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ENHANCEMENT OF NUTRIENTS UPTAKE AND METABOLISM EFFICIENCY OF SORGHUM (*Sorghum bicolor* L) PLANTS BY BIOFERTILIZER UNDER WATER STRESS

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ABSTRACT: **The present study was carried out during two succeeded seasons (1999 and 2000) to evaluate of the effects of *Rhizobium leguminosarum* (R.l) and *Bradyrizobium japonicum* (B.j) inoculation strains, as plant growth ppromoting rhizobacteria (PGPR), with $^1/_2$ NPK recommended dose) on chemical constituents; sugars, chlorophyll, N, P, K, Na, proline, total free amino acids, IAA, ABA, and GA_3 concentrations, of two sorghum cultivars (Dorado and Shandaweel) grown under different soil moisture (100%, 60% and 40% of field capacity). The plants were subjected to four treatments; full NPK recommended dose (control), $^1/_2$ NPK, $^1/_2$ NPK + R.l and $^1/_2$ NPK+ B.j inoculation strain. Also, the qualitative assessment of IAA, hydrogen cyanide (HCN), siderophore production and solubilized phosphorus as affected by the tested rhizobial strains, was conducted. A sample of plants was taken after 70 day from sowing. The results clearly confirmed that inoculation treatments reduced proline accumulation while, increased total sugars, K, total free amino acids in leaves and IAA concentrations in root. Total chlorophyll and nitrogen concentrations increased in leaves of both cultivars. Total nitrogen of leaves was more affected by R.l strain in Dorado than Shandaweel. In addition, ABA concentration increased with reduced soil moisture level after inoculation treatments in Shandaweel, meanwhile, P and GA_3 concentrations did not affected. Therefore, the biofertilizer is considered to be excellent as the effective tools to enhance nutrients**

uptake and reduced chemical fertilizer as well as drought stress damages and consequently the cost and pollution.

Key words: *Bradyrhizobia*, hormones, metabolism, nutrient, PGPR, sorghum, sugar

INTRODUCTION

Sorghum (*Sorghum bicolour* L.) is considered the most important cereal crop in Egypt, after wheat, maize and rice. *Rhizobium* is known to survive in the soil and the rhizosphere of legumes and non-legumes. Non-symbiotic Plant Growth Promoting Rhizobacteria (PGPR) are often used as inoculates, however, they are not as effective as entophytic bacteria (Antuon *et al*., 1998). The PGPR bacteria have the effect plant growth directly by producing and secreting plant growth promoting substances such as, auxins, gibberellins, cytokines, by eliciting root metabolism activity with bacterial surface components and for by supplying biological fixed nitrogen (Chiarini *et al.* 1998). On the other hand, indirect mechanism as suppression of bacterial, fungal and nematode pathogens by production of various metabolites (Kozyrovska *et al*., 1997). Also, increased uptake of nutrients such as N, P, K (Kapulnik *et al*., 1985) has been suggested as one of the mechanism by which PGPR increased growth and crop yield (Höflich *et al*., 1997 and Galal *et al*., 2001).

In addition, *Rhizobium* is more tolerant to drought soils, as well as it is tolerant to the high temperature up to 50°C for more than a few hours (Nutman, 1965). Therefore, resistance to water deficit is manifested in four general ways: timing to avoid water stress, morphological and physiological adaptation as well as metabolic alterations. The last way must be consider for understanding with the relation between PGPR and metabolic activity. This area includes synthesis of protein involved in osmotic adjustment. Proline seem to be the amino acid accumulated in the largest amounts in response to drought stress (Rashad and Ismail, 2000).

Therefore, the present study aimed to investigate the interactive effects of rhizobial inoculation and NPK fertilization doses metabolic activity and nutrients uptake efficiency for two sorghum cultivars grown under different levels of water stress.

MATERIALS AND METHODS

Two pot experiments were conducted at the greenhouse of Biological Nitrogen Fixation Unit, Agricultural Research Center, Giza, Egypt, during 1999 and 2000 growing seasons. Two sorghum cultivars (Dorado and Shandaweel) were supplied by the Field Crops Research Institute, ARC, and were sown on 21st and 18th June 1999 and 2000, respectively. Five seeds of sorghum were sown in each pot (30 cm in diameter contained 10 kg sandy loam soil, which obtained from El-Nubaria as a reclaimed region) and were thinned to leave only two seedlings per pot.

Soil physical properties: sand 66.94%, silt 27.9%, clay 5.86%, textural class (sandy loam), S.P. 41.6%, pH 7.72, E.C (dS m^{-1}) 1.4, $CaCO_3$ 4.2%.

Chemical properties: total nitrogen 0.011%, organic matter 0.52%, Ca^{++} 4.20 meq L^{-1}, Mg^{++} 2.3 meq L^{-1}, Na^{+} 1.49 meq L^{-1}, K^{+} 0.8 meq L^{-1}, HCO^{-}_3 1.23 meq L^{-1}, Cl^{-} 2.5 meq L^{-1}, $SO^{=}_4$ 5.06 meq L^{-1}, P 2.18 ppm, Fe 2.2 ppm, Zn 0.42 ppm and Mn 0.91 ppm. Soil physical and chemical properties were analyzed as described by Piper (1950).

Both pot experiments during the two growing seasons included four treatments (for each cultivar) with four replicates as follows: 1- *NPK recommend dose (control) 2- $^1/_2$ NPK dose 3- $^1/_2$ NPK+ *R. leguminosarum*. 4-$^1/_2$ NPK + *B. japonicum*. The pots experiment was kept at different water regime levels; 100%, 60% and 40% field (F.C.) using Moisture meter after two weeks from sowing. Table (1) shows the source of 12 strains used in this study. The strains were purified and maintained on yeast extract mannitol agar (Vincent, 1970), and stored at -70°C in yeast extract mannitol of both containing 20% glycerol. 10 ml of both culture (x10^9 cell/ml) was added four times to each pot, one at planting and three subsequent applications at one-week intervals. The uninoculated sorghum plants received only bacterial culture

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RQ.F= NPK recommended doses are 120KgN, 48 Kg P_2O_5 and 3Kg K_2O/feddan medium as described by (Sabry *et al.*, 1997). Qualitative assessments of indole acetic acids (de-Britto Alvarez, *et al.* 1995), siderophore (Alexander and Zubeter, 1991), hydrogen cyanide (Baker and Schippers, 1987) and phosphate solublization (Goldstein, 1986) were determined.

A sample of plants was taken after 70 days from sowing and separated into shoots and roots. The crude dry materials of both shoots and roots were prepared and kept for the chemical analyse of reducing, non-reducing and total sugars, total chlorophyll (a+b), proline, total free amino acids, protein, K, P, Na, IAA, ABA and GA_3.

Determination of total chlorophyll (a and b), total free amino acids, proline and plant hormones were carried out on the fresh matter. For pigment determination, the leaves were extracted with dimethylformmid using a spectrophotometer as described by Nornai (1982). Total amino acids were determined in ethanol extract of leave by using ninhydrin reagent (Moore and Stein, 1954). Methanol extracts of the leaves were used for analysis of endogenous plant hormones (IAA, GA_3 and ABA) as described by Vogel (1975). Proline was measured as described by Bates *et al.* (1973).

Determination of total nitrogen, phosphorus, potassium, sodium, total sugars were carried out on the dry material of leaves and roots, hence their dry matter were extracted with hot ethanol (80%) to determine total sugars (reducing + non-reducing sugar). Total sugars were determined by using the phosphomolybdic acid method (A.O.A.C., 1975). For total nitrogen, the modified (Micro-Kjeldahl) apparatus of Parnas and Wagner as described by Pregl (1945) was used. Phosphorus was determined calorimetrically as described by King (1951). Potassium and sodium were determined using the Flame photometer as described by (A.O.A.C., 1975).

RESULTS AND DISCUSSION

It well established that, *R. leguminosarum*–fast growing in rhizosphere and execrate phytohormones in root which transfers up to shoot and possibly increases leaf area, while *B. Japonicum*– slow growing and its effect on growth of root under drought stress conditions.

Data presented in Table (2)

showed that three out of 12 tested strains (ARC 301, ARC 310 and ARC 103), produced hydrogen cyanide (HCN). In this respect, de Britto Alvarez *et al.* (1995) indicated that less than 1% of the 709 isolates obtained from the rhizosphere of tomato produced

Table (1): Strains of rhizobia and bradyrhizobia used in this study.

Bacteria	Strains	Source
Rhizobium leguminosarum		
bv. *phaseoli*	127k 80C, NB554	A (Nottingham Unvi.,UK)
	ARC301, ARC 310	B (ARC, Giza, Egypt)
bv. *trifolii*	SU 157	A
	ARC 101,ARC 103	B
Bradyrhizobium japonicum	USDA SPC4	A
	USDA HH303	C (United State, Dept. of Agric,
	USDA 205	C USA)
	ARC 500	B
	ARC 501	B

Table (2): Qualitative assessments of cyanogens (HCN), siderophores, IAA and soluble phosphate produced by different rhizobia and bradyrhizobia strains

Strains	Relative Rreaction			
	Cyanogens [a]	Siderophore [b]	IAA producers [c]	P-solubilizers [d]
Rhizobium leguminosarum bv. *phaseoli*				
127 K80C	-	+++	+++	++
NB 554	-	++	-	-
ARC 301	+	+++	+++	+++
ARC310	-	-	–	-
bv. *trifolii*				
SU 157	-	++	+	-
ARC 101	+	+++	-	-
ARC103	++	-	-	+
Bradyrhizobium japonicum				
USDA 110SPC4	-	++	+++	+
USDA 205	-	+++	+++	-
USDA HH 303	-	++	-	+
ARC 500	-	-	-	-
ARC 501	-	-	-	-

(-) No, (+) low, (++) moderate, (+++) high reaction.

cyanogens. Moreover, only eight (3%) *R. leguminosarum* of the 266 rhizobia and bradyrhizobial strains produced HCN, as well as production of HCN by *Pseudomonas* is associated with biological control of black root of tobacco (O'Sullivan and O'Gara, 1992). The obtained results in Table 2 also revealed that 67% of the tested strains produced siderophore and about 42% produced IAA. Phosphate soluble was also produced by 42% of the tested strains. These results are in agreement with Antoun *et al.* (1998) who found that, most rhizobia and bardyrhizobia produce siderophore and about 58% of rhizobial strains produced IAA.

The most important finding in the present results (Table 2) is that the qualitative assessments of rhizobial strains strongly emphasized the superiority of only two of *R. leguminosarum* strains; 127 K80C and ARC 301 in addition to another *B. japonicum* strains; USDA 110 spc4 and USDA 205, as a root nodule bacteria. Accordingly, these four super strains were prepared (each two similar strains were mixed as one suspension) to apply as a plant growth promoting rhizobacteria (PGPR) with sorghum plants in our present study. a: A change of colour from yellow to orange-brown of filter paper impregnated with 0.5% picric acid and 2% sodium carbonate indicated the production of cyanide (Baker and Schippers, 1987). b: A bacteria forming an orange halo on chrome azural S agar plate or growing on TSA (10%) agar plates containing 50 mg/l of 8 hydroxyquinoline was considered as positive siderophore producer (Alexander and Zubeter, 1991). C: IAA producing bacteria were separated from organisms producing other indoles (yellow to yellow-brown pigment) by their characteristics pink to red colour produced after exp phosphate agar plates were considered phosphate solubilizers (Goldstein, 1986).

Chemical analysis:

It is important to mention here that the obtained results during both experimental seasons exhibited the same clear and decisive trend. Therefore, the tabulated and discussed data of the various determinations represent the recorded results in the second season (2000).

As shown in Tables (3 and 4), total sugar concentrations seem to be unaffected by both levels of water stress, i.e. 60% and 40%

Table (3): Effect of biofertilizer treatments and three different NPK fertilization doses on the chemical compositions concentrations of the leaves and roots of 70 days-old plants (Shandaweel cultivar) grown under different levels of water stress during 2000 season.

Treatment	Full NPK dose			½ NPK dose			½ NPK+R.l			½ NPK+B.j		
F.C %	100	60	40	100	60	40	100	60	40	100	60	40
Leaves												
Total sugars	36.9	20.5	29.5	22.6	11.6	12.4	35.3	26.7	27.0	36.7	22.6	28.9
T. Chls (a+b)	8.23	6.83	4.63	3.16	3.29	3.88	6.80	9.06	5.44	4.68	6.25	5.73
K	21.0	17.3	16.5	10.0	12.1	9.7	19.2	22.8	24.7	17.4	23.7	22.7
Proline	2.4	4.1	3.5	1.4	0.5	1.5	2.0	0.6	0.8	1.2	0.7	1.8
T.free A.A	8.4	6.9	2.9	5.7	2.4	1.6	4.7	3.0	4.0	6.8	2.3	5.6
N	43	32	33	20	28	23	40	39	42	35	35	38
P	3.3	4.3	3.4	1.2	2.1	1.1	5.7	4.3	6.7	2.0	2.8	9.8
Na	6.4	8.1	9.5	3.8	4.6	3.8	7.4	10.2	8.4	9.0	10.8	11.9
IAA	6.2	17.4	20.4	4.4	5.3	3.4	14.5	19.5	25.2	15.3	24.1	33.4
ABA	8.4	16.1	17.7	12.3	9.8	13.8	12.0	13.6	14.6	10.2	10.8	16.8
GA_3	13.2	14.7	11.8	6.8	0.13	0.21	4.9	7.2	13.6	17.6	9.3	12.4
Roots												
Total sugars	35.2	39.9	32.3	15.7	23.1	24.0	33.4	39.6	36.7	45.2	34.5	44.6
K	9.1	10.1	8.9	5.9	1.6	7.9	8.1	9.0	10.2	10.7	9.9	13.6
T.free A.A	1.8	1.9	3.4	0.86	0.51	1.3	3.5	4.8	4.2	6.4	3.1	3.7
N	25	20	18	16	14	11	24	18	19	18	18	14
P	3.02	2.2	3.5	1.71	0.22	0.44	2.3	3.04	3.02	2.2	2.3	2.7
Na	8.1	6.1	7.3	3.02	3.43	4.6	8.14	8.7	7.7	9.3	9.8	8.14
IAA	11.3	3.6	12.6	1.57	0.19	1.35	14.2	19.5	18.3	12.5	12.7	16.9
ABA	1.0	4.2	5.2	1.18	1.56	4.6	5.34	13.5	6.1	3.57	15.6	18.6
GA_3	4.5	5.2	1.6	0.81	1.03	2.1	0.85	4.7	1.5	0.55	4.8	2.0

T: total, Chls: chlorophyll, A.A: amino acid. T.sugar, K, proline, P, N, Na concentrations expressed as mg/g D.W and other

F.C. in the full-fertilized plants of both cultivars, except the reductions in the Dorado roots at 40% F.C. and in Shandaweel leaves at 60%. Meanwhile, the reduction of 50% of NPK fertilization induced a considerable reduction in sugar concentrations in both cultivars organs at the all F.C. levels. Such reduction was more pronounced at 60% and 40% F.C. when the plants were suffering from the double stresses, i.e. nutrient stress and water stress, especially in Dorado cultivar. When the inoculation treatments were associated with half NPK dose a high positive responses of sugar concentrations in the leaves and roots of both cultivars, were detected.

The most interesting finding here

Table (4): Effect of biofertilizer treatments and three different NPK fertilization doses on the chemical compositions concentrations of the leaves and roots of 70 days-old plants (Dorado cultivar) grown under different levels of water stress during 2000 season.

Treatment	Full NPK dose			½ NPK dose			½ NPK+R.l			½ NPK+ B.j		
F.C %	100	60	40	100	60	40	100	60	40	100	60	40
Leaves												
Total sugars	30.2	27.4	30.2	16.8	7.7	14.4	19.2	21.8	27.5	35.4	26.8	36.8
T. Chls (a+b)	7.57	6.5	6.22	2.56	5.16	4.15	5.52	4.83	7.05	8.44	2.81	7.86
K	18.4	8.45	16.1	6.9	10.2	12.3	20.3	20.4	23.9	15.9	19.1	24.2
Proline	2.2	2.4	3.5	0.3	0.7	0.9	2.6	2.4	2.9	2.0	1.5	2.5
T.free A.A	9.3	6.8	5.4	3.7	3.6	2.4	10.4	8.4	2.0	7.0	5.2	2.4
N	28	25	23	18	14	1	26	30	32	33	22	20
P	5.07	6.76	3.76	2.32	3.04	2.1	4.84	6.84	3.62	3.35	6.58	4.04
Na	6.14	7.98	8.83	5.11	7.31	4.83	8.34	11.6	9.06	7.56	9.89	7.98
IAA	14.6	7.47	6.6	3.4	7.6	5.8	19.8	9.24	2.35	24.7	15.8	17.6
ABA	19.6	20.0	6.64	15.7	2.33	10.4	3.8	13.6	1.20	17.4	7.16	2.10
GA_3	10.7	18.4	21.3	4.6	6.0	9.8	11.5	36.0	23.8	10.5	30.8	28.8
Roots												
Total sugars	38.4	39.2	28.9	25.2	17.2	14.9	31.0	52.0	36.9	49.5	43.9	42.6
K	9.68	5.87	13.1	1.91	3.5	6.4	8.2	6.7	9.3	6.3	7.5	9.2
T.free A.A	7.0	5.4	4.7	1.3	0.5	1.0	8.3	7.9	3.1	6.5	10.5	6.9
N	18.1	16.5	11.2	12.4	7.1	9.0	16.7	12.5	12.3	14.1	12.8	10.7
P	3.41	3.79	2.21	1.81	2.49	0.86	3.01	3.78	1.90	2.88	3.01	1.45
Na	3.34	4.81	5.2	2.91	3.0	2.6	4.51	4.61	3.8	4.7	3.8	4.0
IAA	12.5	14.5	16.1	7.2	8.1	9.3	18.1	16.7	20.4	16.8	18.8	29.9
ABA	3.2	7.9	8.8	4.2	6.7	7.9	5.2	4.3	1.8	6.7	4.8	1.8
GA_3	4.1	2.1	2.5	1.2	0.95	1.1	3.9	2.19	1.90	2.8	7.7	2.9

T: total, Chls: chlorophyll, A.A: amino acid. T.sugar, K, proline, P, N, Na concentrations expressed as mg/g D.W and other

is that sugar concentration in both inoculated organs surpassed even the respective concentrations in the same organs of the control plants (at 100% F.C.) in several cases. The obtained data also confirmed the superiority of *B. japonicum* strain in this regards especially at 100% and 40% F.C. in both cultivars. Moreover, sugar concentrations in the inoculated organ especially the roots ranged between 2 to 3 folds that in the same organs of the plants received only 50% of NPK fertilization. This finding strongly confirmed that inoculation treatments could successfully compensate the double stresses, the reduction in both nutrients as well as water stress due to the increasing of the availability of nutrient in the soil and this enhanced their uptake by the roots. Also, the accumulation of much more quantities of sugars in the roots of water stressed plants increasing their ability to extract more water from the soil solution and consequently did not suffer from the dissection and finally the death. Moreover, it is well known that sugars as osmolytes enable plants to keep better water relation under stress conditions, i.e. salt stress, water stress, nutrients stress and plants to maintain conditions water uptake must decrease internal water potential to maintain sugar. In this regards, Ghallab and Salem, (2001) reported that the high positive responses induced by rhizobial inoculation with respect to total sugar concentration suggest the possibility of the promoting effects of bacteria on photosynthetic activity and consequently photosynthetic products, mainly sugars. It is well documented that sugars and K contribute to the osmotic adjustments in many plant species and osmotic regulation with stressed plants was higher with sugars and K, both of them was found to correlate negatively with the osmotic potential, thus the accumulation of sugars and the other solutes is screening parameter for salinity or drought tolerance. Although K concentration was similarly affected with the different stress treatments, as did with sugar, the data showed that reduced 50% of NPK fertilization severely affected K concentration in both leaves and roots of both cultivars at the all F.C. levels. Moreover, the considerable increments in K concentrations following inoculation was more pronounced in the leaves of both cultivars than the roots ones especially at 40% F.C. suggesting that inoculation treatments increasing K accumulating potential as the water stress increased. In this regard, Lin *et al.* (1983) found that *Azospirillum* inoculation greatly enhanced the uptake of NO^{-}_{3}, NH^{+}_{4}, P, K and Fe as well as accumulation of these nutrients in the stem and leaves of corn and sorghum. Comparing N concentration in both cultivar organs with full-fertilized plants (control), it could be observed that there is a gradual decrease in N concentration by increasing drought level to reach maximum at 40% F.C. Meanwhile, the plants received only 50% of NPK dose showed much lower values in their N concentration than the control ones at the all F.C. levels. Following inoculation treatments, N concentration showed much higher values in both cultivar organs which even greatly exceeded the respective values in the leaves of the full NPK (control plants) with special referring to the superiority of *B. japonicum* strains in both cultivar organs.

As for P concentrations in the same organs, the data reveal that although it fluctuated in the full-fertilized plants with increasing drought stress level, it showed a dramatic drop in the leaves and roots of both cultivar plants received only the half dose of NPK. As would be expected more positive responses in P concentration in both leaves and roots of both cultivars following inoculation were recorded, which, as did with N concentration, even surpassed the control treatment in several cases with marked superiority of *R. leguminosarum* strain followed by *B. japonicum* strain in this respect. The favourable effects of rhizobial inoculation treatment on nutrients concentration was previously evidenced by several workers, Ishac *et al.* (1994) reported that compounds as mg/g F.W . Each value is represent means of three measurements for three plants inoculation with VAM + *R. leguminosarum* increased dry matter as well as N and P content. Moreover, the mixed inoculation of wheat with *Azotobacter* and *Azospirillum* increased the uptake of N, P, K, Fe, Zn, Mn and Cu (Lippman *et al.*, 1995 and Amera and Dahdoh, 1997).

Regarding Na concentration, the data obtained exhibited that a quit different have been observed as result of inoculation with both strains under stress conditions. However, the relative Na to K in Dorado has been considered in both leaves and root when compared with Shandaweel one.

As shown in Tables (3 and 4), the responses of the chlorophyll (a+b) concentration in the leaves of both cultivars, regardless few exceptions, were nearly similar to that described for total sugar concentration in both cultivars (Tables 3 and 4). Such responses were more pronounced with Dorado plants, which are more likely and may be attributed to the very essential role played by chlorophylls in photosynthesis. Increasing sugar accumulation in the leaves and roots of both cultivars strongly indicate a high efficiency of photosynthesis process and consequently increasing photosynthetic products, mainly sugars. On the other hand, it is well documented that rhizobial inoculation enhanced the uptake of ions, which catalyse chlorophyll biosynthesis. Supportive evidences for the obtained results were found in the work of Jadhave *et al.* (1994) who found that the catechol siderophore of a peanut *Rhizobium* isolate, increased plant growth and chlorophyll content compared with plant grown with iron alone. Moreover, Rashad and Ismail (2000) suggested that the biofertilizer application might induce the maintenance of photosynthetic capacity under elevated temperature by increasing chlorophyll biosynthesis or chloroplast size.

On the other hand, the general responses of proline concentration in the leaves of both cultivars, regardless some exception, was similar to that of sugar and chlorophyll, though to lesser extent, it showed gradual increasing with increasing drought level in both cultivars plant received the full fertilization, then it sharply dropped in the leaves of received the full dose of NPK fertilization. In the inoculated treatments, proline showed much higher positive responses, except the unexpected drop in the leaves of Shandaweel cultivar inoculated plants at 60% and 40% F.C. The obtained data also confirmed the favourable effect of inoculation with *R. leguminosarum* tend to increase proline concentration in Dorado leaves, so that proline concentration in the Dorado leaves received the half fertilization dose + inoculated with *R. leguminosarum* strain was more than 8 folds at 100% F.C. and more than 3 folds at either 60% or 40% F.C. when compared with only half NPK treatments plants under the same drought conditions. In according with the present results, Voetberg and Sharp (1991) reported that, proline accumulation is one of the best- characterized osmo-regulatory response which increase up to 100 folds in response to stress. Also, the correlation between growth delay and proline accumulation was previously observed in sorghum seedlings exposed to salinity by

Palovskaya and Khramor (1990). Moreover, Good and Zaplachinski (1994) insured this concept, suggesting that proline is the most actively accumulated amino acid, both in terms of the total amount accumulated and the percentage, increases during drought stress.

As shown in Tables (3 and 4), total free amino acids in the leaves and roots of the full fertilized plant in both cultivars showed gradual decrease as the water stress level increased, except Dorado roots where the reverse trend was recorded. The marked sharp drop in total amino acids concentration was recorded in both leaves and roots (especially the latter ones) of the plants received only 50% NPK dose in both cultivars. When these plants were inoculated especially with *R. leguminosarum* strain in Dorado organs and Shandaweel roots, much higher values of amino acids concentration were recorded which even exceeded the respective values of the full-fertilized control plants at the all F.C. levels except 40% F.C. in Dorado organs. Meanwhile, Shandaweel roots exhibited much more positive response to both inoculation treatments than their leaves, so that, the concentration of amino acids in the inoculated roots were more than double that in the roots of the full-fertilized control plants. In this concern, Good and Zaplachinski (1994) reported that most of the amino acids for example, alanine and aspartic acids showed a linear increasing (average 5-9 folds over the control) with drought stress in Brassica leaves. On the other hand, Kozyrovska *et al.* (1997) suggested that maize grains inoculated with PGPR produced greater yield and possessed a greater percentage of protein.

Therefore, it can be concluded that the favourable effects of the biofertilizer inoculation regarding in increasing the symbiotic N_2-fixation in the soil and the availability of the different nutrients in the soil, enhancement of free amino acids in the plant cells as well as the physiological enzymatic activity and growth rate of roots. Therefore, the high stimulative effects on K, sugars, chlorophyll and free amino acids suggesting also the possibility of the promotive effects of biofertilizer treatments on photosynthetic activity and consequently its products, mainly sugars. Also, increasing available N will enhance the biosynthesis of amino acids, proline, enzymes and proteins.

Concerning the effect of different treatments on the phytohormones, i.e. IAA, GA_3 and ABA in the leaves and roots of both sorghum cultivars, the data in Tables 3 and 4 indicated that IAA concentration showed a marked reduction in the leaves of full-fertilized Dorado control plants at 60% and 40% F.C., meanwhile it increased in the roots of the same plants as the water stress level increased. Moreover, it showed another reduction in the leaves and roots of the plants received only the half dose of NPK fertilization except the leaves at 60% F.C. In Shandaweel cultivar, IAA concentration showed a gradual increases in both leaves and roots of the control plants as the water stress level is increased except the roots at 60% F.C. A considerable increment in IAA concentration following inoculation with both rhizobial strains in the roots and leaves of Shandaweel cultivar, which greatly exceeded the respective values in the full fertilized, control plant organs. As for GA_3, the obtained results clearly indicate that in the leaves of the full-fertilized Dorado plants indicated that GA_3 concentration showed gradual marked increases as the water stress levels is increased, meanwhile the reverse trend was observed in the roots of the same plants. Moreover, a considerable reduction, as compared with the control plants, was recorded in the organs of the plants received 50% of NPK fertilization when, the reduced fertilization was associated with the biofertilizer inoculation a considerable positive effects in GA_3 concentration in the leaves and to lesser extent in the roots so that, its values surpassed the control ones, except in the roots at 100% F.C. On the other hand, GA_3 concentration seems to be unaffected by water stress in the full fertilized leaves and shoots except the marked reduction in the roots at 40% F.C. Also, another considerable reduction in the root and leaves of the plants received 50% only of NPK fertilization, meanwhile following inoculation GA_3 concentration exceeded the content values only in the leaves at 40% F.C., however,. the other increments could not approach the control values, and even showed lower values in the roots than the half-fertilized roots at 100% and 40% F.C. Comparing ABA concentration, it could be noticed that its concentration showed a gradual increase in the Dorado leaves and roots as the water stress increased except the reduced value in the leaves at 100% F.C., however, it concentration greatly fluctuated in the half-fertilized organs as well as in the plants which received the half fertilization + inoculation, thus no clear trend could be drawn in this regard. Nevertheless, the effects of different treatments on ABA concentration were more obvious in Shandaweel organs, it showed a gradual increases in both organs of full-fertilized plant, especially in the leaves, with increasing water stress level. Moreover, although ABA concentration increased in the leaves as affected by 50% NPK fertilization, except at 60% F.C. compared with the full fertilization treatments, a complete response was observed in their roots. When the reduced fertilization was combined with the inoculation much more considerable increases in ABA concentration which greatly surpassed the respective value of the leaves and roots of the control plant in the all cases except the leaves at 60% and 40% F.C.

The promotive effects of rhizobial inoculation on the phytohormonal status in the plants of Dorado and Shandaweel cultivars grown under different levels of water stress have been shown with half NPK dose with either strain inoculation treatment than the control plant. So that did accumulated more IAA and GA_3 as well as ABA in many cases, in the leaves and roots of both cultivars than that in the leaves and roots of control plants, regardless only few exceptions. This strongly confirmed the previous conclusion drawn with the different other constituents, i.e. sugars, chlorophylls, proline, free amino acids, N, P and K as well as dry matter accumulation in the present study. Such promotive effects of biofertilization may be attributed to the production of growth promotive substances from rhizosphere microorganisms such as IAA and GA_3 (Ghulam *et al.*, 1994). In this respect, *Azospirillum* produce several plant hormones in liquid culture, mainly IAA as reported by Fallik *et al.* (1989), gibberellins (Bottini *et al.*, 1989), ABA (Kolp and Martin, 1989) and cytokinins (Horeman *et al.*, 1986).

CONCLUSION

The obtained data in the present study strongly emphasize the superiority of rhizobial inoculation in compensating of 50% of applied NPK fertilizer in addition to increasing to great extent the tolerance of sorghum plants to water stress at its both two levels 60% and 40% F.C. This can be realize from the most interesting and important finding in the results of such inoculation treatments which is that the considerable increments recorded by the inoculated plant under nutrient and water stresses, which even exceeded the unstressed control plants in several cases. Therefore, the obtained data in the present study strongly confirmed the beneficial effects of rhizobial inoculation and suggested that, biofertilizer inoculation can be successfully applied in order to reduced the half of the consumed chemical fertilizers as well as to great extent, increasing sorghum tolerance against drought or water stress. Taking into consideration the economic point of view, such inoculation treatments could be applied to reduce the consumption of chemical fertilizer (by 50%) as well as to saving a great part of the used water for irrigation for sorghum cultivars which in turn minimizing the agricultural costs as well as the pollution of the Egyptian agriculture environment.

REFERENCES

Alexander, D. B. and D. A.Zubeter (1991): Use of chromoazurol S reagents to evaluate siderophore production by rhizosphere bacteria. Biol. Fertil. Soil, 12, 39-45.

Amera, M. A.T. and M. S. A., Dahdoh (1997): Effect of inoculation with Plant Growth Promoting Rhizobacteria (PGPR) on yield and uptake of nutrients by wheat grown on sandy soils. Egyptian J. of Soil Sci. 37: 4, 467-484.

Antoun, H., C.; J., Beauchamp; N. Goussard; R., Chabat and M. Lalande (1998): Potential of *Rhaizobium* and *Bradyrhizobium* species as plant growth rhizobacteria on non-legumes; Effect on radishes (*Raphanous sativus* L.). Plant and Soil, 204: 57-67.

A.O.A.C., (1975): Official

Methods of Analysis of the Association of Official Agricultura Chemists, Washington. 12th Ed.

Baker, A.W. and M. Schippers (1987): Microbial Cyanide production in the rhizosphere in relation to potato yield reduction and Pseudomonas spp.-mediated plant growth-stimulation. Soil Biol. Biochem. 19, 451-457.

Bates, L. S.; L. P., Waldren and I.D.Teare (1973): Rapid determination of free proline for water stress studies. Plant and Soil. 39: 205-207.

Bottini, R.; M. Fulchieri; D.Pearce and R. P. Pharis (1989): Identification of gibberellins A1, A3 and iso-A3 in cultureof *Azospirillium lipoferum*. Plant Physiol. 90:45-47.

deBritto Alvarez, M. A.; A. Gagn and H. Antoun (1995): Effect of compost on rhizosphere microflora at the tomato and on the incidence of plant growth promoting rhizobacteria. Appl. Environ. Microbiol., 61: 194-199.

Chiarini, L.; A. Bevivino; S. Tabacchini and C. Dalmastri (1998): Inoculation of *Burkhoderia cepacia*, *Psedomonas fluoresces* and Enterobacter sp. On *Sorghum bicolour*. Root colonization and plant growth promotion of dual strains inocula. Soil Biol. Biochem. 30. 81.

Fallik, E.; Y. Okon; E. Epstein; A. Goldman and M. Fischer (1989): Identification and quantification of IAA and IBA in *Azospirillum brasilense* inoculated maize roots. Soil Biol. Biochem. 21: 147-153.

Galal, Y. G. M.; I. A. El Ghandour and E. A. Akel (2001): Stimulation of wheat growth and N-fixation through *Azospirillum* and *Rhizobium* inoculation. A field trial with 15N technique. Inter. Conf. Of Plant Nutrition-Food Security and Sustainability of agro-ecosystem. 666-667 Kulwier Publisher.

Ghallab, A. M. and S. M. Salem (2001): Effect of some biofertilizer treatments on growth, chemical composition and productivity of wheat plants grown under different levels of NPK fertilization. Ann. Of Agric., Sci., Ain Shams Univ., Cairo, 46 (2), 485-509.

Ghulam, R.; M. Sajjad Mirza; L. Farooq and A. M. Kauser (1994): Identification of plant growth hormones produced by bacterial isolated from rice, wheat and Kallar grass. Good, A. G., Zaplachinski, T.: The effect of drought stress on free amino acids accumulation and protein synthesis in *Brassica napus*. Physiol. Plant. 90: 9-14.

Goldstein, A.H. (1986): Bacterial solubilization of mineral phosphates, historical perspective and future prospects. Am. J. Alteran. Agric. 1: 51-57.

Good, A.G. and T. Zaplachinski (1994): The effect of drought stress on free amino acids accumulation and protein synthesis in *Brassica napus*. Physiol. Plant., 90: 9-14.

Höflich, G.; W. Wiehe and G. Kuhn (1997): Influence of associative rhizosphere bacteria on nutrient uptake and yield of maize. Arch. Acker. Pflanzenbau. Bodenkd. 41, 323-331.

Horeman, S.; K. J. De Conink; R. Neuray; K. Hermans and K. Vlassak (1986): Production of plant growth substances by Azospirillum sp. and other rhizosphere bacteria. Symbiosis, 2: 341-346.

Ishac, Y. Z., J. S. Angle, M. A. El-Borollosy, M. E. El-Demerdash, M. I. Mostafa and C. N. Fares (1994): Soil moisture, inoculum and soil effects on growth and nodualtion of *Vicia faba* and *Lens esculenta*. Annals. of Agric. Science-Cairo. 39: 2, 581-593. 42:(1), Pp: 51-60.

Jadhave, R. S.; N. V. Thaker and A. Dessi (1994): Involvement of the siderophore of cowpea *Rhizobium* in the ion nutrition of the peanut. World J. Microbiol. Biotech. 10: 360-361.

Kapulnik, Y.; R. Gafni and Y. Okon (1985): Effect of Azospirillum spp. Inoculation on root development and NO_3 uptake in wheat in hydroponic system. Can. J. Bot. 63: 627-631.

King, E. J. (1951): Microanalysis in medical biochemistry. 2nd Ed. Churchill, London.

Kolp, W and W. P. Martin (1989): Response of plant roots to inoculation with *Azospirilum brasilense* and to application of indole-acetic acid. In *Azospirillum*- II. Genetics, Physiology, Ecology, Edited by Kingmaker. Springer-Verlag, Berlin Heidelberg. pp: 215-221.

Kozyrovska, N.; G. Kovtunovych; E. Gromosova; P. Kuharchul; V. Kordyum and D. L.. Wise (1997): Novel inoculation for an environmentally friendly Crop production. Proc. of the third International Symposium of the International Society for Environmental Biotechnology, Boston, USA, 18: 1-4, 79-85.

Lin, W.; Y. Okon and R. W. F. Hardy (1983): Enhanced mineral uptake by *Zea mays* and *Sorghum bicolour* inoculated with *Azospirillum brasilense*. App. Environ. Microbiol., 45:1775-1779.

Lippman, B.; V. Leinhos and H. Bergman (1995): Influence of auxin producing rhizobacteria on root morphology and nutrient accumulation of crops. I. Changes in root morphology and nutrient accumulation in maize (*Zea mays* L.) caused by inoculation with indole-3-acetic acid producing Pseudomonas and Acinetobacter strains or IAA applied exogenously. Ann. Bot. 69:31-36

Martin, P.; A. Glatzle; W. Kolb; H. Omay and N. Schmidt (1989): N_2-fixing bacteria in the rhizosphere quantification and hormonal effects on root development. Festschrift für Phlanzenernahrung und Bodenkunde. 152, 3: 237-245.

Moore, S. and Stein, W. H (1954): A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem.,11:907-913.

Nornai, R. (1982): Formulae for determination of chlorophyllous pigments extracted with N, N-Dimethy.lformamide. Plant Physiol., 69: 1371-1381.

Nutman, P. S (1965): The relation between nodule bacteria and the legume host in the rhizosphere and the process of interaction. In Ecology of Soil-borne Plant pathogens. Ed. K. E. Baker and W. C Synder Univ. California Press. Berkeley and Los Angeles.

Palovskaya, M. M. and V.A. Khramov (1990): Growth of sorghum seedling and their proline content under the effect of salts. Akad. Sol. Skokhozyaia tvennykh Nauk.

Piper, G. S (1950): Soil and Plant Analysis. 1st Ed. Inter-science Publishes, N.Y.

Pregl, L F (1945): Quantitative Organic Micro-Analysis. 4th Ed. J. and A. Churchill, LTD. London.

Rashad, M. H. and E.A. Ismail, (2000): Heat tolerance and bio-chemical fertilizer in wheat. I Chemical constituents and yield performance. X^{th} International Colloquium for Optimization of Plant Nutrition, April, Cairo (In press).

Sabry, S. R.; S. A. Saleh and A A. Ragab (2000): Response of wheat (*Triticum aestivum* L.) to inoculation with *Azorhizobium caulinodans* and VA-Mycrorrhizas under different nitrogen fertilizer levels. Zagazig J. Agric. Res. 27: 1, 145-158.

Sabry, S. R.; S. A. Saleh; C. A. Batchlor; S. L. Kothari; M. R. Davey and E.C. Cocking, (1997): Endophytic establishment of *Azospirillum caulinodans* in wheat. Proc. R. Soc. L and. B. 264: 341-346.

O' Sullivan, D. J. and F. O' Gara (1992): Traits of fluorescent *Pseudomonas spp*, involved in suppression of plant root pathogens. Microbiol. Rev., 56: 662-676.

Vincent, J. M (1970): A manual for the practical study of root bacteria, IBP Handbook No. 15, Blackwell Sci. Publications, Oxford and Edinburgh, Pp: 58-72.

Voetberg, G. S. and R. E. Sharp (1991): Growth of the maize primary root at low water potential. III. Role of increased proline deposition in osmotic adjustment. Plant Physiol., 96: 1125-1130.

Vogel, A. I (1975): A textbook of practical organic chemistry. Publish by English Language book Society and Long man Group Limited, 3rd Ed., 966.

Watrud, L. S.; F. T. Perlak; M. T. Tran; K. Kusans; E. J. Mayer; M. A. Miller-Wideman; M. G. Obukowicz; D. R. Nelson; J. P. Kreitinger and J. R. Kaufman (1985): Cloning of the *Bacillus thuringiensis* sub-sp. Kurstaki deltsendotoxin gene into *Pseudomonas fluorescence*. Scientific issues. Ann. Soc. Microbiol. Washington, D.C. 40-44.

زيادة كفاءة امتصا ص العناصر والتحولات الغذائية لنبات الذرة الرفيعة بواسطة التسميد الحيوى تحت الإجهاد المائى

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أجـــرى هذا البحث خلال موسمي ١٩٩٩، ٢٠٠٠ لتقيم تأثير بكتيريا الريزوبيا *Rhizobium legumnioserum* , *Bradyrhizobium japonicum* كمشـــجع لكـــلا من نمو النباتات و بكتـــيريا الريزوســـفير علـــى المكونات الكيميائية ومنها السكريات، الكلوروفيل، النيتروجين، الفســـفور، البوتاســـيوم والصـــوديوم، البر ولين، الأحماض الامينية ، اندول حامض الخليك، الابسيســـك، والجبريلـــيك فى صنفى نبات الذرة الرفيعة (شندويل، دورادو) الذين ينمو تحت مســـتويات مختلفة من رطوبة التربة (١٠٠، ٦٠، ٤٠% من السعة الحقلية). عوملت النباتات بـــأربع معـــاملات مـــن التسميد وهما تسميد كامل من NPK ، نصف التسميد الكامل، نصف التســـميد الكامل مع اللقاح البكتيرى *Rhizobium legumnioserum* ونصف التسميد الكامل مـــع *Bradyrhizobium japonicum* . تـــم التقدير الوصفى لكلا من اندول حامض الخليك هيدروجيـــن ســـيناميد وإنـــتاج السيدروفور، الفسفور الذائب المعامل اتز باللقاح ا لريزوبيا . وأخـــذت العيـــنات النباتـــية بعد ٧٠ يوم من الزراعة. أوضحت النتائج أن المعاملات البكترية خفضـــت تـــراكم البر ولين على الرغم من إنها أدت إلى زيادة السكريات الكلية، البوتاسيوم، الأحمـــاض الامينـــية الكلية فى الأوراق واندول حامض الخليك فى الجذر. تركيز الكلوروفيل والنيتروجيـــن زاد فى الأوراق كلتى الصنفين. كما أن النيتروجين فى الأوراق تأثراكثر باللقاح البكتيرى البكتيرى *Rhizobium legumnioserum* فى صنف دورادو عن صنف شندويل. بالاضـــافة إن حـــامض الابسيسيك زاد مع نقص نسبة الرطوبة فى التربة بعد المعاملة باللقاح البكتـــيرى فى صنف شندويل. بينما مستوى الفسفور وحامض الجبريليك لم يتأثر. لذلك يعتبر التســـميد الحـــيوى كمصدر منشط لزيادة امتصاص العناصر من التربة ونقص الأضرار نتيجة الإفراط فى التسميد المعدنى والجفاف كما أنة يقلل التلوث البيئى والتكاليف.