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Address:-Ashok Yakkaldevi 258/34, Raviwar Peth, Solapur - 413 005 Maharashtra, India
Cell : 9595 359 435, Ph No: 02172372010 Email: ayisrj@yahoo.in Website: www.ror.isrj.org

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ORIGINAL ARTICLE

BIOFERTILIZATION OF MAIZE GROWN UNDER SALINITY STRESS WITH *AZOTOBACTER* AND ITS IMPACT ON ROOT ANATOMICAL STRUCTURE

[1]MahdiM. Almutawa, [1]Afnan F. Najjar, [1]Fahad A. Al-Fassi and [1&2]Abu-Bakr M. Gomaa

[1]Biological Sciences Department, Faculty of Science, King Abdulaziz University, Saudi Arabia.
[2]Agricultural Microbiology Department, National Research Centre, Cairo, Egypt.

Abstract:

A pot experiment was conducted in the greenhouse of the Faculty of Science, King Abdulaziz University on maize plants to study the impact of different salinity levels, i.e. 0, 1500 and 3000 ppm either in the presence or absence of Azotobacter chroococcumon maizeroots anatomical analysis. The mineral fertilization treatment with NPK (as a positive control) was resulted in smaller compacted and pressed cortical cells on the outer layers of cortical zone; however, the inner cortical zone was completely devoid of any traces of cortical cells. The area occupied by vascular tissue clearly revealed complete disruption of connective tissue. The interaction between NPK and salinity level of 1500 resulted in abnormal outline shape of root-cross section which tended to increase in length by 10% more than the treatment with NPK alone.Both salinity levels reduced stele diameters (length and width) with 4.76 and 23.81% respectively in comparison with the control treatment of mineral fertilization with NPK. The same trend was recorded with the number and thickness of metaxylem vessels where the reduction percent reached 47.05 and 3.38% consecutively with the treatment that received 3000 ppm salinity level against the NPK treatment.Inclusion of Azotobacter with both salinity levels resulted in normal shape of root cross-section. Such response was also detected on vascular tissue with 9 metaxylem vessels. Furthermore, biofertilization with Azotobacter combined with salinity level of 1500 ppm was resulted in more clear and healthy shape of root cross-section. Such effect was recognized in all tissue (cortical zone, pericycle, metaxylem vessels and connective tissue) of root-cross section. The outer and inner cortical layers were detected by 10 layers of cortical cells which were very clear in shape. The excellent shape, arrangement and photometric analysis were recognized in treatment of Azotobactercombined with salinity level of 1500 ppm. This positive influence of biofertilization with Azotobacter was attributed to its properties in biological nitrogen fixation and production of plant growth promoting substances.

KEYWORDS:

Biofertilization, Azotobacter, salinity, maize, roots anatomical structure.

INTRODUCTION

The problem of soil salinity is of immense importance particularly for those countries that lies in arid to semi-arid zones. Generally, high evapotranspiration due to high temperature in the semi-arid and arid zones is the basic cause for salt accumulation on the soil surface. The evaporation rate is generally high and exceeds that of precipitation. Thus, the insufficient rainfall together with high evaporative demand and shallow ground water in most locations enhances the movement of salts to the soil surface. Improper irrigation practices and lack of drainage have aggravated the problem leading to significant reductions in crop productivity. Also, indiscriminate use of synthetic fertilizers has led to the pollution and contamination of the soil, polluted water basins, destroyed micro-organisms and friendly insects, making the crop more prone to diseases and reduced soil fertility (Aly*et al*., 2001; Ebrahim&Aly, 2004; Merzaeva and Shirokikh, 2010 and Shahzadi*et al*., 2012).

Salinity occurs through natural or human induced processes that result in the accumulation of dissolved salts in the soil water to an extent that inhibits plant growth. Sodicity is a secondary result of salinity in clay soils, where leaching through either natural or human induced processes has washed soluble salts into the subsoil and left sodium bound to the negative charges of the clay due to an increase in its concentration. There is competition for fresh water among the municipal, industrial and agricultural sectors in several regions. The consequence has been a decreased allocation of fresh water to agriculture (Tilman*et al.*, 2002).

Application of bio-fertilizers in agriculture became unavoidable to maximize plants salt tolerance and at the same time to minimize the nonstop addition of high doses of chemical fertilizers in which enormous amounts of deleterious heavy metals and other environmental pollutants might be present (Gomaa, 1995). Use of biofertilization reduced the negative effect of salinity on soybean plants cultivated in salt affected soil (Gaballah*et al*., 2011). Gomaa *et al.* (2008) pointed out that bio-fertilization of wheat with Candida tropicalis significantly increased leaf area and spikes numbers of the tested wheat cultivars under salinity stress.

The present investigation aims at studying the role of bio-fertilization of maize plants with Azotobacterchroococcum in the presence of different salinity levels on the differentiation of vascular system of maize roots.

MATERIALS AND METHODS

Isolation of Azotobacter bacterium:

The rhizoshere soil of sorghum plants was obtained from cultivated soil located in Rabigh area, KSA. The rhizoshere soil was air dried and sieved to pass through 2.0 mm mesh. Decimal serial dilutions of the soil prepared in a sterile distilled water up to 10^{-6} one ml of each dilution was transferred into sterile Petri dishes (where three replicates were prepared from each dilution) then a freshly prepared Ashly's agar medium (SubbaRao, 1977) was cooled and poured into the into sterile Petri dishes. The plates were stirred to mix the dilutions and the medium. The plates were left on the bench till solidification and then incubated at 30℃ for 5 days. The emerged Azotobacter colonies of different morphological characteristics were picked up and subjected to further purification process on Ashby's agar medium (SubbaRao, 1977) to get pure isolates.

The Azotobacter strain was identified according toBergey’s Manual of Determinative Bacteriology (1974).

MAIZE ROOTS ANATOMICAL ANALYSIS:

Adventurous roots samples were prepared as follows: middle parts fromZea mays adventitious roots were cut into parts each of them was equal toone centimeter in length. These parts were killed and fixed in FA(A solution consists of Formalin, acetic acid and Ethyl alcohol). These parts were washed and dehydrated in series of ascending concentrations of Ethyl alcohol varying from 50% to 100%. The samples were embedded in paraffin wax (m.p 58 -60) using xylol as a solvent (Johansen, 1940). Using Rotary microtome sections were cut at 15µm thickness, then mounted on slides with the aid off egg-albumin as an adhesives (Sass, 1958). Wax was dissolved in xylol and the slides were passed through descending series of ethyl- alcohol varying from 100% to 50% in descending order. The sections were stained with safranin and light green. The colored sections were kept as permanent preparations on slides with Canada balsam as mounting medium. Sections were examined and photomicrographs were prepared by pentagon camera on

Olympus microscope.

RESULTS AND DISCUSSION

Identification of Azotobacter strain:

The isolated Azotobacter strain was identified according to Bergey's Manual of Determinative Bacteriology (1974) as Azotobacter chroococcum. Fig. (1) illustrates the isolated Azotobacter colony morphology while Fig. (2) indicates the Azotobacter cells shape andits arrangement in addition to the slime layer around the cells.

Fig. 1: The isolated Azotobacterchroococcum colonies.

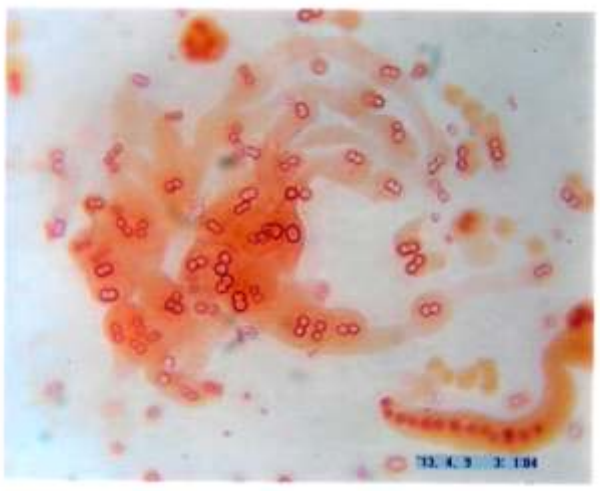


Fig. 2: The cells of isolated Azotobacterchroococcum strain stained with safranin stain.

The maize roots anatomical analysis:

The influence of biofertilization with Azotobacter and application of mineral fertilization (NPK) either alone or in combination with tested salinity levels, i.e. 1500 and 3000 ppm on the anatomical structure (root diameter cross section and cortical layers) of three-month-old maize roots was presented in Table (1) and illustrated in Fig. (3a-c) and Fig. (4a-c).

Regarding the mineral fertilization as such or in combination with both applied salinity levels (1500 and 3000 ppm), the data presented in Table (1) show that application of the high dose of salinity level

(3000 ppm) in combination with the recommended dose of mineral fertilizers (NPK) resulted in reduction percent reached 25 and 29 respectively in length and width of maize roots diameter cross section in comparison with the treatment that received NPK only. The outer layers of cortical zone was compacted and pressed with the epidermal layer, whereas, complete disruption of inner cortical zone was observed (Fig. 3c). This response was associated with the reduction in cortical zone that recorded30% less than the treatment with NPK alone. On the contrary, application of 1500 ppm salinity level accompanied by the mineral fertilizers (NPK) increased both length of root diameters cross section and the thickness of cortical layer; the percentages of increase over the NPK (alone) treatment reached 10 and 100 consecutively.

Table 1: The combined effect of Azotobacter and salinity levels on three-month-old maize roots anatomical structure.

Character / Treatment	Root diameter cross section				Cortical layer			
	Length (µm)		Width (µm)		Number		Thickness (µm)	
	Absolute	± % of NPK	Absolute	± % of NPK	Absolute	± % of NPK	Absolute	± % of NPK
NPK (100%)	1600	0	1600	0	*	*	320	0
NPK+1500 ppm	1760	10	1120	-30	*	*	640	100
NPK+3000 ppm	1200	-25	1136	-29	*	*	224	-30
Azotobacter(Azot.) as control	Absolute	± % of Azot.	Absolute	± % of Azot.	Absolute	± % of Azot.	Absolute	± % of Azot.
Azot.	1120	0	1080	0	*	*	200	0
Azot.+1500 ppm	1600	42.86	1560	44.44	*	*	360	80
Azot.+3000 ppm	1520	35.71	1400	29.63	11	*	240	20
Azotobacter as control	Absolute	± % of Azot.	Absolute	± % of Azot.	Absolute	± % of Azot.	Absolute	± % of Azot.
Azot.	1120	0	1080	0	*	*	200	0
NPK (100%)	1600	42.86	1600	48.15	*	*	320	60
NPK (100%) as control	Absolute	± % of NPK	Absolute	± % of NPK	Absolute	± % of NPK	Absolute	± % of NPK
NPK (100%)	1600	0	1600	0	*	*	320	0
Azot.	1120	-30	1080	-32.5	*	*	200	-37.5
Azot.+1500 ppm	1600	0	1560	-2.5	10	*	360	12.5
Azot.+3000 ppm	1520	-5	1400	-12.5	11	*	240	-25

*not determined

With regard to the biofertilization with Azotobacter either alone or in combination with both tested salinity levels (1500 and 3000 ppm), Fig. (4a-c) shows that inclusion of Azotobacter with both salinity levels resulted in normal shape of root cross-section. Such response was also detected for vascular tissue with 9 metaxylem vessels (Table 2). Furthermore, the treatment with Azotobacteraccompanied by 1500 ppm salinity level resulted in more clear and healthy shape of root cross-section as presented in Fig. (4b). Such effect was recognized in all tissue (cortical zone, pericycle, metaxylem vessels and connective tissue) of root-cross section. The outer and inner cortical layers were detected by 10 layers of cortical cells which were very clear in shape. The combined impact of Azotobacter and salinity levels either 1500 or 3000 ppm augmented both length and width of maize root diameter cross sections and thickness of the cortical layers in comparison with the treatment of Azotobacter as such; the highest increases were recorded with the low salinity level (1500 ppm) that recorded 42.86%, 44.44% and 80.00% respectively over the Azotobacter treatment. These positive effects of biofertilization with Azotobacter could be attributed to the biological nitrogen fixation and the plant growth hormones produced by the applied Azotobacter chroococcum strain. This explanation was in accordance with Lin et al. (1983) and Okon and Kapulink (1986) who stated that association of diazotrophs with plant roots may increase permeability of the host roots to N, P and K-ions. Furthermore, Gomaa (1995) indicated that the Azotobacter supernatant contained plant growth hormones positively affected the hypocotyl growth of cucumber, pea, lettuce, tomato and onion seedlings.

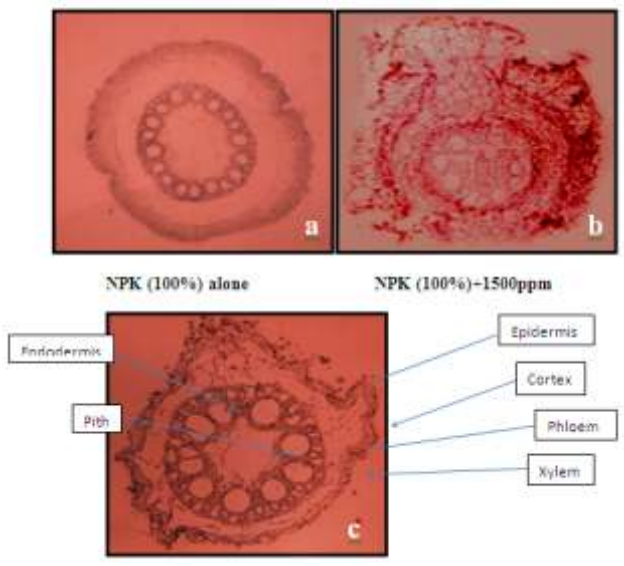
As for the effect of biofertilization with Azotobacter either alone or in combination with the tested salinity levels in comparison with the recommended doses of mineral fertilization with NPK, Table (1) indicates thatAzotobacter in the presence of 1500 ppm increased the thickness of the cortical layer by 12.5% over the NPK (100%) treatment while the application of 3000 ppm with Azotobacter reduced the thickness of the cortical layer by 25% less than the NPK (100%) treatment.

Table 2: The combined effect of *Azotobacter* and salinity levels on three-month-old maize roots anatomical structure.

Character / Treatment	Stele diameter				Metaxylem vessels			
	Length (µm)		Width (µm)		Number		Thickness (µm)	
	Absolute	± % of NPK	Absolute	± % of NPK	Absolute	± % of NPK	Absolute	± % of NPK
NPK (100%)	84	0	760	0	17	0	104	0
NPK+1500 ppm	80	-4.76	576	-24.21	9	-47.05	104	0
NPK+3000 ppm	64	-23.81	600	-12.05	9	-47.05	96	-3.85
Azotobacter(Azot.) as control	Absolute	± % of Azot.	Absolute	± % of Azot.	Absolute	± % of Azot.	Absolute	± % of Azot.
Azot.	600	0	560	0	9	0	120	0
Azot.+1500 ppm	920	53.33	880	57.14	9	0	120	0
Azot.+3000 ppm	640	6.67	560	0	10	11.11	104	-13.33
Azotobacter as control	Absolute	± % of Azot.	Absolute	± % of Azot.	Absolute	± % of Azot.	Absolute	± % of Azot.
Azot.	600	0	560	0	9	0	120	0
NPK (100%)	840	40	760	35.71	17	88.89	104	-13.33
NPK (100%) as control	Absolute	± % of NPK	Absolute	± % of NPK	Absolute	± % of NPK	Absolute	± % of NPK
NPK (100%)	840	0	760	0	17	0	104	0
Azot.	600	-28.57	560	-26.32	9	-47.05	120	15.38
Azot.+1500 ppm	920	9.52	880	15.79	9	-47.05	120	15.38
Azot.+3000 ppm	640	-23.81	560	-26.32	10	-41.18	104	0

*not determined

The impact of various treatments on root maize stele diameter and metaxylem vessels was presented in Table (2) and Figs (3a-c and 4a-c). With regard to mineral fertilization (NPK) either alone or in combination with both tested salinity levels, i.e. 1500 and 3000 ppm (Fig. 4a-c), it was observed that both salinity levels reduced stele diameters (length and width) with 4.76 and 23.81% respectively in comparison with the control treatment of mineral fertilization with NPK. The same trend was recorded with the number and thickness of metaxylem vessels where the reduction percent reached 47.05 and 3.38% consecutively with the treatment that received 3000 ppm salinity level against the NPK treatment. Table (2) also reveals that application of *Azotobacter* in the presence of 1500 ppm salinity level increased the maize root stele diameter (length and width) with 53.33 and 57.14% consecutively when compared with the treatment of *Azotobacter* as such. At the high salinity levels (3000 ppm) in the presence of Azotobacter, the thickness of metaxylem vessels was reduces by 13.33% in comparison with the *Azotobacter* only.



NPK(100%) +3000ppm

Fig. 3(a-c): Effect of NPK alone and in interaction with salinity levels on maize root anatomical structure at the age of three months old. (All Figs. X=50).

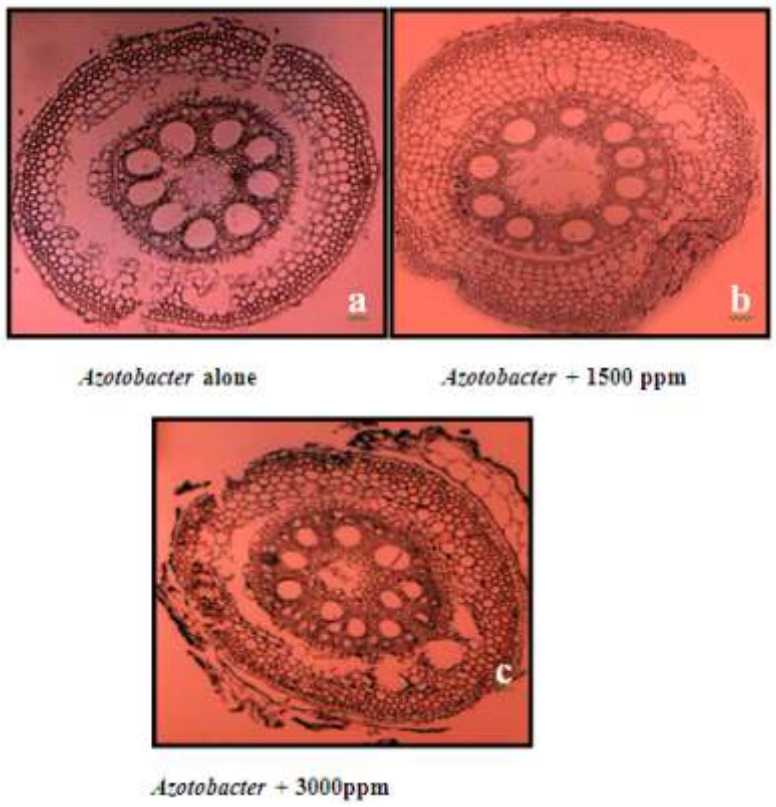


Fig. 4(a-c): Effect of *Azotobacter* alone and in interaction with salinity level of 1500 and 3000 ppm on maize Root anatomical structure at the age of three-month-old.

As for the comparison between the biofertilization with *Azotobacter* and the mineral fertilization with NPK, Table (2) also shows that the stele diameter measures and the metaxylem vessels were increased due to the mineral fertilization except the metaxylem vessels which reduced by 13.33%.

Regarding the effect of biofertilization with *Azotobacter* either alone or combined with salinity levels on maize roots stele diameter and metaxylem vessels and comparing the results with those obtained by the mineral fertilization, Table (2) indicates that *Azotobacter* combined with 1500 ppm salinity level increased both length and width of stele diameter with 9.52 and 15.79% over the NPK treatment. On the other side, inclusion of 3000 ppm salinity level with *Azotobacter* reduced both length and width of stele diameter with 23.81 and 26.32% in comparison with the mineral fertilization treatment. As for the metaxylem vessels, it was recorded that the mineral fertilization surpassed the other treatments in metaxylem vessels number while the opposite was true regarding the thickness of metaxylem vessels (Table 2).

In conclusion, application of *Azotobacter* chroococcum as a biofertilizer mitigated the harmful effect of salinity on maize plants due to its property in biological nitrogen fixation and plant growth promoting substances production.

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Abu-Bakr M. Gomaa
Biological Sciences Department, Faculty of Science, King Abdulaziz University, Saudi Arabia.
Agricultural Microbiology Department, National Research Centre, Cairo, Egypt.