinity stress levels on the seedling vigour index of the maize cultivars. However, there was a significant difference at  $p < 0.05$  between the interaction of cultivars and primers, and the interaction between the primers and the various salinity stress levels. SWAN-LSR-Y showed the highest seedling vigour index (298.33), while the treatment with ascorbic acid mostly improved the seedling vigour index of the plant primers, which significantly differ from salicylic acid treated seeds. Seedling vigour index was lowest at 300

mM salt concentration. Ascorbic acid recorded the highest values of seedling vigour index among the primers. This agreed with the study

of Hamama & Murniati (2010), who showed that priming with ascorbic acid significantly affect seedling vigour index.

**Table (1): Effect of primers on the growth attributes of the maize seedlings under salt stress.**

		Speed of germination	Germination percentage (%)	Plumule length (cm)	Radicle length (cm)	Seedling vigour index
Cultivars	SWAN-LSR-Y	4.04 <sup>a</sup>	55.33 <sup>a</sup>	4.42 <sup>b</sup>	5.52 <sup>a</sup>	298.33 <sup>a</sup>
	BR9928-OMR-SR-Y	3.08 <sup>b</sup>	52.33 <sup>a</sup>	5.05 <sup>a</sup>	5.69 <sup>a</sup>	293.95 <sup>b</sup>
	OMR-LSR-SY	3.29 <sup>b</sup>	43.50 <sup>b</sup>	5.03 <sup>a</sup>	5.72 <sup>a</sup>	248.48 <sup>b</sup>
	p-value	<0.01	<0.002	<0.002	0.74	<0.03
Primers	H <sub>2</sub> O (control)	3.25 <sup>b</sup>	45.55 <sup>b</sup>	5.21 <sup>a</sup>	6.06 <sup>a</sup>	276.89 <sup>ab</sup>
	KNO <sub>3</sub>	3.80 <sup>a</sup>	55.33 <sup>a</sup>	5.21 <sup>a</sup>	6.06 <sup>a</sup>	301.14 <sup>a</sup>
	ASA	3.85 <sup>a</sup>	56.44 <sup>a</sup>	4.85 <sup>ab</sup>	5.65 <sup>a</sup>	311.21 <sup>a</sup>
	SA	2.99 <sup>b</sup>	44.22 <sup>b</sup>	4.80 <sup>ab</sup>	6.17 <sup>a</sup>	232.59 <sup>a</sup>
	p-value	<0.01	<0.002	<0.01	<0.00	<0.01
Stress levels (mM)	0	4.77 <sup>a</sup>	58.33 <sup>a</sup>	8.20 <sup>a</sup>	8.78 <sup>a</sup>	492.73 <sup>a</sup>
	50	4.13 <sup>ab</sup>	54.44 <sup>a</sup>	7.05 <sup>b</sup>	7.80 <sup>b</sup>	392.17 <sup>b</sup>
	100	3.49 <sup>bc</sup>	52.22 <sup>a</sup>	5.58 <sup>c</sup>	6.74 <sup>c</sup>	323.16 <sup>c</sup>
	150	3.06 <sup>c</sup>	50.55 <sup>a</sup>	2.65 <sup>d</sup>	3.92 <sup>d</sup>	167.28 <sup>d</sup>
	300	1.90 <sup>d</sup>	36.39 <sup>a</sup>	0.68 <sup>e</sup>	1.04 <sup>e</sup>	25.37 <sup>e</sup>
	p-value	<0.00	<0.00	<0.00	<0.00	<0.00
Cultivar×Primers	p-value	<0.03	0.99	<0.00	<0.00	0.05
Primer×Stress	p-value	<0.03	Ns	<0.03	<0.01	ns
Cultivar×Stress	p-value	Ns	Ns	ns	ns	ns
Cultivars×Primers×Stress	p-value	Ns	Ns	<0.02	ns	ns

N.B: Values with the same superscript across the treatments are not significant at p<0.05; H<sub>2</sub>O, Water; KNO<sub>3</sub>, potassium nitrate; ASA, ascorbic acid; SA, salicylic acid; ns, not significant.

**Biochemical parameters**

**Reactive Oxygen Species (ROS) concentration**

**Hydrogen peroxide concentration**

There was a significant (p<0.05) interaction between the different levels of salinity stress, the primers and the cultivars on the hydrogen peroxide concentration of the maize seedlings (Table 3). There was no significant different between the interaction between the stress and the cultivars. The highest H<sub>2</sub>O<sub>2</sub> concentration was obtained in the cultivar OMR-LSR- SY. Among the primers used for the priming of seeds, H<sub>2</sub>O<sub>2</sub> concentration was lowest in the

maize plants primed with water. It should be noted that the highest level of hydrogen peroxide was recorded in the 300 mM concentration of salinity stress and lowest in the 50 mM concentration. As reported by Ali *et al.* (2021), seed priming was found to have significant effects on hydrogen peroxide concentration in rice seedling which aligned well with the results of this study.

**Superoxide anion radical concentration**

Superoxide radical concentration was significantly affected at p<0.05 by the interaction between the cultivars, primers and salinity stress levels. The interaction between the

stress levels, cultivars, primers, primers and stress were also highly significant (Tables 2 and 3). This is in line with the report of Kazemi *et al.* (2017), who showed that, there was a significant effect of place and prime on the level of superoxide dismutase in maize. The least concentration of superoxide radical was achieved in the cultivar BR9928-OMR-SR-Y.

stress levels, cultivars and primers, cultivars and Priming with KNO<sub>3</sub> recorded the highest superoxide anion radical among the primers, there was no significant difference between the other primers including the control (H<sub>2</sub>O). This aligned with the study of Ali *et al.* (2021) who reported that seed priming has significant effects on superoxide dismutase activities of rice.

**Table (2): Effect of primers on lipid peroxidation and reactive oxygen species content of three maize cultivars under salt stress.**

		MDA content (x 10 <sup>-6</sup> ) (µmol.mg <sup>-1</sup> .Fw)	Superoxide radical conc. (µmol.mg <sup>-1</sup> .Fw)	H <sub>2</sub> O <sub>2</sub> content (µmol.mg <sup>-1</sup> .Fw)
Cultivars	SWAN-LSR-Y	9.00 <sup>b</sup>	0.0002 <sup>b</sup>	4.80 <sup>a</sup>
	BR9928-OMR-SR-Y	8.00 <sup>b</sup>	0.0001 <sup>c</sup>	3.56 <sup>b</sup>
	OMR-LSR-SY	1.20 <sup>a</sup>	0.0003 <sup>a</sup>	5.29 <sup>a</sup>
	p-value	0.00	<0.00	<0.00
Primers	H <sub>2</sub> O (control)	9.00 <sup>b</sup>	0.0002 <sup>b</sup>	4.06 <sup>b</sup>
	KNO <sub>3</sub>	13.00 <sup>c</sup>	0.0002 <sup>a</sup>	4.37 <sup>ab</sup>
	ASA	6.00 <sup>c</sup>	0.0001 <sup>b</sup>	4.62 <sup>ab</sup>
	SA	10.00 <sup>b</sup>	0.0001 <sup>b</sup>	5.16 <sup>a</sup>
	p-value	0.00	<0.00	0.85
Stress levels (mM)	0	13.00 <sup>a</sup>	0.0002 <sup>a</sup>	3.99 <sup>b</sup>
	50	10.00 <sup>bc</sup>	0.0001 <sup>b</sup>	3.85 <sup>b</sup>
	100	10.00 <sup>b</sup>	0.0001 <sup>b</sup>	4.63 <sup>ab</sup>
	150	70.00 <sup>cd</sup>	0.0001 <sup>b</sup>	4.77 <sup>ab</sup>
	300	80.00 <sup>b</sup>	0.0001 <sup>b</sup>	5.52 <sup>a</sup>
Cultivar×Primers	p-value	0.00	0.00	<0.07
	p-value	Ns	0.00	0.00
Primer×Stress	p-value	Ns	0.00	ns
	p-value	Ns	0.00	0.00
Cultivar×Stress	p-value	Ns	0.00	0.00
	p-value	Ns	0.00	0.00
Cultivars×Primers*Stress	p-value	Ns	0.00	0.00

N.B: Values with the same superscript across the treatments are not significant at p<0.05; H<sub>2</sub>O, Water; KNO<sub>3</sub>, potassium nitrate; ASA, ascorbic acid; SA, salicylic acid; ns, not significant.

## Oxidative stress parameter

### Lipid peroxidation

There was a significant ( $p < 0.05$ ) interaction in the different levels of salinity stress, the primers and the cultivars on the malonaldehyde (MDA) content or lipid peroxidation level of maize seedlings (Table 3). Significantly level of lipid peroxidation was obtained in the OMR-LSR-SY and SWAN-LSR-Y maize cultivars. Among the primers, maize plants primed with ascorbic acid showed the highest lipid peroxidation. The values obtained for lipid peroxidation was highest in the maize plants irrigated with 0 mM (control) salt concentration. This aligned with the work of de Oliveira *et al.* (2012) who reported a significant effect of priming on malondialdehyde concentration by salinity in sorghum seedling shoot and root. It was observed that oxidative stress and lipid peroxidation is caused by malondialdehyde (Ali *et al.*, 2021).

## Enzymatic antioxidant system

### Superoxide Dismutase (SOD) activity

Significantly highest level of SOD activity was obtained in the OMR-LSR-SY cultivar. SOD activity increased with increasing salt stress as the highest level of SOD activity was obtained in maize seedling irrigated with 300 mM of salt (Table 3). An increase in SOD activity in response to salt stress was observed in *Cicer arietinum* and *mulberry* (Kukreja *et al.*, 2005). The results of this study generally showed that priming of the maize seeds with ascorbic acid, salicylic acid and  $\text{KNO}_3$  upregulated the SOD activity when compared to the control. However, among the primers,  $\text{KNO}_3$  treatment seeds showed the highest SOD activity. This result is in line with the findings of Saed-Moocheshi *et*

*al.* (2014), where they reported the highest-level SOD activity in maize seeds primed with  $\text{KNO}_3$  under salinity and drought stress conditions. Lara *et al.* (2014) also reported the effectiveness of priming of tomato seeds with  $\text{KNO}_3$  in upregulating SOD activity.

### Catalase activity

The interaction between maize cultivars, primers, and salinity levels was significant ( $p < 0.05$ ) for catalase activity. The OMR-LSR-SY cultivar had the highest catalase activity, and  $\text{KNO}_3$  priming resulted in the highest activity among primers (Table 3). Catalase scavenges reactive oxygen species and has a very high affinity for hydrogen peroxides; it acts by decomposing the peroxides into molecular oxygen (Mittler, 2002). In this research, there was a gradual decrease in catalase activity observed with increasing salt stress. This submission agrees with findings of Moghaddam *et al.* (2020) where catalase activity increased with increasing salinity in germinating seeds of *Vigna radiata*. Hu *et al.* (2012) observed a considerable inhibition in the activities of catalase (CAT) and ascorbate peroxidase (APX) in Bermuda grass (*Cynodon dactylon*). In this study, the least mean values obtained for catalase activity was obtained in maize seeds primed with salicylic acid followed by ascorbic acid. Ascorbic acid also reduced catalase activity in pea under stress condition (Kukreja *et al.*, 2005). Ascorbic acid, as an antioxidant, has a high affinity for superoxide ion and prevents the accumulation of  $\text{H}_2\text{O}_2$ ; hence, the activity levels of catalase and ascorbate peroxidase reduced as the removal of ROS molecules has been scavenged by ascorbic acid antioxidants properties (Dolatabadian *et al.*, 2008). According to Mittler (2002), APX has a much



higher affinity to H<sub>2</sub>O<sub>2</sub> than CAT suggesting that they have different roles in the scavenging of this ROS, with APX being responsible for maintaining the low levels of hydrogen peroxide while CAT is responsible for the removal of its excess. This reduction in catalase could also be attributed to the inhibition of catalase activity by salicylic acid (Shim *et al.*, 2003).

**Ascorbate Peroxidase (APX) activity**

Significant differences (p<0.05) were observed in the interaction between maize cultivars, primers, and salinity levels on ascorbate peroxidase activity in maize plants. The SWAN-LSR-Y cultivar exhibited the highest level of ascorbate peroxidase activity, and KNO<sub>3</sub> priming showed the greatest impact on

upregulating the activity (Table 3). Increasing salinity stress resulted in reduced ascorbate peroxidase activity, with the lowest activity observed at 300 mM salt concentration. These findings are consistent with previous reports on salt stress in wheat genotypes (Sairam *et al.*, 2005).

**Reduced glutathione concentration**

Significant interactions were observed between salinity stress levels, primers, and cultivars on reduced glutathione concentration in maize seedlings. OMR-LSR-SY and SWAN-LSR-Y cultivars had the highest concentrations, with KNO<sub>3</sub> treatment showing the highest concentration (Table 3).

**Table (3): Detailed effect of the interaction between stress levels, primers and the cultivars on the superoxide anion (μmol.mg<sup>-1</sup>FW) content of maize seeds.**

		GSH content x 10 <sup>-3</sup> (μmol.min <sup>-1</sup> .mg <sup>-1</sup> )	Catalase activity (μmol.min <sup>-1</sup> .mg <sup>-1</sup> )	APC activity (μmol.min <sup>-1</sup> .mg <sup>-1</sup> )	SOD activity (μmol.min <sup>-1</sup> .mg <sup>-1</sup> )
Cultivars	SWAN-LSR-Y	4.90 <sup>a</sup>	41.41 <sup>a</sup>	4.42 <sup>b</sup>	5.52 <sup>a</sup>
	BR9928-OMR-SR-Y	4.00 <sup>b</sup>	52.33 <sup>a</sup>	5.05 <sup>a</sup>	5.69 <sup>a</sup>
	OMR-LSR-SY	4.90 <sup>a</sup>	43.50 <sup>b</sup>	5.03 <sup>a</sup>	5.72 <sup>a</sup>
	p-value	<0.00	<0.002	<0.002	0.74
	H <sub>2</sub> O (control)	4.60 <sup>ab</sup>	45.55 <sup>b</sup>	5.21 <sup>a</sup>	6.06 <sup>a</sup>
Primers	KNO <sub>3</sub>	4.80 <sup>a</sup>	55.33 <sup>a</sup>	5.21 <sup>a</sup>	6.06 <sup>a</sup>
	ASA	3.80 <sup>ab</sup>	56.44 <sup>a</sup>	4.85 <sup>ab</sup>	5.65 <sup>a</sup>
	SA	4.70 <sup>a</sup>	44.22 <sup>b</sup>	4.80 <sup>ab</sup>	6.17 <sup>a</sup>
	p-value	0.06	<0.002	<0.01	<0.00
	0	4.00 <sup>c</sup>	58.33 <sup>a</sup>	8.20 <sup>a</sup>	8.78 <sup>a</sup>
Stress levels (mM)	50	3.90 <sup>c</sup>	54.44 <sup>a</sup>	7.05 <sup>b</sup>	7.80 <sup>b</sup>
	100	4.90 <sup>ab</sup>	52.22 <sup>a</sup>	5.58 <sup>c</sup>	6.74 <sup>c</sup>
	150	4.30 <sup>bc</sup>	50.55 <sup>a</sup>	2.65 <sup>d</sup>	3.92 <sup>d</sup>
	300	5.20 <sup>a</sup>	36.39 <sup>a</sup>	0.68 <sup>e</sup>	1.04 <sup>e</sup>
	p-value	0.008	<0.00	<0.00	<0.00
Cultivar×Primer	p-value	0.00	0.99	<0.00	<0.00
	s				
Primer×Stress	p-value	0.05	ns	<0.03	<0.01
Cultivar×Stress	p-value	0.00	ns	Ns	ns
Cultivars×Primer×Stress	p-value	0.00	ns	<0.02	ns

N.B: Values with the same superscript across the treatments are not significant at p<0.05; H<sub>2</sub>O, Water; KNO<sub>3</sub>, potassium nitrate; ASA, ascorbic acid; SA, salicylic acid; ns, not significant.

Maize plants irrigated with 300 mM salt solution had higher glutathione concentrations. Glutathione plays a vital role in maintaining the GSH/GSSG ratio and has various functions in cellular processes and stress responses (Noctor & Foyer, 1998; Kukreja *et al.*, 2005). Its high activity here aligns with previous reports on salt-tolerant cultivars of potato (Das & Roychoudhury, 2014). In this study, KNO<sub>3</sub> priming increased the activities of SOD, CAT, APX, and GSH, highlighting the importance of potassium (K<sup>+</sup>) in enhancing antioxidant potential.

## Conclusion

The results of the study found that the germination attributes such as speed of germination, germination percentage and seedling vigour index of all the three maize cultivars subjected to salt stress improved upon priming with KNO<sub>3</sub>, ascorbic acid and salicylic acid. An indication that the priming agents confer salt tolerance on the maize plants, however, further studies on tolerance mechanisms need to be done at the molecular level. The priming agents employed in the present study could provide farmers with quicker and cost-effective approach in mitigating salt stress in maize crops, as it provides an alternative to conventional methods for salt tolerance and the cultivars response to salinity.

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## Contributions of Authors

**B.U.O;** Conceptualization.

**A.S.A;** Methodology.

**A.R.L. and H.A:** formal analysis and data curation.

**L.B.A and B.U.O:** Writing—original draft preparation.

**A.A and U.F.O;** writing review and editing.

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## Conflict of interest

The authors declared that they have no conflict of interest.

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## تحسين القدرة الأولية الكيميائية للبذور على الإنبات ونمو الشتلات في تراكيب وراثية من الذرة تحت إجهاد الملوحة

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**المستخلص:** هدفت هذه الدراسة إلى معرفة مستوى التحمل واستخدام البادئات (H<sub>2</sub>O، KNO<sub>3</sub>)، حمض الأسكوربيك وحمض الساليسيليك)، في تخفيف الإجهاد لتراكيب وراثية تم انتاجها حديثا من الذرة الصفراء SWAN-LSR-Y، BR9928-OMR-، SR-Y و OMR-LSR-SY). تم التحقيق من دور SOD و APX و CAT و GSH وبيروكسيد الدهون، لقياس الاستجابة الكيميائية الحيوية لبذور الذرة الصفراء. بذور الذرة الصفراء المعاملة بـ KNO<sub>3</sub> وحمض الأسكوربيك ادى الى تحسن الإنبات والقدرة المضادة للأكسدة ضد ROS في تخفيف إجهاد الملوحة، بينما يبطئ حمض الساليسيليك من الإنبات. تم اتباع نفس الاتجاه في مؤشر قوة البذور وطول الجذر للبذور المحضرة بـ حمض الأسكوربيك، والذي سجل أعلى القيم. لوحظ أن المجموعة الضابطة تحتوي على أعلى مؤشر قوة للبذور، بينما أظهرت البذور المعاملة بـ حمض الساليسيليك أقل مؤشر قوة في بذور الذرة. أظهرت زيادة إجهاد الملوحة تأثيرات سلبية على جميع مقاييس النمو. من بين تراكيب وراثية من الذرة المختبرة، أظهرت SWAN-LSR-Y أكبر قدر من تحمل إجهاد الملوحة، من حيث الإنبات. تظهر الأنشطة الأنزيمية العالية بشكل ملحوظ ونسبة بيروكسيد الدهون المسجلة في البذور المحضرة بـ حمض الأسكوربيك و KNO<sub>3</sub> أهميتها في أنشطة التمثيل الغذائي للنبات

**الكلمات المفتاحية:** النشاط الأنزيمي، الأكسدة، الذرة الصفراء، الملوحة.