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Original article

Ability of mycorrhiza arbuscular and endophyte species to improve salinity tolerance in chickpea (*Cicer arietinum* L.)

A. Oskoueian[1], A. Nezami[2*], A.R. Bagheri[3], A. Lakzian[4]

1. Ph.D Student Dept. of Agrotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
2. Professor, Dept. of Agrotechnology, Ferdowsi University of Mashhad, Crop Physiology, Mashhad, Iran
3. Professor, Dept. of Biotechnology and Plant Breeding, Ferdowsi University of Mashhad, Genetics and Plant Breeding, Mashhad, Iran
4. Professor, Dept. of Soil Science, Ferdowsi University of Mashhad, Soil Biology, Mashhad, Iran

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Extended abstract

Introduction

Low yield and instability, is one of the most important issues in chickpea cultivation. The adverse environmental conditions have affected the crop yield; in this regard, one of the most important factors is salinity stress that reduces crop yield. Meanwhile, microorganisms have a high ability to mitigation the adverse effects of salinity. In addition, the coexistence of beneficial bacteria and fungi creates a potential for a decrease of salinity stress impacts on plants. Mycorrhizal fungi belonging to the branch Glomeromycota, one of the oldest living organisms introduced to coexist with plants on land and in salinity. These fungi are widely found in saline soils. Research has shown that mycorrhizal arbuscular fungi increase salinity tolerance and prevent yield loss. Studies have shown that the coexistence of mycorrhizal arbuscular fungi with crop roots increases the activity of antioxidant enzymes and this expansion of activity to the plant helps to reduce the effects of salinity stress. With regard to the beneficial effects of mycorrhizal fungi on reducing salinity effects in crops, this study aimed to evaluate salt tolerance in chickpea using native mycorrhizal fungi to improve soil properties and its sustainable production under saline conditions.

Materials and Methods

This study was performed in 2016 as factorial based on completely randomized block design with three replications in the research glasshouse of the College of Agriculture, Ferdowsi University of Mashhad. Salinity stress treatments included four levels (tap water [control], 6, 6 and 9 dS.m-1 sodium chloride) and mycorrhiza species at three levels (native mass, *Piriformospora indica* as endophyte, and *Gigospera margareta*). Four weeks after applying salt stress, maximum quantum efficiency of PSII photochemistry (F'v/F'm) II, stomatal conductance, SPAD index, relative water content (RWC) of leaves, and membrane stability index in the youngest fully expanded leaf were measured. In addition, morphological traits, including plant height, lowest branch height, number of branch number, and number of leaves per plant were measured. At the end of the experiment, the shoot fresh and dry weight, length, volume and dry weight of root were measured, finally root colonization was assessed.

Results and Discussion

The maximum quantum efficiency of PSII photochemistry (F'v/F'm) affected by different levels of salinity and mycorrhiza application. The highest and lowest maximum quantum efficiency of PSII

*Correspondent author Ahmad Nezami; E-Mail: Nezami@um.ac.ir

photochemistry (F'v/F'm) levels were related to 9 $dS.m^{-1}$ salinity treatment with mycorrhiza *Piriformospora* indica and control treatment with *Gigospera margareta* species, the difference between which was 4.5 times. In addition, the highest amount of gas exchange was observed in the *Piriformospora indica* species. The highest SPAD index was related to treatment with *Piriformospora indica* fungi in non-stress conditions and the highest salinity stress level. Moreover, application of *Piriformospora indica* fungal species increased RWC by 4.54% and 9.20%, compared to the use of mycorrhiza native mass and *Gigospera margareta* species, respectively. Application of *Piriformospora indica* showed superiority in membrane stability index relative to *Gigaspora margareta* in all treatments of salinity stress, with the exception of 9 $dS.m^{-1}$ treatment. However, no significant difference was observed between mycorrhiza treatments in 9 dS.m-1 of salinity stress. Root inoculation with *Piriformospora indica* increased plant height by 12.7%, compared to mycorrhiza native mass. At all levels of salinity stress, *Piriformospora indica* increased shoot fresh weight, compared to native mass and *Gigospera margareta* treatments. Furthermore, the least and highest decrease in root length was observed in *Piriformospora indica* and *Gigospera margareta* treatments, respectively. Among mycorrhiza fungi treatments, *Piriformospora indica* produced the highest root volume, compared to native mass and *Gigospera margareta* treatments with a difference of 10.9% and 36.4% between them. In addition, in non saline treatment with *Piriformospora indica* had the highest percentage of root colonization (54.66).

Conclusion

According to the results of the study, most traits evaluated in the study were affected by increased intensity of salinity stress. In addition, increased salinity had a negative impact on root development due to increased soil osmotic potential and toxicity, which ultimately reduced plant growth. Moreover, mycorrhiza inoculation had a significant, positive effect on the photosynthetic system of photosystem II, shoot and root dry weight, ratio of shoot to root, root length and percentage of colonization, root volume, root fresh weight, RWC and membrane stability index. Inoculation of commercial species of mycorrhiza under salt stress increased plant salinity tolerance.

***Keywords*:** Colonization, membrane stability, root, stomatal conductance

Table 1. Chemical properties of the soil study

pH	EC	N	P	K
	$dS.m^{-1}$	%	----$mg.kg^{-1}$------	
7.6	0.8	0.01	1	125

Table 2. Source of variation, degree freedom and mean squares of study traits in chickpea under salinity stress and mycorrhiza fungi

S.O.V	df	Plant height	Lowest branch height	No. of secondary branches	No. of leaf per plant	Shoot dry weight
Block	2	11.08	0.02	0.19	21.02	0.01
Salinity (S)	3	224.2^{**}	0.10^{ns}	0.69^{*}	542.2^{**}	1.10^{**}
Mycorrhiza (M)	2	136.8^{**}	0.02^{ns}	1.36^{**}	197.5^{**}	0.53^{**}
S×M	6	3.78^{**}	0.10^{ns}	0.10^{ns}	9.68^{ns}	0.01^{**}
Errir	22	4.99	0.14	0.13	11.09	0.01
CV%		*8.05*	*18.04*	*14.01*	*7.63*	*3.02*

^{ns}, * and **: non-significant and significant at 5 and 1% level of probability, respectively

Table 3. Effect of salinity and mycorrhiza fungi on study traits in chickpea.

Salinity (dS.m^{-1})	**No. of secondary branches**	**No. of leaf per plant**
0.5	2.77^{a}	50.37^{a}
3	2.77^{a}	49.77^{a}
6	2.66^{a}	40.00^{b}
9	2.22^{b}	34.44^{c}
Mycorrhiza		
Local ecotype	2.66^{b}	40.83^{b}
Gigospera margarita	24.41^{c}	41.81^{b}
Piriformospora indica	31.16^{a}	48.29^{a}

Means with common letter(s) in each column are not statistically significant at 5% probability levels based on Duncan's test

Table 4. Effect of salinity and mycorrhiza fungi of study traits in chickpea.

Salinity	**Mycorrhiza**	**Plant height**	**Shoot dry weight**
dS.m^{-1}		cm	g.plant^{-1}
0.5	**Local ecotype**	31.33^{bc}	5.66^{bc}
	Gigospera margarita	31.00^{c}	4.06^{e}
	Piriformospora indica	36.33^{a}	6.77^{b}
3	**Local ecotype**	31.33^{bc}	4.68^{d}
	Gigospera margarita	26.33^{de}	3.83^{e}
	Piriformospora indica	33.66^{ab}	6.08^{b}
6	**Local ecotype**	26.33^{de}	4.18^{e}
	Gigospera margarita	23.00^{ef}	2.81^{g}
	Piriformospora indica	29.00^{cd}	5.27^{c}
9	**Local ecotype**	21.66^{f}	3.32^{f}
	Gigospera margarita	17.33^{g}	2.44^{g}
	Piriformospora indica	25.66^{de}	4.13^{e}

Means with common letter(s) in each column are not statistically significant at 5% probability levels based on Duncan's test.

Table 5. Source of variation, degree freedom and mean squares of maximum quantum yield of PSII photosystems (F'_v/F'_m), Stomatal conductance, Spad, relative water content (RWC) and membrane stability index (MSI) in chickpea under salinity stress and mycorrhiza fungi.

S.O.V	**df**	**F'_v/F'_m**	**Stomatal conductance**	**Spad**	**RWC**	**MSI**
Block	2	0.0001	148	7.37	5.09	0.69
Salinity (S)	3	0.526**	994**	140**	270**	57.6**
Mycorrhiza (M)	2	0.005**	73.5**	101**	254**	10.6**
S×M	6	0.007**	15.24^{ns}	4.80*	11.0^{ns}	0.87^{ns}
Error	22	0.0001	11.8	1.48	11.70	0.85
CV%		1.85	7.75	7.81	4.71	4.10

$^{ns, *}$ and**:non-significant and significant at 5 and 1% level of probability, respectively.

Table 6. Effect of salinity and mycorrhiza fungi on stomatal conductance, relative water content (RWC) and membrane stability index (MSI) in chickpea.

Salinity	Stomatal conductance	RWC	MSI
$dS.m^{-1}$	$mmol.m^{-2}.s^{-1}$	----------%----------	
0.5	51.67^{a}	79.20^{a}	$25/79^{a}$
3	43.10^{b}	70.31^{c}	21.56^{c}
6	34.69^{c}	74.15^{b}	22.88^{b}
9	$27/37^{d}$	$66/38^{d}$	19.79^{d}
Mycorrhiza			
Local ecotype	39.88^{a}	72.55^{b}	22.71^{a}
Gigospera margarita	36.46^{b}	67.89^{c}	21.48^{b}
Piriformospora indica	41.27^{a}	77.09^{a}	23.33^{a}

Means with common letter(s) in each column are not statistically significant at 5% probability levels based on Duncan's test

Table 7. Effect of salinity and mycorrhiza on maximum quantum yield of PSII photosystems (F'_v/F'_m) and spad in chickpea.

Salinity ($dS.m^{-1}$)	Mycorrhiza	F'_v/F'_m	Spad
0.5	**Local ecotype**	0.730^{b}	72.66^{ab}
	Gigospera margarita	0.720^{b}	67.93^{e}
	Piriformospora indica	0.750^{a}	74.66^{a}
3	**Local ecotype**	0.560^{d}	67.66^{e}
	Gigospera margarita	0.500^{e}	62.68^{fg}
	Piriformospora indica	0.590^{c}	68.33^{de}
6	**Local ecotype**	0.430^{f}	70.33^{cd}
	Gigospera margarita	0.390^{h}	64.00^{f}
	Piriformospora indica	0.420^{g}	71.00^{bc}
9	**Local ecotype**	0.160^{i}	62.00^{fg}
	Gigospera margarita	0.140^{j}	61.11^{g}
	Piriformospora indica	0.170^{i}	64.00^{f}

Means with common letter(s) in each column are not statistically significant at 5% probability levels based on Duncan's test

Table 8. Source of variation, degree freedom and mean squares of study traits in chickpea under salinity stress and mycorrhiza fungi.

S.O.V	df	Root length	Root volume	Root dry weight	Root/Shoot	Colonization
Block	2	0.19	0.05	0.004	0.07	0.79
Salinity (S)	3	130^{**}	3.17^{**}	0.05^{**}	0.51^{*}	558^{**}
Mycorrhiza (M)	2	5.77^{**}	3.06^{**}	0.03^{**}	0.01^{ns}	538^{**}
S×M	6	1.33^{*}	0.12^{ns}	0.001^{ns}	0.14^{ns}	13.8^{**}
Error	22	0.46	0.06	0.001	0.15	2.99
CV%		*4.19*	*7.93*	*9.16*	*10.44*	*4.47*

^{ns}, * and **:non-significant and significant at 5 and 1% level of probability, respectively

Table 9. Effect of salinity and mycorrhiza fungi of study traits in chickpea.

Salinity	Root volume	Root dry weight	Shoot/Root
dS.m^{-1}	cm^{-3}	g.plant^{-1}	
0.5	4.11^{a}	0.54^{a}	4.03^{a}
3	3.29^{b}	0.48^{b}	3.84^{ab}
6	3.04^{b}	0.45^{b}	3.45^{b}
9	2.72^{c}	0.36^{b}	3.76^{ab}
Mycorrhiza			
Local ecotype	3.38^{b}	0.44^{b}	3.74^{a}
Gigospera margarita	2.75^{c}	0.41^{b}	3.81^{a}
Piriformospora indica	3.75^{a}	0.52^{a}	3.76^{a}

Means with common letter(s) in each column are not statistically significant at 5% probability levels based on Duncan's test

Table 10. Effect of salinity and mycorrhiza fungi of study traits in chickpea.

Salinity	Mycorrhiza	Root length	Colonization
dS.m^{-1}		cm	%
0.5	**Local ecotype**	20.33^{b}	47.66^{c}
	Gigospera margarita	20.33^{b}	40.33^{d}
	Piriformospora indica	21.66^{a}	54.66^{a}
3	**Local ecotype**	16.66^{c}	45.08^{c}
	Gigospera margarita	17.33^{c}	32.3^{8f}
	Piriformospora indica	17.33^{c}	50.00^{b}
6	**Local ecotype**	16.33^{c}	36.66^{e}
	Gigospera margarita	14.33^{d}	27.66^{g}
	Piriformospora indica	16.66^{c}	40.00^{d}
9	**Local ecotype**	11.00^{f}	30.75^{f}
	Gigospera margarita	11.00^{f}	25.23^{g}
	Piriformospora indica	12.66^{e}	33.66^{f}

Means with common letter(s) in each column are not statistically significant at 5% probability levels based on Duncan's test

Table 11. Coefficient of correlations of study traits of chickpea.

No.	Traits	1	2	3	4.	5.	6.	7.
1.	F'v/F'm	1						
2.	Stomatal conductance	0.89**	1					
3.	Spad	0.74**	0.74**	1				
4.	RWC	0.64**	0.58**	0.86**	1			
5.	MSI	0.80**	0.72**	0.83**	0.74**	1		
6.	Plant height	0.81**	0.78**	0.74**	0.70**	0.73**	1	
7.	Lowest branch height	0.26ns	0.20ns	0.24ns	0.05ns	0.28ns	0.34ns	1
8.	No. of secondary branches	0.47**	0.42*	0.58**	0.54**	0.47**	0.61**	0.16ns
9.	No. of leaf per plant	0.80**	0.80**	0.59**	0.57**	0.60**	0.78**	0.27ns
10.	Shoot dry weigh	0.87**	0.84**	0.77**	0.73**	0.76**	0.89**	0.25ns
11.	Root length	0.96**	0.88**	0.79**	0.70**	0.82**	0.83**	0.22ns
12.	Root volume	0.73**	0.74**	0.81**	0.78**	0.78**	0.87**	0.26ns
13.	Root dry weight	0.77**	0.76**	0.81**	0.74**	0.70**	0.83**	0.31ns
14.	Shoot/Root	0.26ns	0.25ns	0.01ns	0.09ns	0.78**	0.19ns	-0.11ns
15.	Colonization	0.80**	0.79**	0.82**	0.74**	0.75**	0.90**	0.29ns

Table 11. Continued

No.	Traits	8.	9.	10.	11.	12.	13.	14.	15.
8.	No. of secondary branches	1							
9.	No. of leaf per plant	0.46**	1						
10.	Shoot dry weigh	0.56**	0.89**	1					
11.	Root length	0.47**	0.78**	0.88**	1				
12.	Root volume	0.62**	0.66**	0.86**	0.76**	1			
13.	Root dry weight	0.61**	0.76**	0.87**	0.80**	0.83**	1		
14.	Shoot/Root	-0.05ns	0.31ns	0.32ns	0.24ns	0.12ns	-0.18ns	1	
15.	Colonization	0.62**	0.77**	0.91**	0.79**	0.90**	0.84**	0.21ns	1

ns, * and **:non-significant and significant at 5 and 1% level of probability, respectively.