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Response of Soil Microflora and Major Elements Content of Wheat Plants to Potassium Fertilization and/or *Azospirillum brasilense* Inoculation

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IN A POT experiment, the effect of various doses of potassium and/or *Azospirillum brasilense* inoculation on rhizosphere microflora, growth and macronutrient content of wheat plant was studied. It was shown that 150 mg/kg soil induced the maximum significant increase in *Azospirillum* population and nitrogenase activity in the free soil, rhizosphere and rhizoplane in either inoculated or uninoculated wheat plants. This was followed by a significant drop with further increase in KCl reaching maximum at 300 mg/kg Soil. A reliable increase in the bacterial and Actinomycetes count was observed at high KCl concentrations in all rhizosphere samples. The reverse was observed with the fungus population. Also, the biomass gain by wheat plants, nitrogen and phosphorus contents increased with increasing KCl level up to 200 mg/kg soil then progressively dropped till we reached 250 and 300 mg/kg soil. On the other hand, the potassium content progressively increased with increasing KCl level, to the same extent. Irrespective of inoculation, the maximum gain of biomass, nitrogen, potassium and phosphorus were attained at 150 mg KCl/kg soil. Accordingly, soil amendment with potassium, in the appropriate concentration, provides an optimum condition for the interaction of *A. brasiliense* with the other soil microorganisms in the rhizosphere of wheat plants leading to maximum growth incorporation of the nutrient elements.

Keywords: *Azospirillum brasilense,* Potassium fertilizer and wheat, Wheat plant and microflora, Nitrogen fixation and wheat.

It is well known that plants release various organic compounds, via their roots, to the surrounding medium. These compounds are regarded the main source of nutrients to the microorganisms in the rhizosphere. These, in turn, stimulate photosynthetic and growth processes of the plants (Merbach and Roppel, 1992). This is attributed to the following activities: a-Secretion of vitamins and phytohormones (Polonskaya, 1995), b-Production of aniotibiotics that inhibit growth of the pathogenic fungi (Garagula *et al.*, 1989), and c- Conversion of mineral elements to a form available for plant roots. Under stress factors such as mineral deficienct, exosmosis and linear growth of roots are enhanced (Panikiv, 1991).

The microbial population of the root zone of plants strongly influences their development and crop quality (Whipps and Lynch, 1986). The effects of the physical and biological properties of the soil on a plant are, to a greater extent, mediated by the rhizosphere microorganisms (Foster, 1985).

The interaction of plants with associative nitrogen-fixing and growth -stimulating bacteria is well documented. It is difficult to predict accurately the plant reaction to bacterial inoculation, which varies from positive to neutral to negative (Kloepper *et al.*, 1989; Maiorova *et al.*, 1996). The activity of the introduced bacteria greatly depends on their interaction with the resident microorganisms. Nitrogen-fixing bacteria easily form associations with various microorganisms, which provide them with readily available sources of carbon and phosphate and utilize part of the nitrogen fixed (Belimov and kezhemyakor 1992). Diazotrophs adapt better to ambient conditions and have some nutritional advantage over other bacteria. Nitrogen-fixing Azospirilli are antagonists to phytopathogenic microorganisms (Redkina, 1990). On the other hand, under strong competition for root excretions as the sole substrate, many rhizosphere bacteria also produce antibiotics and other inhibitors of microbial growth (Suslow and Schrath,1982). A rapid decrease in the numbers of *Azospirillum* cells on wheat (Bashan, 1986) and maize (Fallik and Okon, 1988) roots following their introduction demonstrated their poor ability to compete with rhizosphere microflora and led to a decrease in inoculation efficiency.

Due to its stimulatory effect on the activity of K^+ - dependent ATPase and other enzymes, potassium favours the synthesis of high molecular compounds in the roots and can change the permeability of the cell membranes (Evans and

Wildes, 1971). These processes are closely connected with the qualitative and quantitative composition of root exometabolites which are used by associative nitrogen fixers to cover their nutritive and energy requirements and define, to a great extent, the structure of the microbial community in the rhizosphere (Kravchenko, 1989). A high content of potassium in the rhizosphere of wheat was reported to decrease the number of bacterial cells exhibiting growth on media with glucose + amino acids. The share of bacteria that require more complicated media for their growth increased significantly under these conditions (Simek *et al.*, 1993). Soil mineral deficiency stimulate the competition between the rhizosphere microorganisms and plant roots. This could be attributed to more efficient consumption of dissolved minerals by microorganisms and increased rate of exosmosis of organic compounds by the roots (Pechurkin *et al.*, 1997).

The aim of this work was to study the response of the microbial population and nitrogenase activity in the rhizosphere of wheat plants to potassium fertilization and/or *Azospirillum* inoculation with reference to some parameters of the growing wheat plants.

Material and Methods

Wheat seeds (*Triticum vulgare* L. cultivar Giza 164), kindly supplied by the Agriculture Research Center, Giza, Egypt, were surface sterilized by soaking in 0.1% mercuric chloride for 1 min, then thoroughly rinsed with running water for 1 hr. Fifty uniform seeds were placed in 15-cm diameter Petri dishes containing two wetted filter papers with 20 ml water, and germinated for 48 hr. Seeds were then sown in 20-cm plastic pots (5 seeds/pot) containing 4kg clay loam soil (pH 7.4) containing 0.8, 1.2, 0.6 mg total nitrogen, phosphorus and potassium per 100g oven-dry soil respectively.

Azospirillum brasilense cd strain, obtained from the Biofertilizer Unit, Soil and Water Research Institute, Agriculture Research Center, Giza was grown for 3 days at 27°C on agar Dabereiner'S malate medium, supplemented with sucrose and yeast extract (DAS medium) (Berestetskii *et al.*, 1985). Cells were thoroughly washed with sterile water to give a suspension of 10^6 cells / ml. Half the pots was inoculated with 10 ml of the above suspension per 1kg soil.

All pots were irrigated to field capacity during the experimental period, under greenhouse conditions at 20 - 28°C. Ten days post sowing, the seedlings were thinned to 3 uniform plants/pot. 50, 100, 150, 200, 250 and 300 mg KCl /1 kg soil was applied to the pots before planting.

Plants were harvested 45 days post sowing and their total dry weight was assayed. Their total nitrogen and phosphorus contents were determined colorimetrically as described by Raveh and Avnimelech (1979) and Murphy and Riley (1962) respectively. Potassium content was assayed by flame photometry.

For analysis of the rhizosphere microorganisms, roots of 4 replicate samples of each treatment, were washed of adherent soil by placing them in 100 ml sterile tap water and shaking at 180 rpm for 3 min. The soil, washed off, represented the rhizosphere samples. The roots were then weighed and homogenized in 100 ml sterile tap water, under aseptic conditions. Microorganisms, remaining on the roots represent the rhizoplane flora. Four replicate samples (5g each) of the surrounding free soil were placed in 100 ml sterile tap water.

Prior to microbiological analysis, all samples of soil, rhizosphere or rhizoplane were sonicated for 30 seconds, in a bath sonicator (Model B 220 USA). Sequential ten fold dilutions were prepared for each sample and plated on soil extract agar, starch casein agar and Czapek agar medium for bacterial, actinomycetal and fungal population (Johnson and Gurl, 1972); whereas *Azospirillum* was assayed on DAS medium. Each inoculation was performed in 5 replicates. Colony forming units (CFU) were counted 3 days (Bacteria) and 7 days (Actinomycetes or fungi) post inoculation.

The experimental design was complete randomized block with four replicates for each treatment. Statistical analysis was carried out according to Snedecor and Cochran (1980) using L.S.D. to compare the significance of the results.

Results

Table 1 shows that KCl, in presence or absence of inoculated *Azospirillum* significantly increased the latters count in either free soil, rhizosphere or rhizoplane; more prominently under the former condition. The effect continued with increased KCl level to 150 mg / kg soil followed by a significant drop, reaching maximum at 300 mg / kg soil. The *Azospirillum* count was doubled in the non-inoculated soil and tribled or even more in the inoculated soil. The rhizoplane count was much higher, in both cases, than either the rhizophere or free soil count. In the meantime, the relative drop in *Azospirillum* count with increasing KCl level over 150 mg/kg soil, was hardly, if at all, affected by *Azospirillum* inoculation.

TABLE 1. Efect of potassium chloride (mg/kg soil) and / or *Azospirillum brasilense* inoculation on *Azospirillum* population and nitrogenase activity in the rhizosphere rhizoplane, and surrounding soil of wheat plants.

Treatment	K Cl	*Azospirillum* population (CFU x 10^5 cells)			Nitrogenase activity (nmol C_2H_4 /g day)		
		I	II	III	I	II	III
Uninoculated soil	0	0.8	1.4	1.2	1.6	2.6	2.3
	50	0.9	1.7	1.6	1.8	2.8	2.6
	100	1.3	2.6	2.8	2.1	3.5	3.4
	150	1.5	2.8	2.9	2.3	4.1	3.6
	200	1.0	1.8	1.7	1.9	3.3	2.9
	250	0.6	1.6	1.4	1.4	2.9	2.5
	300	0.3	0.8	0.5	0.8	1.6	1.3
L.S.D.	5%	0.5	0.6	0.6	0.7	1.0	0.9
	1%	0.9	1.1	0.9	0.9	1.6	1.6
Inoculated soil	0	10.2	14.6	13.7	36.0	55.0	40.0
	50	12.4	19.2	17.4	42.0	62.0	52.0
	100	22.6	45.9	44.8	75.0	145.0	125.0
	150	31.2	63.8	60.2	95.0	182.0	158.0
	200	14.5	46.2	42.5	48.0	152.0	118.0
	250	8.3	20.2	27.0	27.0	85.0	58.0
	300	3.8	8.6	6.2	18.4	40.0	36.0
L.S.D.	5%	3.9	4.7	4.3	9.7	14.2	13.8
	1%	7.5	7.2	7.6	17.4	27.6	24.3

I: per 1g dry weight free soil

II: per 1g dry weight rhizosphere soil

III: per 1g root fresh weight (rhizoplane)

Similar results were obtained for the nitrogenase activity, where the maximum activity was attained at 150 mg KCl / kg soil that was almost doubled by inoculation of the organism. The relative increase in nitrogenase activity was insignificantly, if at all, affected by the type of sample, being 1.44, 1.58 and 1.57 in the surrounding soil, rhizosphere and rhizoplane respectively.

It is interesting to note that although the nitrogenase activity was highest in the rhizosphere yet the relative increase in its activity was greater in the rhizoplane especially following inoculation. The relative suppressive effect of large KCl doses was far greater in the surrounding soil and least in the rhizosphere; indicating that the nutritional status plays a role in counteracting the suppressive effect of excess potassium on the nitrogen fixing microorganisms colonizing the rhizosphere zone.

Table 2 shows that the natural bacterial count was largest in the rhizoplane and least in the surrounding soil. These numbers slightly but progressively increased in the surrounding soil by increasing KCl concentration; a response that was significantly furthered in the rhizosphere and rhizoplane. Although, at 250 or 300 mg KCl /kg soil, the total bacterial count in the rhizosphere was more or less similar to that of the rhizoplane, yet the relative increase was far greater in the former than the latter and almost unaffected by *Azospirillurn* inoculation. It might be recalled that these KCl levels reduced the *Azospirillurn* count to the half (Table 1). On the other hand, at 150 mg/kg soil (the highest *Azospirillurn* count), the relative bacterial count was least.

Occurrence of actinomycetes in the surrounding soil, rhizosphere or rhizoplane samples was not affected by KCl supplementation. The resuts in Table 2 showed vertical insignificant increase in actinomycetes counts by the increase in KCl levels, which indicates the tolerant ability of actinomycetes towards different concentrations of KCl. On the other hand, fungi populations were affected by KCl concentration. Table 2 indicated reduction in fungi count by progressive increase in KCl concentration, irrespective of type of tested sample. These conclusions apply to the inoculated and uninoculated samples for actinomycetes and fungi.

Table 3 shows that the biomass gain by the wheat plants increased with increased soil KCl doses up to 150 mg/kg soil then gradually dropped, irrespective of inoculation with *Azospirillum*, which furthered increased dry weight gain. The maximum gain was attained at 150 mg/kg soil. This applies to the nitrogen content of the wheat plants. Phosphorus content showed slow rate of increase parallel to the increase in KCl levels up to 150 mg KCl/kg soil. Increasing KCl level increased P content of wheat plants in both inoculated and uninoculated treatments. On the other hand, the potassium content progressively increased irrespective of inoculation reaching maximum at 150mg KCl/kg soil, then dropped without significant differences in both inoculated and uninocuated samples.

TABLE 2. Effect of potassium chloride (mg/kg soil) and / or *Azospirillum brasilense* inoculation on the microbial Population in the rizosphere, rhizoplane and surrounding soil of wheat plants.

Treatment	KCl	Bacterial populations (CFU x 10^{5})			Actimoyccics populations (CFU x 10^{5})			Fungal populations (CFU x 10^{3})		
		I	II	III	I	II	III	I	II	III
Uninocuted soil	0	3.2	9.6	15.8	2.4	34.7	24.6	5.2	11.3	12.9
	50	2.9	11.2	18.6	2.2	33.8	23.2	5.0	10.6	12.7
	100	3.0	14.4	20.9	2.2	32.9	22.8	5.1	10.3	12.3
	150	3.2	15.9	24.4	2.1	33.2	23.8	4.9	9.7	11.6
	200	3.4	19.0	26.2	2.6	34.9	25.6	4.7	9.3	11.0
	250	3.7	24.8	30.4	2.9	38.8	28.8	3.9	8.5	10.5
	300	3.8	30.2	32.6	3.2	39.9	32.6	3.8	7.9	9.8
L.S.D.	5%	0.5	3.9	4.1	0.5	3.8	3.1	0.6	1.3	1.2
	1%	0.8	4.7	5.0	0.7	5.1	4.9	1.2	2.1	2.0
Inoculated soil	0	3.8	13.4	17.6	3.8	40.2	30.2	5.9	11.9	13.5
	50	3.6	17.1	20.4	3.5	39.8	28.9	5.6	11.6	13.2
	100	4.2	21.8	26.2	3.4	38.2	27.5	5.3	10.9	12.9
	150	4.5	23.2	27.8	3.8	38.8	28.7	4.6	10.8	12.1
	200	4.3	25.2	30.6	4.0	40.6	31.6	4.5	10.2	11.8
	250	5.1	32.6	36.2	4.6	44.8	33.9	4.4	9.6	10.9
	300	5.3	40.5	37.4	5.0	45.3	35.8	4.2	8.8	10.2
L.S.D.	5%	0.6	4.2	5.2	0.7	4.2	3.5	0.7	1.1	1.4
	1%	0.9	5.6	6.8	1.3	6.2	5.7	1.5	1.8	2.5

TABLE 3. Effect of potassium chloride (mg/kg soil) and / or *Azospirillum brasilense* inoculation on wheat plant dry weight (g/plant) and its total nitrogen, phosphorus and potassium content (mg/1g dry weight).

Treatment	K Cl	Dry weight	N	P	K
Uninoculated soil	0	0.60	32	2.6	27
	50	0.71	33	2.7	39
	100	0.81	36	3.0	53
	150	0.85	41	3.2	56
	200	0.73	36	3.1	52
	250	0.64	30	2.2	50
	300	0.53	25	2.0	46
L.S.D.	5%	0.07	3	0.4	12
	1%	0.10	7	0.9	23
Inoculated soil	0	0.69	38	2.8	30
	50	0.83	44	2.9	40
	100	0.94	47	3.2	51
	150	0.98	49	3.4	55
	200	0.94	45	3.3	50
	250	0.75	39	2.3	49
	300	0.60	30	2.1	47
L.S.D.	5%	0.80	5	0.5	10
	1%	1.40	9	0.7	15

Discussion

The results of this work outlined the range of potassium salt doses that provide for the maximum survival and nitrogenase activity of *Azospirillum* in close association with wheat plants. Addition of potassium in appropriate concentration seemed to alter the root exudate composition in favour of better growth of *Azospirillurm*. One cannot explain the lower reltive rate of multiplication of uninoculated soil *Azospirillum* compared with that after soil incoculation to shortage of root exudates. It might be attributed to changes in balance between the soil microbiota for the welfare of *Azospirillum* flourishing which may be affected by the exudates of the plant roots. Such fluctuations reflect themselves on the nitrogenase activity which is dependent on the *Azospirillum* content.

It might be recalled that Kraffczyk *et al.* (1984) reported that raising the potassium concentration from 48.4 mg to 290 mg per pot, doubled the acid/carbohydrate ratio and to a lesser extent that of malic acid (1.9 fold) whereas oxalic acid content dropped to the half. Okon *et al.* (1976) reported that malic acid, as opposed to oxalacetic and carbohydrates, is a preferential carbon source for *A. brasiliense*. Also, in the rhizosphere of wheat plants with different levels of genome ploidy, *A. brasilense* populations more effectively utilized root exudates with a low content of amino acids and carbohydrates and a high content of organic acids primarily malic acid (Kravchenko *et al.* 1993). Kravchenko *et al.* (1996) found that 30-600 mg/l potassium in the liquid medium of *A. brasiliense* produced a negligible effect on the number and nitrogenase activity of *Azospirillum*. The extended use of naturally acidic mineral fertilizers as KCl on dernopodzoic medium loam soil had negative effects on soil quality: soil acidity increased: cellulolytic activity decreased, total number of amino acids decreased and mcroorganisms activity decreased (Mineev *et al.*,1998).

The results in Table 2 indicate the high sensitivity of rhizosphere than the rhizoplane bacteria to the high level KCl amendment whereas the free soil bacteria were almost insensitive. Furthermore, they add further evidence for the competition between the different soil microbiota; since the relative increase in the bacterial count, at the optimum KCl concentrtion for maximum *Azospirillurn* count (in either inoculated or uninoculated soil) was almost equally lower than at the most inhibitory KCl level for growth of *Azospirillum* where bacterial count was, at least, doubled.

Furthermore, the results show the slight antagionsitic property between the components of the soil microbiota, in response to KCl application. The stimulatory effect of low KCl doses to *Azospirillum* growth (in either inoculated or uninoculated soil) seemed to slightly suppress the flourishing of either Actinomycetes or fungi in the different soil areas; a response that continued, to the same extent, regardless the drop in *Azospirillum* count, as a result of increased number of soil bacteria. The variations in the microfloral communities can be attributed to changes in root exosmosis under different levels of KCl whereas the nutritional status seemed of minor effect on the response of these microbiota.

In this connection, Panikove (1991) stated that mineral deficiency enhanced exosmosis and linear growth of roots. Kravchenko *et al.* (1996) suggested that the variations in potassium concentrations, within the rhizosphere of barley plants, cause changes in root metabolites which create a peculiar ecochemical zone in the rhizosphere. Lugauskas *et al.* (1997) recorded that potassium fertilizers helped the spread of *Streptomyces* and the amount of bacteria in the soil was reduced when high amounts of fungus and *Streptomyces* species were applied in the treatments.

Table 3 emphasized the role of a wide range of K fertilization (50 -200 mg/kg soil) on dry weight gain, above which the excess K (up to 300 mg/kg soil) was suppressive. *Azospirillum* inoculation invariably affected the response of wheat plants to K amendment. Despite the apparent increase in the nitrogen content of *Azospirillum* supplemented wheat plants, yet the relative nitrogen gain was unaffected by the soil-fixed nitrogen by *Azospirillum.*

Azospirillum inoculation significantly increased dry weight gain and nitrogen content without affecting the phosphorus and potassium content, of the non -potassium amended samples to values almost equivalent to those supplemented with 50 mg KCl/kg soil. In the meantime, *Azospirillurm* inoculation lowered the relative accumulation of potassium in the wheat plants.

Accordingly, one may conclude that *Azospirillurn* inoculation may replace potassium fertilization at the rate of 50 mg KCl/kg soil. Maximum values of above-ground biomass, N, P and K incorporation into the green mass were recorded at moderate concentration (150 mg/kg soil) in both inoculated and uninoculated plants, but seemed with inhibitory effects on application of larger potassium doses (300 mg KCl/kg soil). These responses may be attributed to indirect effect of the different doses of KCl on the interaction of root exudates and microfloral population within the rhizosphere.

It may be recalled that Rankov and Dimitrov (1987) demonstrated that application of 720 kg/ha P_2O_5 and K_2O_2, either singly or in combination, speeded up the growth of most microorganisms and enhanced the intensity of nitrification in tomato rhizosphere. Algawadi and Gatir (1992) reported that inoculation of *Azospirillum brasilense* and *Pseudornonas dtrara* or *Bacillus polymyxa* on the rhizosphere of sorghum plants signifcantly increased growth and nitrogen and phosphorus uptake compared with single inoculation of individual organisms. Kravchenko *et al.* (1996) reported that application of moderate doses of potassium fertilizer increased growth and macroelements, incorporated in the plant shoot were enhanced but, at higher doses, these actions were reversed. In pot experiments, Sarwar *et al.* (1998) found out that inoculation of rice plants with *Azosprillum lipoferrum*, in silty clay loam soils, produced significantly higher growth and yield and increased the nitrogen, phosphorus and potassium content of the rice plant.

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استجابة الكائنات الدقيقة بالتربة ومحتوى العناصر الكبرى لنبات القمح للتسميد بالبوتاسيوم والتلقيح بالازوسبيريلم برازلينس

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فى تجربة أصيص، تم دراسة تأثير إضافة التركيزات المختلفة للبوتاسيوم فى وجود أو غياب التلقيح بالاوزسبيريلم برازلينس على تعداد الكائنات الدقيقة بمناطق المحيط الحيوى للجذر وكذلك النمو ومحتوى العناصر الكبرى لنبات القمح.

وأوضحت النتائج أن التركيز ١٥٠ مجم/كجم تربة انتج أعلى زيادة معنوية فى عدد الازوسبيريلم ونشاط إنزيم النيتروجينيز وذلك فى التربة البعيدة عن الجذور والتربة القريبة من الجذور والتربة الملاصقة للجذور فى كلا من نباتات القمح الملقحة والغير ملقحة بالازوسبيريلم، ومع زيادة تركيز كلوريد البوتاسيوم انخفض عدد الازوسبيريلم ونشاط إنزيم النيتروجينيز وكان أكثر الانخفاضات عند التركيز ٢٠٠ مجم / كجم تربة.

كذلك أوضحت النتائج وجود زيادة واضحة فى عدد البكتريا والاكتينوميستات مع انخفاض فى عدد الفطريات وذلك مع التركيزات المرتفعة من كلوريد البوتاسيوم. أيضا أوضحت النتائج زيادة المحتوى الحيوى والمحتوى النيتروجينى والفسفورى والبوتاسى مع زيادة تركيز كلوريد البوتاسيوم فى التربة وحتى ٢٠٠ مجم / كجم تربة ثم انخفاض تدريجى مع زيادة كلوريد البوتاسيوم حتى ٣٠٠ مجم / كجم تربة وكان أعلى مستوى للمحتوى الحيوى والمحتوى النيتروجينى والفسفورى والبوتاسى عند التركيز ١٥٠ مجم /كجم تربة من كلوريد البوتاسيوم وذلك فى وجود أوغياب التلقيح بالازوسبيريلم.

وبناء على ذلك فإن إضافة التركيز المناسب من البوتاسيوم يؤدى إلى الظروف الملائمة للتفاعل التبادلى بين الازوسبيريلم برازلينس والكائنات الدقيقة فى المحيط الحيوى لنبات القمح مما يؤدى إلى أعلى نمو واستفادة من العناصر المغذية.