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Research Article
Mycorrhiza and Seed Priming Effect to Improve the Balance of Sodium and Potassium and Some Changes in Antioxidants in the Leaves of Maize (*Zea mays* L.) Under Soil Salinity

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Abstract

Background and Objective: In order to improve the growth and resistence maize (*Zea mays* L.) under soil salinity, application of seed priming and mycorrhiza that can be beneficial for a better alternative, once it increases the production of some antioxidants, the balance of sodium and potassium, reduces the absorb of sodium in plant and increase the emergence rate and emergence percentage in plants under soil salinity. This study aimed to evaluate the effects of seed priming and mycorrhiza on improving the balance of sodium and potassium and some changes in antioxidants in the leaves of maize under soil salinity. **Materials and Methods:** This combined analysis experiment was laid out in a randomized complete block design as factorial with three replications in both year and place of saline and non-saline on hybrid corn NS640. The tertiary factor was inoculation and non-inoculation with mycorrhiza years places (*Glomus mossea*), the fourth factor was priming with NaCl solution, salicylic acid, tap water and non-prime (control). The proper solutions concentration and priming duration were determined in separate experiments. Two-way analysis of variance was done using the PROC GLM procedure of the SAS. **Results:** The results showed inoculation with mycorrhiza and seed priming treatments in comparison to non-inoculation and non-prime the enzymes catalase and peroxidase, superoxide dismutase, soluble proteins and potassium increased in leaves in both places and maximum of these characteristics were obtained by seed priming with salicylic acid and inoculation with mycorrhiza especially in saline soil. The sodium amount in inoculation with mycorrhiza and prime with salicylic acid, than non-prime and non-inoculation in both places was decreased specially in saline environment. Colonization percentage and rate of emergence has been increased in inoculation with mycorrhiza and prime with salicylic acid in both environments, specially in non-saline environment. **Conclusion:** Maize seed priming with salicylic acid and inoculation with mycorrhiza play an important role in the plant response to salt stress.

Key words: Catalase, colonization, peroxidase, salicylic acid, superoxide dismutase

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Corn has relatively short growing season and high performance[1]. Khuzestan province located in Southwestern Iran has around 1.2 million hectares of arable land. Corn cultivated fifty thousand hectares and produced more than 250,000 t[2]. Almost thirty percent of corn planted was in soil with high salinity. Seed priming by different methods an application of with mycorrhiza fungi is one approach that can improve emerge and yield in seeds planted in saline lands. According to reports seeds prime in environmental stress such as salinity, seedling growth with more vigor produces[3]. Janda *et al.*[4] reported that prime with salicylic acid and tap water stimulates growth of corn and increased production of antioxidants. Prime with salicylic acid at a concentration of 1.4 mM in corn increased resistance to salinity stress[5]. Similar results in tomatoes has been obtained and due to increase the activity of enzymes such as aldose-rodactaze and ascrobate-peroxidase and concentrations of osmolytes such as soluble proteins in leaves[6]. Chang and Sung[7] reported that hydro and osmo-prime with salt than others prime treatments to improve the appearance of sweet corn seedling. Hydro-prime will speed up germination, establishment and enhanced the yield of the barley[8]and beans[9]. Wu *et al.*[10] reported that prime with salicylic acid to reduce the negative effects of salinity and significantly increased resistance of maize seedlings by the content of carotenoids and antioxidants, content of proline and osmolytes. When the seeds have been primed by different methods and inoculated with mycorrhiza has been improved biomass and yield in saline conditions[11]. Mycorrhiza increased uptake of nitrogen, phosphorus, magnesium and other elements[12] and also improves soil structure, increased plant resistance to stress conditions such as drought and salinity[13]. Plants have been inoculated with mycorrhiza increased growth and yield, potential of osmotic and maintain ionic balance normal level that increased resistance to stress conditions[11]. Increase the activity of antioxidants such as catalase, peroxidase and superoxide dismutase were higher in mycorrhiza plants that reduced the lipid peroxidation because antioxidants reduced the effects of reactive oxygen species before they damage the membrane lipids and reduce lipid peroxidation[14]. Other research in mycorrhiza plants in salinity showed that the content of sodium in the root to shoot more and decreased the sodium to potassium ratio in leaf than non-mycorrhiza[15]. This study aimed to evaluate the effects of priming and mycorrhiza on improving the balance of sodium and potassium and some changes antioxidants in the leaves of maize under soil salinity.

MATERIALS AND METHODS

Site description: This study was conducted during the two years 2014/15 and 2015/16 at two locations in the Gotv and City in Khuzestan province with the height of 76 m.a.s.l. and coordinates 32 degrees North latitude and 48 degrees East longitude. Both the soils profile locations in Table 1. Electrical conductivity of irrigation water for both locations was 1.1 dS m^{-1}. The regions climate was semi-arid and dry. Meteorological data from weather stations in the city, which was 5 km from the study, were obtained (Table 2).

Experimental details: To prepare the field, it was irrigated before conducting the experiment and after the field got wet enough, it was a 30 cm deeply by mold board plowing in a few days before sowing and followed by a disking to slice plant residue and incorporate fertilizers into the soil. In both years, planting date was 20 July and harvesting date was 11 December. After preparing the soil for surviving soil conditions sampling do. Fertilize were according to soil test results. Phosphorus and potassium and 1/3 nitrogen fertilizers before planting strips were used and the remaining nitrogen fertilizer was applied at four leaf stage and flowering. Each plot contained six rows with each row between two rows of 75 cm

Table 1: Soil Analysis with different parameters

Type of soil	Organic materials (%)	pH	EC (dS m^{-1})	Soil texture	Available phosphorus (ppm)	Available potassium (ppm)	N(%)
Non-saline	1.4	8.0	2.5	Loamy	13	220	0.28
Saline	0.89	8.4	7.0	Loamy	9	195	0.178

Table 2: Meteorology data of different months

Years	Parameters	July	August	September	October	November	December
2014/15	The average temperature (°C)	36.6	36.4	33.7	27.8	20.1	16.3
	Average rainfall (mm)	0.0	0.0	0.5	22.1	14.7	71.9
	Total sunshine (h)	339.6	344.3	333.9	334.3	211.1	190.4
2015/16	The average temperature (°C)	36.6	37.8	35.1	39.7	21.2	14.5
	Average rainfall (mm)	0.0	0.0	0.0	0.0	54.9	93.6
	Total sunshine (h)	305.6	369.0	310.8	253.0	189.0	169.1

and a length of 5 m, depth of planting each seed 4-6 cm was considered. The distance between the two plants 16 cm and plant density per hectare was 74000 plants. Seed priming was performed before planting and seeds for plantings transferred to the field. Mycorrhiza strips underneath the seeds were placed 20 g m^{-2}. The inoculum contained 150 spores per gram which was prepared by Turan company.

Sampling: To measure the amount of soluble proteins, sodium, potassium and the antioxidant enzymes (catalase, peroxidase, superoxide dismutase) in the 4-6 leaf stage sampling was performed using the upper leaves and to keep fresh samples were placed in containers of liquid nitrogen and transferred to the laboratory for analysis. The laboratory located at Faculty of Agriculture, University of Shahid Chamran, Ahvaz. In addition to calculating the root colonization at flowering (tasseling) stage of plant roots and transported to the laboratory approximately one gram samples were stained color. Phillips and Hayman[15] was used for root staining. The percentage of root colonization was determined by the intersection of grid lines.

Chemical analysis

Protein extraction and enzymatic extraction: Some of the fresh materials (shoots) were worn with liquid nitrogen inside a porcelain mortar. Centrifuge samples at 12000 rpm for 10 min at 4°C was distributed between the supernatant at 20°C until testing was held. This solution was used to measure enzyme and protein.

Catalase (CAT), peroxidase (POD), superoxide dismutase (SOD): Activities of antioxidant enzymes were measured using the methods of Samantary[16].

Sodium and potassium were measured by photometry: In this method, the elements in the solution by flame or atoms are excited and thermal energy electrons and atomic nuclei pushed farther into orbit. The radiation intensity was directly related to the concentration and thus determines the light intensity comparison.

Emergence percentage (EP) were calculated using Eq. 1 with the following equation[17]:

$$EP = \frac{n}{N} \times 100 \quad (1)$$

Where:
n = Total number of emerged seedlings
N = Total number of planted seeds

Seedling emergence rate (ER) were calculated using Eq. 2[18].

$$ER = \frac{\sum n}{\sum (t_x)(n_x)} \quad (2)$$

Where:
n = Total number of seeds emerged
n_x = Number of newly emerged seeds at time t_x
t_x = Average seed emergence time

Statistical analysis: This combined analysis experiment was laid out in a randomized complete block design as factorial with 3 replications in two years 2014/15-2015/16 in two places of saline and non-saline on hybrid corn NS640. The third factor was inoculation and non-inoculation with mycorrhiza (*Glomus mossea*), the fourth factor in four level osmo-primes with NaCl solution, prime with salicylic acid, prime with tap water and non-prime (control). The NaCl solution (1280 mg NaCl solved in 1 L distilled water) and salicylic acid concentration and duration as well as the duration of tap water were determined in preliminary tests in laboratory. The concentration of salicylic acid 0.5 mM within 14 h, osmo priming with NaCl solution at a concentration of 2 dS m^{-1} (1280 mg NaCl soluted in 1 L distilled water) within 22 h, tap water within 18 h were selected as the best combination of on-farm seed priming. Two-way analysis of variance was done using the PROC GLM procedure of the SAS package 9.1 (SAS Institute, Cary, NC, USA) and test the significance of variance sources, while LSD test (p = 0.05) was used to compare the differences among treatment means.

RESULTS AND DISCUSSION

Results showed that the effects of place, mycorrhiza, prime, place × prime, place × mycorrhiza and mycorrhiza × prime, prime × place × mycorrhiza on the emergence rate was significant (Table 3). The comparison of means indicated that the maximum emergence rate in non-saline environments, inoculation with mycorrhiza and prime with salicylic acid derived that showed increase of about 37% compared to non-inoculation and non-prime. Emergence rate in non-saline in comparison to saline environment was more. The most rate of emergence in saline environments reached, in this place, inoculation with mycorrhiza and prime with salicylic acid in comparison to non-prime and inoculation with mycorrhiza showed increase 27.5% and in comparison to non-inoculation and non-prime showed increase 70% (Table 4). In both saline and non-saline environment, inoculation and non-inoculation with mycorrhiza, prime

Table 3: Significance levels of analysis of variance combined over two locations (saline and non-saline) and two growing seasons (2014/15 and 2015/16)

Source of variation	df	Emergence rate	Emergence percentage	Catalase	Peroxidase	Superoxide dismutase	Soluble proteins	Sodium	Potassium	Colonization percentage
Place (PL)	1	***	***	***	***	***	***	***	***	***
Year (Y)	1	ns	***	ns	**	ns	ns	***	***	***
Y×PL	1	ns	*	ns	*	*	ns	ns	*	***
Rep (Y×PL)	8									
Mycorrhiza (M)	1	***	***	***	***	***	***	***	***	***
Priming (P)	3	***	***	***	***	***	***	***	***	***
PL×M	1	***	***	*	**	***	***	***	***	***
PL×P	3	***	***	***	**	*	**	***	*	ns
Y×M	1	ns	*	ns	**	ns	ns	ns	ns	**
Y×P	3	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y×PL×M	1	ns	*	ns	ns	ns	ns	ns	ns	**
Y×PL×P	3	ns	ns	ns	ns	ns	ns	ns	ns	ns
M×P	3	***	ns	ns	***	ns	ns	ns	ns	**
PL×M×P	3	***	ns	*	ns	***	*	ns	*	*
Y×M×P	3	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y×PL×M×P	3	ns	ns	ns	ns	ns	ns	ns	ns	ns
Rep×M×P(Y×PL)	56									
CV (%)		12.15	9.7	14.08	16.4	11.8	7.5	9.6	10.2	8.3

*Significance at 0.05 probability level, **Significance at 0.01 probability level, ***Significance at 0.001 probability level, ns: Non significant

Table 4: Interaction place×mycorrhiza×prime on the amount of catalase, superoxide dismutase, potassium, soluble protein, colonization percentage, emergence rate

Place	Mycorrhiza	Priming	Catalase ($\mu M\ H_2O_2\ mn^{-1}$/protein)	Superoxide dismutase (μg^{-1} FW)	Potassium (mg g^{-1})	Soluble protein (mg g^{-1})	Colonization percentage	Emergence rate
Non-saline	Non- inoculated	Tap water	0.45^{a}	175.00^{f}	10.98^{i}	12.11^{k}	30.80^{fg}	0.153^{cd}
		NaCl	0.52h	173.50^{f}	10.85^{i}	11.90^{k}	30.10^{fg}	0.157^{cd}
		SA	0.55^{g}	179.50^{f}	11.73^{gh}	12.70^{e}	31.30^{ef}	0.163^{bcd}
		Control	0.38^{i}	152.80^{g}	10.26^{j}	10.70^{l}	27.70^{gh}	0.135^{ef}
	Inoculated	Tap water	0.76^{ij}	205.16^{e}	11.93^{g}	12.90^{i}	51.60^{cd}	0.175^{ab}
		NaCl	0.64^{efg}	202.00^{e}	11.80^{g}	12.50^{j}	52.60^{b}	0.169^{abc}
		SA	0.79d^{e}	221.20^{d}	12.93^{f}	13.90^{g}	54.90^{a}	0.185^{a}
		Control	0.50^{gh}	187.00^{f}	11.26^{hi}	12.15^{k}	47.50^{c}	0.148^{de}
Saline	Non-inoculated	Tap water	0.802^{de}	240.50^{c}	14.30^{d}	15.40^{e}	26.50^{hi}	0.122^{fg}
		NaCl	0.87^{cd}	245.16^{c}	14.10^{d}	15.00^{f}	24.00^{i}	0.116^{g}
		SA	1.07^{b}	260.30^{b}	15.45^{c}	15.80^{d}	29.60^{fgh}	0.131^{fg}
		Control	0.60^{fg}	225.16^{d}	13.60^{e}	13.40^{h}	20.40^{j}	0.094^{h}
	Inoculated	Tap water	1.016^{bc}	264.60^{b}	16.26^{b}	16.90^{b}	40.80^{d}	0.1485^{de}
		NaCl	1.011^{bc}	273.30^{ab}	16.05^{b}	16.20^{c}	40.80^{d}	0.148^{de}
		SA	1.3^{a}	283.60^{a}	17.70^{a}	17.73^{a}	44.70^{c}	0.153^{cd}
		Control	0.79d^{e}	238.50^{c}	15.48^{c}	15.10^{ef}	34.30^{e}	0.120^{fg}
LSD = 0.05			0.169	13.94	0.4824	0.327	3.25	0.0163

Number followed by the same letter in the same column are not significantly different by LSD $\alpha = 5\%$

treatments increased emergence rate than non-prime and prime with salicylic acid to the tap water and NaCl solutions is further increased. It seems that one of the reasons mycorrhiza symbiosis and help to water uptake and phosphorus, which makes faster seeds germination. Zou *et al.*[19] found that the coexistence lead to adequate food prepared specially phosphorous, increased the level of absorption by increasing hypha growth and water absorption by decrease of osmotic effects. Prime with salicylic acid reduced osmotic potential lead to water increasing absorption and more active enzymes that eventually lead to more germination rate because of better activity in some enzymes in the seeds simplify access to food during germination in seeds prime and these seeds can to complete better the germination process in the short term and environmental stress such as salinity and well tolerated[20]. Harris *et al.*[3] reported that hydro-priming would speed up germination, seedling establishment in rice and corn make faster development, earlier flowering and maturity ultimately increased yield and resistant to drought and salinity. Results showed that the effects of place, year, year×place, mycorrhiza, year×place×mycorrhiza, prime×place and year×mycorrhiza on emergence percentage was significant (Table 3). The comparison of means indicated the most percentage of germination obtained in the 1st year, non-saline

Table 5: Interaction year × place × mycorrhiza on the amount of colonization percentage, emergence percentage

Years	Place	Mycorrhiza	Colonization percentage	Emergence percentage
2014/2015	Non-saline	Non-inoculated	27.00^{e}	86.58^{ab}
		Inoculated	50.40^{b}	88.91^{a}
	Saline	Non-inoculated	24.00^{e}	72.90^{d}
		Inoculated	39.60^{c}	81.16^{c}
2015/2016	Non-saline	Non-inoculated	34.66^{d}	84.83^{b}
		Inoculated	53.75a	87.00^{ab}
	Saline	Non-inoculated	25.83^{e}	68.50^{e}
		Inoculated	43.30^{c}	79.40^{c}
LSD = 0.05			3.25	2.33

Number followed by the same letter in the same column are not significantly different by LSD α = 5%

Table 6: Interaction year × mycorrhiza on the amount of peroxidase, superoxide dismutase, emergence percentage

Years	Mycorrhiza	Peroxidase (μM H_2O_2 mn^{-1}/protein)	Superoxide dismutase (μg^{-1} FW)	Emergence percentage
2014/2015	Non-inoculated	0.075^{e}	209.04^{b}	79.75^{b}
	Inoculated	0.127^{a}	236.68^{a}	85.04^{a}
2015/2016	Non-inoculated	0.073^{c}	208.60^{b}	76.6^{c}
	Inoculated	0.102^{b}	235.75^{a}	83.2^{a}
LSD = 0.05		0.023	13.94	2.33

Number followed by the same letter in the same column are not significantly different by LSD α = 5%

Table 7: Interaction place × mycorrhiza on the amount of peroxidase, sodium, soluble proteins

Place	Mycorrhiza	Peroxidase (μM H_2O_2 mn^{-1}/protein)	Sodium (mg g^{-1})	Soluble protein (mg g^{-1})
Non-saline	Non-inoculated	0.05^{d}	1.68^{c}	12.20^{d}
	Inoculated	0.09^{c}	1.31^{d}	13.10^{c}
Saline	Non-inoculated	0.115^{b}	3.61^{a}	18.15^{a}
	Inoculated	0.14^{a}	2.50^{b}	16.08^{b}
LSD = 0.05		0.023	0.1725	0.327

Number followed by the same letter in the same column are not significantly different by LSD α = 5%

place and inoculation with mycorrhiza. In the 1st year emergences percentage was further to the 2nd year. The most percentage of emergence in the 1st year in non-saline and inoculation with mycorrhiza achieved and showed increasing of 2.6% in comparison to the non-inoculation. In the 1st year in saline environment, inoculation with mycorrhiza increased 11.3% in compared to the non-inoculation (Table 5). In this study, in both years the most emergence percent was observed in non-saline environment. Results and difference emergence percentage between inoculation and non-inoculation with mycorrhiza represent a positive impact of mycorrhiza on the saline environment in compared to the non-saline because of mycorrhiza role in reduce absorb sodium, increasing uptake phosphorus and water in salty stress redound to further percentage of emergence. In the 1st year to the 2nd year in August average temperatures was lower and help to further emergence (Table 2). The positive effect of prime on germination time proved for germination, especially in terms of stress, this could be due to faster absorption of water by the seeds pre-treated[21].

The results showed that the effects of place, mycorrhiza, prime, place × mycorrhiza, place × mycorrhiza × prime and prime × place on the catalase amount was significant (Table 3). The comparison of means indicated the maximum amount of enzyme in saline environment, inoculation with mycorrhiza and prime with salicylic acid achieved that showed an increase 64.5% in comparison to non-inoculation and non-prime. In saline environment than non-saline more enzymes in the leaves had accumulated (Table 4). The results showed that the effects of place, year, year × place, mycorrhiza, prime, mycorrhiza × prime, prime × place, year × mycorrhiza, mycorrhiza × place on the amount of the peroxidase was significant (Table 3). The means comparison showed the maximum amount of enzyme in the 1st year and inoculation with mycorrhiza obtained in compared to non-inoculation showed an increase 69% (Table 6). The means comparison showed the maximum amount of enzyme in saline and inoculation with mycorrhiza achieved that in compared to non-inoculation showed an increase 21.7% (Table 7). The means comparison indicated the maximum amount of enzyme in saline place and prime with salicylic acid obtained that in comparison to non-prime showed an increase 75%. In saline place amount of enzyme in the leaves than to non-saline place was further (Table 8). The comparison of means indicated the most amount of enzymes in inoculation with mycorrhiza and prime with salicylic acid obtained in comparison to non-inoculation and non-prime in this environment showed an increase 80% (Table 9). The results

Table 8: Interaction place×prime on the amount of emergence rate, emergence percentage, catalase, peroxidase, superoxide dismutase, soluble protein, sodium, potassium

Place	Priming	Catalase ($\mu M\ H_2O_2\ mn^{-1}$/protein)	Peroxidase ($\mu M\ H_2O_2\ mn^{-1}$/protein)	Superoxide dismutase (μg^{-1} FW)	Soluble protein ($mg\ g^{-1}$)	Sodium ($mg\ g^{-1}$)	Potassium ($mg\ g^{-1}$)	Colonization percentage	Emergence Rate	Emergence percentage
Non-saline	Tap water	0.57^{c}	0.08^{cd}	192.50^{d}	12.30^{e}	1.44^{e}	11.40^{e}	41.00^{a}	0.164^{a}	87.60^{b}
	NaCl	0.58^{c}	0.07^{d}	187.70^{de}	12.20^{ef}	1.57^{de}	11.30^{e}	42.00^{a}	0.163^{a}	86.50^{b}
	SA	0.62^{c}	0.087^{cd}	200.37^{d}	13.10^{d}	1.26^{f}	12.30^{d}	42.16^{a}	0.174^{a}	90.080^{a}
	Control	0.51^{c}	0.06^{d}	177.00^{e}	11.90^{f}	1.70^{d}	10.70^{f}	41.60^{a}	0.142^{b}	83.08^{c}
Saline	Tap water	0.85^{b}	$0.12a^{b}$	252.5^{bc}	15.90^{ab}	2.94^{b}	15.20^{b}	30.16^{c}	0.13^{b}	76.30^{d}
	NaCl	0.89^{b}	0.10^{bc}	259.2^{ab}	15.80^{b}	3.08^{b}	15.07^{b}	33.40^{bc}	0.127^{b}	74.60^{d}
	SA	1.13^{a}	0.14^{a}	272.00^{a}	16.20^{a}	2.60^{c}	16.50^{a}	33.80^{b}	0.137^{b}	81.50^{c}
	Control	0.71^{bc}	0.08^{cd}	238.80^{c}	15.40^{c}	3.59^{a}	14.50^{c}	33.40^{bc}	0.052^{c}	69.50^{e}
LSD = 0.05		0.213	0.0253	13.94	0.327	0.1715	0.482	3.25	0.0163	2.336

Number followed by the same letter in the same column are not significantly different by LSD $\alpha = 5\%$

showed that the effects of place, year×place, mycorrhiza, prime×place, mycorrhiza×prime×place was significant on the level of the superoxide dismutase (Table 3). The comparison of means indicated the maximum amount of enzyme in saline environment, inoculation with mycorrhiza and prime with salicylic acid was achieved that in comparison to inoculation with mycorrhiza and non-prime showed increase 18.9% and in comparison to non-inoculation and non-prime showed an increase 25.9%. In the saline place concentrations of the enzyme in comparison to the non-saline was more (Table 4). The comparison of means indicated the maximum amount of enzyme in the 1st year and saline place obtained in comparison to non-saline showed an increase 34.5% (Table 10). The different stresses produce reactive oxygen species (ROS) that has deleterious effects on growth and physiology in plant[22]. These cells need to be protect from oxidative damage, evidence prove the prime can increase amount enzymes such as catalase, peroxidase and superoxide dismutase on seeds and leaves that prevent of destroy ROS until cope with stress and improve plant activity[7]. This study revealed the most catalase amount in saline place, inoculation with mycorrhiza and prime with salicylic acid obtained than non-saline place more enzyme accumulated in the leaves. The results indicated the most amount of peroxidase in the 1st and 2nd year obtained in inoculation with mycorrhiza. The enzyme concentration in the 1st-2nd year was further, in saline place concentration of enzymes in comparison to the non-saline was more and prime with salicylic acid in both environments lead to the further accumulation of enzyme. In saline environment superoxide dismutase accumulation in comparison to the non-saline place is more and in this place, enzyme amount in inoculation with mycorrhiza and prime with salicylic acid treatment was more and the most enzyme amount achieved in the 1st year and saline environment. Growth places is one of the important factor in the production and accumulation of antioxidants in plants, the unfavorable environment lead to increase the enzyme amount as a resistance mechanism in the plant. In the 1st-2nd year temperature was favorable for plant growth. Results indicated increase germination rate and biomass lead to more enzymes also prime with salicylic acid, NaCl solution and tap water reduce osmotic potential and maintain cell membranes and continue the activity of enzymes in seeds and seedlings which lead to an increase in germination rate and biomass production and accumulation of antioxidants in the plant. Salinity stress increase levels of antioxidant enzymes in plants[23]. The use of salicylic acid in plants under salinity can reduce the toxic effects and increase resistance to stress in wheat[11] and tomatoes[6]. In salinity stress, plants that have been

Table 9: Interaction mycorrhiza × prime on the amount of peroxidase, colonization percentage, emergence rate

Mycorrhiza	Priming	peroxidase (μM H_2O_2 mn^{-1}/protein)	Colonization percentage	Emergence rate
	Tap water	0.076^{c}	26.4^{cd}	0.1379^{cd}
	NaCl	0.07^{cd}	28.4^{c}	0.137^{cd}
Non- inoculated	SA	0.082^{c}	27.5^{c}	0.147^{bcd}
	Control	0.049^{d}	24.1^{d}	0.0537^{e}
	Tap water	0.12^{b}	44.7^{b}	0.157^{ab}
Inoculated	NaCl	0.108^{b}	47.0^{ab}	0.153^{abc}
	SA	0.144^{a}	48.5^{a}	0.164^{a}
	Control	0.08^{c}	45.9^{ab}	0.132^{d}
LSD = 0.05		0.023	3.25	0.0163

Number followed by the same letter in the same column are not significantly different by LSD α = 5%

Table 10: Interaction year × place on the amount of superoxide dismutase

Year	Place	Superoxide dismutase (μg^{-1} FW)
2014/2015	Non-saline	191.70^{c}
	Saline	257.90^{a}
2015/2016	Non-saline	187.04^{c}
	Saline	241.20^{b}
LSD = 0.05		13.94

Number followed by the same letter in the same column are not significantly different by LSD *a* = 5%

primed with salicylic acid increased antioxidant enzymes amount (catalase, peroxidase, superoxide dismutase) and increased plant resistance[24]. The uses of salicylic acid increased the rate of photosynthesis, maintain membrane stability, improved growth parameters, the efficiency of photosynthesis and increased production of antioxidant enzymes in response to salt stress in plants[20].

Results indicated the effects of place, mycorrhiza, prime, place × prime, place × mycorrhiza, place × mycorrhiza × prime on the soluble proteins was significant (Table 3). The comparison of means showed the maximum amount of soluble proteins in saline environment, inoculation with mycorrhiza and prime with salicylic acid achieved that showed increase 17.4% in comparison to inoculation with mycorrhiza and non-prime and in comparison to non-inoculation and non-prime indicated increase 32.3%. In saline environment than non-saline more soluble proteins in the leaves had accumulated. In non-saline environment, inoculation with mycorrhiza and prime with salicylic acid showed increase 14.4% in comparison to inoculation with mycorrhiza and non-prime (Table 4). In plants that are under stress one of the ways to counter rising is osmolytes (soluble carbohydrates, soluble proteins, etc.). Osmolytes addition to adjust osmotic potential that prevent of oxygen free radicals, detoxification and swept the reactive oxygen species[25]. In salty stress, soluble proteins increase in maize salt-sensitive varieties[26]. Ashraf *et al.*[27] represent the wheat under salinity to the results similar achieved. Soluble proteins involved in the regulation of the osmotic plant[28]. The results showed that in the saline environment, inoculation with mycorrhiza and prime with salicylic acid showed a further increase of soluble protein in the leaves that play role in reducing the negative effects of salty stress and help the better plants growth. In wheat under salt stress, prime with salicylic acid increased the amount of soluble proteins and amino acids like proline and decrease the protein content that lead to decrease of osmotic potential and reduce tension[14]. Wu *et al.*[12] reported that prime with salicylic acid decreased negative effects of salinity stress and increased the resistance of corn seedlings by increasing of carotenoids and antioxidant also significantly increased the proline and soluble proteins and other osmolytes.

The results indicated that the effects of place, year, mycorrhiza, prime, prime × place and place × mycorrhiza on amount of sodium was significant (Table 3). The means comparison showed the most sodium amount obtained in saline place and non-prime in comparison to prime with salicylic acid showed increase 27.5%. The lowest amount of sodium in non-saline environment and prime with salicylic acid stored (Table 8). The means comparison indicated the most means sodium obtained in the saline place and non-inoculation with mycorrhiza in comparison to inoculation with mycorrhiza showed increase 30.7% (Table 7). The results indicated the effects of place, year, mycorrhiza, prime, place × mycorrhiza, place × priming and prime × place × mycorrhiza on potassium concentration was significant (Table 3). The means comparison showed the most amount of potassium in saline environment, inoculation with mycorrhiza and prime with salicylic acid obtained in comparison to inoculation with mycorrhiza and non-prime showed increase 14.3% approximately and in comparison to non-inoculation and non-prime indicated increase 30.1% (Table 4). The results from the present study indicated the plants grew in the saline place in comparison to the same plants in non-saline place more accumulated and when inoculation with mycorrhiza take place or in both environments prime with salicylic acid in comparison to non-prime is reduced the entrance sodium to the plant because of the mycorrhiza prevent the entrance of sodium in exchange and increased absorb of potassium to the

plant, the competition between sodium and potassium in favor of potassium acts and maintain the sodium in hypha and root that this potassium increased the resistance to salty stress. In saline place the sodium and potassium increased in the leaves but inoculation with mycorrhiza and enhancing of the symbiosis and prime lead to increase of entrance the potassium ions also reduced entrance the sodium ions. Zhu *et al.*[29] reported the corn plants that inoculation with mycorrhiza reduced sodium uptake, salinity increased sodium absorption in the plant and reduce the absorption of phosphorus, potassium, calcium and magnesium in the *Androgaphis paniculta*. Sodium increase lead to decrease the entrance of potassium because of sodium ions competed with potassium ions to connect to a situation. Colonization of plants slowly increased the amount of potassium ions under salinity stress[30]. In mycorrhiza plants concentration of sodium ion at the root was 51% more than non-mycorrhiza plants[30]. Mycorrhiza plants to reduce the damage of salinity and increased the strength and absorption of nutrients by the hypha of fungi and reduces the toxic effects on plant tissue[28].

The results indicated the effects of place, year, place × year, mycorrhiza, place × mycorrhiza, year × mycorrhiza, year × place × mycorrhiza, place × mycorrhiza × prime on the colonization percentage was significant (Table 3). The comparison of means indicated the most colonization percentage in non-saline environment, inoculation with mycorrhiza and prime with salicylic acid obtained in comparison to non-prime and inoculation showed increase 15.5%. In saline environment, the most colonization percentage obtained in inoculation with mycorrhiza and prime with salicylic acid in comparison to inoculation and non-prime showed increase 30.3% and in comparison to non-inoculation and non-prime showed increase was more two fold (Table 4). The means comparison showed the most colonization percentage in the 2nd year, in non-saline place and inoculation with mycorrhiza achieved in comparison to non-inoculation with mycorrhiza showed increase of 55%. In the 2nd year, saline place and inoculation with mycorrhiza in comparison to non-inoculation showed increase 67.6%. In both saline and non-saline environments colonization percentage was more in the 2nd year (Table 5). Colonization depends on some factors such as temperature, humidity, pH and salty on the soil and etc. These results from the present study indicated in both saline and non-saline environments, inoculation with mycorrhiza and prime with salicylic acid lead to enhancing the colonization, this increase was more in non-saline environment. Sheng *et al.*[31] showed the salinity has been suspended the colonization and growth hypha in corn sensitive to salt stress[32]. Zou *et al.*[19] observed that in citrus fruits that have a symbiotic mycorrhiza overcome the salinity. Colonization will lead to sufficient food supply, especially phosphure, increase the level of absorption by increasing growth hypha and water absorption by reducing the osmotic potential were performed also confirmed that symbiotic with mycorrhiza occurs naturally in saline environment and salt stress in some plants such as bananas, vetch, tomatoes, olives, corn, lotus and palm trees reduced symbiotic with mycorrhiza[33-34]. The results showed in the 2nd year colonization percentage and coexistence is more that can be caused by the presence of mycorrhiza (*Glomus mossea*) in the environment and land cultivation during the 2nd year and adapted to the conditions environment.

CONCLUSION

These results demonstrated that seed priming and inoculation with mycorrhiza treatments specially priming with salicylic acid improving some traits in maize such as production of antioxidant, the amount of potassium in leaves, seedling establishment and soluble proteins as well as reducing sodium, helps to increase resistance in maize under saline so that priming with salicylic acid and inoculation with mycorrhiza was the best combination treatments and approach for the maize cultivation development under saline soil.

SIGNIFICANCE STATEMENTS

This study discovers the interaction of seed priming and inoculation with mycorrhiza on maize (*Zea mays* L.) that can be beneficial for increase the production of some antioxidants (catalase, peroxidase, superoxide dismutase), the balance of sodium and potassium, reduces the absorbance of sodium in plant and increase the emergence rate and emergence percentage in plants under saline soil. This study will help the researcher to uncover the priming maize seeds with salicylic acid and inoculation with mycorrhiza which play an important role in the plant response to salt stress that many researchers were not able to explore. Thus, a new theory on these priming combination and possibly other combinations that induction resistance in maize, may be arrived at.

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