International Journal of Environmental Studies and Researches (2022),1 (1):107-121

Effect of Salinity Stress on Plant growth and Soil Enzymes in Wheat Rhizosphere

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Abstract

In greenhouse experiment, the influence of salinity stress with Nacl at concentration 2000, 4000and 6000 mg / Kgindvidually and in compination with inoculation by Azospirillumbrislenseon wheat (*Triticum aestivum* L.) plant growth during winter season and microbial enzyme activities were investigated under sandy soil conditions. Wheat plants(Misr 3) were cultivated in pots contain 8Kg sandy soil mixed with 10% compost , irrigation with saline water after 25 day from sowing .The inoculation treatment using A. brislense was added twwec before sowing and after 15 day from sowing . Results, showed that, salinity signifcantly decreased plant fresh and dry weights of wheat plants,espicilly with 6000 mg / Kg . However, inoculation with A.brislense increased plant fresh and dryweights compared to control or all treatment under salinity .The results gained denoted that increased salinity from 2000 to 6000 mg / Kg decreased enzymeactivities i.e. dehydrogenase, urease, and phosphatase activities in the rhizosphere soil especially at 6000 mg / Kg Nacl. A. brislense inoculation improved soil enzymes compared to control and alleviated salinity stress.

Keywords: Dehydrogenase,Enzyme activities, Phosphatase,Urease , salinity, Sandy soil.wheat

Introduction

Salinity is a significant problem worldwide, and Egypt is one of the countries that is particularly affected **Al-Naggar *et al.* (2015)**. Wheat (*Triticum aestivum* L.) is a major cereal crop with a global yield of more than 8.8 million tons **FAO (2020**). Egypt's wheat yield is insufficient for human use. To reduce the gap between consumption and production, wheat growing was expanded to newly reclaimed fields. However, salinity stress (which is expected in the Egyptian North) significantly impacts wheat growth and yield, and yields might drop dramatically, making crop farming unprofitable. (**Mujeeb-Kazi et al., 2019; Zeeshan *et al.*, 2020).** Plants change morphologically, physiologically, biochemically, and molecularly due to salinity **Abd El-Hamid *et al.* (2020)**.

Wheat (*Triticum aestivum* L.), also known as a monocot grass is an important staple food of one-third population of the world as it brings higher carbohydrates and calories to human body (**Hasanuzzaman *et al.*, 2017)**. To ensure the food security for over 9.1 billion global population in 21st century, it has been predicted to enhance the production of wheat by 60% (around 5 t ha−1) from its recent yield of 3.3 t ha−1 till 2050 (**Daryanto *et al.*, 2016)**. On the other end, unfortunately, wheat grain yield losses are increasing up to 60% (**El-Hendawy *et al.*, 2017)** due to the adverse effects caused by soil salinity exposure of about 20% of world's agricultural land (**Upadhyay *et al.*, 2019**). In addition, agricultural soils in hot arid regions accumulate higher amount of salts after the evaporation in the summer and subsequent cultivation of wheat in the winter, thus, causes severe damage at germination and seedling stage than the developmental phases (**Rady *et al.*, 2019**). Therefore, in the scenario of emerging climatic variations, it is very important to understand the salinity tolerance mechanisms to improve wheat production. .Initially, salts accumulate in the plant root zone, reduces plant water uptake from soil due to osmotic gradient which consequently interrupts plant turgor, termed as osmotic stress (**Naveed *et al.*, 2020**). Thereafter, various transporters play their role in plant roots for transport and extensive accumulation of Na^+ and Cl^- ions in cell apoplast and/or cytoplasm via symplastic pathway causing ion toxicity (**Munns *et al*, 2006**). The higher Na^+ flux causes inhibition of Ca^{2+} and K^+ uptake, which ultimately disturbs cell functioning such as photosynthetic capacity, activities of antioxidant enzymes, protein biosynthesis and functioning, hormone release, and plant metabolism, thereby hampering crop growth and yield (**Kamran *et al.*, 2020)**. Rhizosphere microorganisms, particularly beneficial bacteria and fungi, can improve plant performance under stress environments and, consequently, enhance yield both directly and indirectly (**Dimkpa *et al.*, 2009).** Some plant growth-promoting rhizobacteria (PGPR) may exert a direct stimulation on plant growth and development by providing plants with fixed nitrogen, phytohormones, iron that has been sequestered by bacterial siderophores, and soluble phosphate (**Hayat *et al.*, 2010).** Others do this indirectly by protecting the plant against soil-borne diseases, most of which are caused by pathogenic fungi .The problem of soil salinization is a scourge for agricultural productivity worldwide. Crops grown on saline soils suffer on an account of high osmotic stress, nutritional disorders and toxicities, poor soil physical conditions and reduced crop productivity.

In this study, we evaluated the effect of salinity stress indivadually and combaned inoculation with *Aospirillum brislense* on wheat plant growth and enzyme activities, i.e. dehydrogenase ,uruase,phosphatase acid and alkaline in the rhizosphere soil.

Materials and Methods

Sandy soil from the Farm of Environmental Studies and Research, Institute, Sadat City University, was used. Surface soil samples (0-30cm -depth) were collected from the assigned locations. The samples were air-dried, ground, mixed well and sieved through a 2 mm - sieve. The sieved soils were subjected to initial analyses for pertinent physical and chemical properties following the standard methods described by **Page *et al*. (1982)** and **klute (1986)**. The obtained data were recorded in **Table 1** and the analsis of used compost **Table 2.**This study was carried out during the usual winter growth season (2021).

Plant growth medium

The air dried and seived soil was placed in plastic pots of 30 cm – diameter and 25 cm – depth. Each pot was filled with 8 kg of the soil, contain mixture of (compost) with sandy soil (1:10 v:v) . Plants were hand-irrigated with fresh water and fertilized with the standard recommendations of the ministry of agriculture in Egypt for two weeks until plant establishment was assured in the growing media then

Table 1. Physical and chemical properties of experimental soil.

$CaCO_3$%	Organic matter,%	Particle size distr., %			Texture class
		San	Silt	Clay	
1.90	0.03	88.5	4.8	6.61	Sandy

PH*	EC** dSm-1	Soluble cations (meq/L)				Soluble anions (meq/L)			
		Ca^{++}	Mg^{++}	K^{+}	Na^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	SO_4^{--}
7.63	1.82	0.36	0.32	0.13	0.56	-	0.41	0.36	0.61

Table 2. Chemical properties of the organic fertilizers used.

Organic fertil.	**pH ***	**E.C ** dSm-1**	**Organic C%**	**Total N %**	**C:N ratio**	**Total P %**	
Compost	7.62	2.46	17.28	1.21	14.3	0.52	0.61

salinity treatments were applied. Three levels of salinity namely 2000, 4000 and 6000 ppm were established by dissolving Rashidi salt in fresh water until reaching the targeted concentrations. Plants were divided into groups then irrigated with the targeted saline level. Irrigation was carried out daily and in each irrigation cycle, enough drain was allowed to ensure adequate leaching and until reaching the targeted level of salinity in the drain.

Preparation of cultures

Azospirillum brasilense stored at -80 °C was grown for two days at 30 °C on agar plates with Ty medium. The plated cells were suspended in 5 ml of Ty medium and cultured overnight at 30 °C on a shaker. The optical density (OD)of the overnight culture was measured at 595nm using a spectrophotometer and OD was adjusted to 0.4, 1ml of the cell suspension having OD of 0.4 was centrifuged at 6000 rpm for 5 minutes, then the supernatant was discarded and the cells were washed two times with 1ml 10M MgSO4. The supernatant was discarded and the cells were resuspended in 1ml 10M $MgSO_4$. The washed cells suspension was diluted to 10-12 in 10mM $MgSO_4$, and 200µl of the bacterial suspension, corresponding to approximately 10^3 to 10^4 cells was used to inoculate each plant growing in plate as single inoculm and 100µl of the bacterial suspension.

Plant materials and growth conditions:-

Wheat cultivar Misr 3 was kindly provided by Wheat Research Depart., Field Crops Research Institute, Agricultural Research Center, Egypt.

Assay of Enzyme Activities and Bacterial counts in Soil

Activities of the enzymes under study were determined according to the following methods:

- Dehydrogenase: colourimetrically, for the 2,3,5- triphenyl formazan (TPF) produced from the reduction of 2,3,5- triphenyl tetrazolium chloride (TTC), using acetone for extraction **(Thalmann, 1967).**

- Phosphatases: colourimetrically, using a modified universal buffer (MUB) at pH 6.5 for acid phosphatase and at pH 11 for alkaline phosphatase (**Tabatabai,1982**).

Results and Discussion

Fresh and dry weights

The prominent remark noticed from **Table 3** is that, increased salinity levels from NaCl from 2000 ppm to 6000 ppm led to decreased fresh and dry weight of wheat plants ,Also, water content was decreased with increasing NaCl levels. Data presented in **Table 3** show the salinity 2000 ppm decreased fresh weight of plant to 1.95 g/plant while 6000 ppm decreased fresh weight to 1.46 g/plant at 30 days from sowing at the same the weight of wheat plant at 60 days was decreased. The most decrease was observed with adding 6000 ppm NaCl. Inoculation of wheat plants by Azospirillum

Table 3. Effect of salinity levels on fresh and dry mater of wheat plants.

Treatment	30 day after sowing			60 day after sowing		
	Fresh (g/plant)	**Dry (g/plant)**	**Water content**	**Fresh (g/plant**	**Dry (g/plant)**	**Water content**
Control	2.06	1.08	0.98	4.48	3.18	1.3
2000 ppm	1.95	0.88	1.07	3.36	2.06	1.3
4000 ppm	1.66	0.67	0.99	2.98	1.56	1.42
6000 ppm	1.46	0.58	0.88	1.85	0.97	0.88
Azospirillum	2.18	1.69	0.49	5.69	4.16	1.53
Azo+2000ppm	2.08	1.19	0.89	4.88	3.75	1.13
Azo+4000ppm	1.89	0.98	0.91	3.85	2.64	1.01
Azo+6000ppm	1.72	0.76	0.96	2.58	1.45	1.13
LSD at 0.05	0.4152	0.143	-	0.288	0.186	-

enhanced growth .Fresh and dry weight .Also, Azospirillum inoculation led to increase in water content in plant. Data presented in **Table 3** declared that Azospirillum inoculation increased fresh and dry weight compared to control. Also, Azospirillum inoculation decreased the harmful or damaging effect of NaCl on wheat plants. Soil salinity detrimentally affects the various morphological characteristics of wheat plants including seedling growth, plant height, shoot, and root length, the number of roots, leaves, leaf area, fresh and dry weight, root/shoot ratio, and chlorophyll content.

Ahmad *et al.* (2013a) observed that the early maturity of wheat due to salinity stress reduced the crop height and leaf area and found that plumule length was the most sensitive during early growth stages. **Orhan (2016)** reported that halotolerant and halophilic bacteria strains increased the root and shoot length and total fresh weight of wheat under severe salt stress (200 mM NaCl).Also, **El-Akhdar *et al* (2019**) found that ,inoculation with associative nitrogen fixation *Azospirillum* enhanced wheat plant and decreased the deleterious effect of salt stress on wheat plants.

Dehydrogenase enzyme

Dehydrogenase activity is frequently used as a measurement of the overall microbial in soil. Data presented in **Table 4** show the effect of salinity levels (2000,4000 and 6000 ppm NaCl) and inoculation with Azospirillum with salinity.Data show the dehydrogenase activity in the rhizosphere of wheat soil was reduced with adding NaCl when gave 34.68 µg TPF/100 g dry soil/h without adding any salt control but adding 2000 ppm show lower decrease.On the other hand adding 4000 or 6000 ppm led to more decrease of DHA especially with 6000 ppm that appeared greatest decrease in DHA. When gave mean 21.06 µg TPF.

Concerning *Azospirillum* inoculation, data showed the positive effect of the DHA compared to control when gave the higher results value 41.36 (µg TPF/100 g dry soil/h) at 30 day from sowing. Also, the adding *Azospirillum* as inoculum but improve DHA, Also, decrease the deleterious effect of NaCl on the values DHA .Generally, excessive amount of salts in the soil may have an adverse effect on soil microbial activity (**Sinagh *et al*., 2018**).**Rath *et al.* (2016)** found that increased salinity has a strong inhibitory effect on a range of microbial processes in soil. These results agree with **Nia *et al.*,(2012)** who found that Azospirillumstrains alleviate salinity stress with wheat plants.

Urease activity

Soil enzyme activation are closely refuted to soil proportion, soil types and environment of conditions. Urease promotes the hydrolyze of nitrogen. Containing organic matter specially and is closely related to the formation and availability of N in the soil . Date presented in **Table (5)** indicate the effect of salinity stress on urease activity in the rhizosphere soil of wheat plant at 0, 15 , 30. 45 and 60 day from sowing. Adding NaCl decrease the activity of soil enzyme with time. The accumulation of salinity in the rhizosphere inhibited microbial activity in the rhizosphere soil and led to decrease urease activity with time experiment.

Table 4. Effect of salinity on dehydrogenase activity (mg TPF g^{-1} dry soil/h).

Treatment	**Days after sowing**					**Mean**
	0	**15**	**30**	**45**	**60**	
control	34.68	35.95	39.26	36.28	60	41.23
2000 ppm	34.12	36.18	38.38	35.17	35.21	35.81
4000 ppm	28.98	29.36	27.66	25.38	23.52	26.98
6000 ppm	23.48	21.48	19.85	20.57	19.96	21.06
Azospirillum	36.75	38.15	41.36	39.37	37.11	38.54
Azo+2000 ppm	33.28	35.96	39.17	36.18	34.26	35.77
Azo+4000 ppm	30.46	31.88	35.15	32.21	31.18	32.17
Azo+6000 ppm	26.18	29.34	31.28	29.36	27.11	28.65
L.S.D. at 0.05	0.891	1.251	1.133	0.842	1.237	-

Table 5. Effect of salinity on urease activity in soil (mg NH^4 g^{-1} dry soil/24 h).

Treatment	**Days after sowing**					Mean
	0	**15**	**30**	**45**	**60**	
Control	28.59	31.65	32.49	29.66	27.29	29.93
2000 ppm	26.65	28.18	29.65	27.57	25.86	27.58
4000 ppm	24.83	25.25	24.88	23.59	21.75	24.06
6000 ppm	23.11	21.88	19.65	20.18	17.69	20.50
Azospirillum	29.65	33.18	35.61	34.12	30.28	32.56
Azo+2000 ppm	27.15	31.26	32.28	31.36	27.36	29.88
Azo+4000 ppm	25.24	27.68	28.99	26.86	22.54	26.26
Azo+6000 ppm	23.96	23.11	24.17	22.11	19.36	22.54
L.S.D. at 0.05	1.789	1.032	2.005	1.132		26.66

Phosphatase activity

Acid phosphatase activity

Data presented in **Table 6** and illustrated in declared that increased salinity in rhizosphere wheat soil led to decreased the enzyme activity, control treatment gave the mean 146-28 ugpg-1 dry soil/h. The highest effect of saliniy was found with 6000 ppm when gave 121.32 µg p g-1 dry soil/h. on the other hand, inoculation wheat by Azospirillium improved the microbial activity in the rhizosphere soil and increased alkaline phosphatase when gave 148.75 µg P g-1 compared with other treatments. Also, the incorporation Azospirillum alleviates salinity stress on plant and improved microbial activity.

Table 6. Effect of salinity on acid phosphatase activity in soil (µg P g^{-1} dry soil/h).

Treatment	**Days after sowing**					**Mean**
	0	**15**	**30**	**45**	**60**	
Control	151.33	164.42	176.64	184.54	191	173.58
2000 ppm	148.46	156.56	161.65	168.86	174	161.90
4000 ppm	136.51	143.42	148.48	156.88	162	149.45
6000 ppm	131.48	136.43	139.89	141.46	156	141.05
Azospirillum	156.21	165.32	179.32	185.11	194.52	176.09
Azo+2000 ppm	151.69	169.55	170.36	174.65	176.34	168.51
Azo+4000 ppm	146.11	154.89	162.11	166.72	169.66	159.89
Azo+6000 ppm	139.42	143.16	154.32	158.44	161.33	151.33
L.S.D.at 0.05	3.136	2.331	2.247	2.283	2.511	-

Treatment	**Days after sowing**					**Mean**
	0	**15**	**30**	**45**	**60**	
Control	151.33	164.42	176.64	184.54	191	173.58
2000 ppm	148.46	156.56	161.65	168.86	174	161.90
4000 ppm	136.51	143.42	148.48	156.88	162	149.45
6000 ppm	131.48	136.43	139.89	141.46	156	141.05
Azospirillum	156.21	165.32	179.32	185.11	194.52	176.09
Azo+2000 ppm	151.69	169.55	170.36	174.65	176.34	168.51
Azo+4000 ppm	146.11	154.89	162.11	166.72	169.66	159.89
Azo+6000 ppm	139.42	143.16	154.32	158.44	161.33	151.33
L.S.D.at 0.05	3.136	2.331	2.247	2.283	2.511	-

Low salinity levels, but the addition of potassium decreased the toxic effects. In the Ko parcel, the reductions in soil respiration, phosphatase and ß-Glucosidase were 70%, 61.5% and 61%, respectively, when the salinity of the irrigation water was increased from 0.65 to 6.5 dS m-1 . The reductions for the same indices were lesser at K1 (58%, 52% and 53%) and K2 parcels (54%, 47% and 51%), respectively.

Azospirillium inoculation increased microbial activity may be Azospirillum fix nitrogen release growth promoting ie, gibroline and dissolved phosphorus in soil . Also Azospirillum alleviate salinity stress on wheat and increased acid phosphatase activity in the rhizosphere

Data presented in **Table 6** show the effect of salinity stress and the inoculation of Azospirillun on acid phosphatase in the wheat rhizosphere soil. The same trend with alkaline was found with acid phosphatase increased salinty from 2000 to 6000 ppm NaCl. These data was agree with those obtained by **Okure *et al*. (2000)** they found that , the increase in soil salinity had a negative effect on soil's microbiological activity. Soil microbial respiration and the two enzyme activities were inhibited even at low salinity levels, but the addition of potassium decreased the toxic effects. In the Ko parcel, the reductions in soil respiration, phosphatase and ß-Glucosidase were 70%, 61.5% and 61%, respectively, when the salinity of the irrigation water was increased from 0.65 to 6.5 dS m^{-1} . The reductions for the same indices were lesser at K1 (58%, 52% and 53%) and K2 parcels (54%, 47% and 51%), respectively.

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Alkaline phosphatase activity

Data presented in **Table 7** and declared that increased salinity in rhizosphere wheat soil led to decreased the enzyme activity, control treatment gave the mean 146-28 ugpg-1 dry soil/h. The highest effect of saliniy was found with 6000 ppm when gave 121.32 µg p g^{-1} dry soil/h. On the other hand, inoculation wheat by Azospirillium improved the microbial activity in the rhizosphere soil and increased alkaline phosphatase when gave 148.75 µg P g^{-1} compared with other treatments. Also, the incorporation Azospirillum alleviate salinity stress on plant and improved microbial activity. Results presented in **Tables 6 and 7** show that theacid and alkaline phospatase enzymes were heighest after60 day from sowing compared with other days, may be the plant was the maximum growth and the root exudate was heigher than other times.

Table 7. Effect of salinity stress on alkaline phosphatase activity in soil (µg P g^{-1} dry soil/h).

Treatment	**Days after sowing**					**Mean**
	0	**15**	**30**	**45**	**60**	
Control	131.12	137.24	142.46	156.36	164.22	146.28
2000 ppm	128.11	130.69	135.63	141.56	148.36	136.87
4000 ppm	121.39	126.48	129.48	132.48	131.68	128.30
6000 ppm	118.28	120.05	119.59	121.56	126.69	121.23
Azospirillum	136.25	138.36	144.42	159.36	165.37	148.75
Azo+2000 ppm	132.16	134.12	141.36	152.26	148.89	141.75
Azo+4000 ppm	129.36	131.26	138.58	146.22	132.88	135.66
Azo+6000 ppm	121.69	126.48	129.46	131.24	129.75	127.72
L.S.D. at 0.05	1.300	2.375	2.759	2.407	2.151	**-**

Conclusion

It was concluded from the present study that salts stress (Nacl4000 and 6000 ppm) adversely affects the growth and microbial enzymes. Inoculation by *Azospirillum* alleviates salinity stress on wheat plant growth and increased enzyme activities in the rhizosphere soil.

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