

FOLIAR APPLICATION OF SOME MICRONUTRIENTS ON THE ADAPTATION AND GENETIC BEHAVIOR OF SOME SOYBEAN CULTIVARS UNDER DESERT CALCAREOUS SOIL

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ABSTRACT

Two field experiments were carried out on calcareous soils at Maryut experimental station, Desert Research Center during 2000 and 2001 seasons, to study the effect of genetic variability and some different foliar application treatments on growth traits, yield, yield components of some soybean (*GLYCINE MAX L.*), cultivars. A split plot design with three replications was used. The main plots were devoted at random to four spray-foliar application treatments, i.e. ZnSO₄ 0.1%, MnSO₄ 1.0%, FeSO₄ 0.5% and tap water as control and four soybean cultivars, i.e., Clark, Crawford, Giza 35 and Giza 21 in the sub plots. Also subunits composition of the two soybean seed major storage proteins (B-conglycinin (7S) and glycinin(11S)) as well as the other uncharacterized subunits were studied for the 4 different cultivar. The obtained results could be summarized as follows:

Generally, foliar application treatments may correct the metabolic disturbance under stress condition of Maryut Agric. Research Station (calcareous and saline soil). Spray foliar applications by ZnSO₄ 0.1%, MnSO₄ 1.0%, and FeSO₄ 0.5% led to improve growth parameters, yield, yield components and nutritious value through increasing intensities of B-conglycinin and glycinin. of soybean cultivars as compared with the control at 75 days (SAS) and harvesting date. Foliar application of ZnSO₄ 0.1% surpassed the other spray treatments for increasing all studied parameters of soybean cultivars under investigation.

Genetic variability were detected clearly among the four soybean cultivars in growth traits, yield, yield components and seed storage protein subunits. Giza 21 cultivars exhibited a significant higher values and considered the highly yielding ability and nutritive value under calcareous soil of Maryout followed by Giza 35, Crawford and Clark descendingly. Also ZnSO₄ 0.1%, combined with Giza21 cultivar interaction achieved the best treatment.

Keywords: Adaptation - Calcareous soils - Foliar application - Growth - Yield - SDS protein banding patterns - Genetic variability - Soybean cultivars

INTRODUCTION

Soybean is considered as one of the most important legume crops and one of the highly nutritious source of food for man and animals. In addition, its oil is the major industrial food product of legume oil crops all over the world. Soybean production in Egypt is one of the main problems that needed extensive agriculture researches for improving its production under reclaimed and desert regions. In these regions calcareous soils deficiency of nutrients is always expected due to the existence of nutrients mostly in forms of low availability (Badawy, 1990), this represent an obvious limiting factor for growth, yield and biochemical aspects of some crops. Micronutrients considered an essential elements for plant life., which act as plant growth

hormones and play a role in the production and functioning of several enzymatic systems in plant. So they have vital functions in the overall plant metabolism (Badawy 1990). In addition Gettier *et al.*, (1985) & Mascognia and Cox (1985) reported that application of Mn and Zn on soybean increased yield of seeds in the reclaimed soil. In addition application of Fe and Mn nutrients to the growing plants in calcareous soil enhanced the growth and increased the corresponding plant content of each nutrient (Gaber, 1979).

Soybean seeds contain up to 40% protein and 20% oil. Glycinin (11S) and B. conglycinin (7S), the two main classes of multi-subunit seed storage proteins, account for approximately 70% of total protein in a typical soybean seed (Meinke *et al.*, 1981).

Cysteine plus methionine account for 3 to 4.5% of the amino acid residues of glycinin, a family of five different proteins (Nielsen *et al.*, 1989, and Fukushima 1991). Thus the sulfur- amino acid content of glycinin is similar to that of their high- quality dietary proteins (Paek *et al.*, 1997).

B-conglycinin is very deficient in sulfur containing amino acids (Kitamura *et al.*, 1984). Which sulfur-amino acids account for less than 1% of the amino acid residues of B. conglycinin (Sebastiani *et al.*, 1990). The mature subunit of B. conglycinin is composed of approximately 416 amino acid residues and lacks both methionine and cysteine (Coates *et al.*, 1985). Hence the B. subunit is primarily responsible for the low sulfur amino acid content of the combined soybean seed storage proteins. The various subunits associated with glycinin and B-conglycinin are commonly visualized by polyacrylamide gel electrophoresis.

Genotypic variation among soybean cultivars was shown in their growth traits and its components (Mohammed, 1994; Sharief and Salama, 1996). Also such variation was clearly observed in nodule number weight, plant dry weight and the ability to fix N₂ in symbiosis (Pazdernik *et al.*, 1996 and 1997), in seed protein content (Tomkins and Shipe, 1997), and in response to adverse condition (water stress) (Serraj and Sinclair, 1997).

Therefore, the objective of this investigation were aimed to assess the effect of genetic variability among for soybean cultivars and microelements effects such as Zn, Mn, and Fe foliar application on the performance of productivity in addition to subunits composition of the two soybean seed major storage proteins as well as the uncharacterized subunits of different soybean cultivars under stress condition of Maryout.

MATERIALS AND METHODS

Two field experiments were performed in Maryut Agricultural Research Station, Desert Research Center, at Maryut region, 35 Kms. west Alexandria during two successive growing seasons. (2000 and 2001), to study the effect of four foliar application treatments (ZnSO₄ 0.1%, MnSO₄ 1.0%, FeSO₄ 0.5%) and tap water as a control on growth, yield, oil yield and the genetic variability among four soybean cultivars i.e. Clark, Crawford, Giza 35 and Giza 21, on protein banding patterns. The soil of the experimental station is highly calcareous soil (32%), mechanical and chemical analysis of

soil as well as irrigation water are presented in Table (1 a and b). Such mechanical and chemical analysis were determined according to Richards (1954) and Jackson (1958). Each experiment included 16 treatments, i.e. the combination of four foliar application and four Soyabean cultivars, which were arranged in a split plot design with three replications. The main plots were occupied by the different spray-foliar application treatments while Soybean cultivars in the sub plots.

Table 1: Mechanical and chemical properties of Maryut Farm soil and chemical analysis of irrigation water.

a). Physical analysis

Depth (cm)	CaCO ₃ (%)	Particle size distribution (%)				Texture class
		Coarse sand	Fine Sand	Silt	Clay	
0 - 40	32.00	9.57	46.40	19.22	24.81	Sand clay loam

b). Chemical analysis of Maryut Farm soil and irrigation water

Depth (cm)	E.C. mmhos/cm	pH	O.M. (%)	Soluble anions (meq/L.)				Soluble cations (meq/L.)			
				HCO ₃ ⁻	CO ₃ ⁻	Cl ⁻	SO ₄ ⁻	Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺
0-40	4.00	7.8	0.49	3.20	-	25.21	18.24	15.25	5.30	24.70	0.82
Irrigation water	2.80	7.5	-	8.54	-	15.32	6.20	6.20	7.75	16.60	0.33

Each basic unit included 5 ridges, 60 cm apart and 3.5 m in length, comprising an area of 10.5 m² (1/400 fed.). Seeds of Soybean cultivars were obtained from Agricultural Research Center and were planted in hills distanced at 20 cm apart. Sowing dates were on May10 and 12 in 2000 and 2001 seasons, respectively. Irrigation was applied directly after sowing. The plants were thinned to one plant per hill at 45 days after sowing DAS. Calcium superphosphate (15.5% P₂O₅) at the rate of 100 kg/fed. was applied during tillage operations. Potassium sulphate (48%K₂O) at the rate of 50 kg/fed. was added before the first irrigation, while ammonium nitrate (33.5% N) at the rate of 45 kg N/fed. was added in two equal doses at 45 and 75 DAS, respectively. Twice foliar-spraying application were applied after 60 and 75 days from sowing using Tween 20 as wetting agent.

Two samples of plants at 75 DAS and at harvesting days were randomly taken from each plot and separated into roots and shoots to determine the following characters; plant height, No. of branches/plant, No. of nodules/plant, fresh and dry weights /plant in addition, No. of pods/plant, 100 – seed weight, seed weight/plant, seed yield kg/fed & straw yield kg/ fed., Samples were dried in an oven at 65°C, to determine dry weight of plants. Also seeds samples were randomly taken for assessment of oil % and protein banding patterns as follows:

Extraction of oil:

Oil was extracted by Soxhlet apparatus using petroleum ether (boiling point 60-80 °C) for 6-8 h. according to the method described in A.O.A.C (1985).

SDS-Protein electrophoretic analysis.

Extraction of total proteins:

Mature seeds of ten plants from each treatment were sampled at random each plant seeds were ground in a mill., seed coats were removed before grinding. . one gm of each seed powder was defatted with cold acetone.

Total proteins were extracted for 1 hour using 0.05 M Tris-HCl buffer pH 8.0 containing 2% (W/V) sodium dodecyl sulfate and 0.78 ml 2-mercaptoethanol and shaken vigorously then heated in a 70°C on water bath for 3 minutes (Ogawa *et al.*, 1989). After centrifugation at 13.500 Xg for 3 minutes, the supernatant was taken for loading on gel electrophoresis .

Gel electrophoresis:

Protein in the seed extracts were separated electrically in sodium dodecyl sulfate polyacrylamide gel.

Electrophoresis was performed at 10mA per gel at room temperature for 7 hours or until the bromophenol blue tracking dye ran off the bottom of the gel. The gel was stained in 2 ml mL⁻¹ coomassie blue R-250 (Sigma chemical Co., St. Louis) in water- methanol- acetic acid (4 : 5 : 1) and destained in water - methanol - acetic acid (8 : 1 : 1) for calibrating the molecular weights of the different bands, The following marker proteins were used phosphorylase (MW 92000), Bovine albumin (MW 67000), ovalbumin (MW 45000), Carbonic anhydrase (MW 29000), soybean trypsin inhibitor (MW 21000), cytochrome C (MW 12500) and Myoglobin (MW 6500) .

Data were computed using the statistical analysis of variance according to Steel and Torrie (1960). Combined analysis for the two seasons was practiced as results followed similar trend. The mean values were compared at 0.05 level of probability by least significant difference (L.S.D).

RESULTS AND DISCUSSION

1- Growth Traits

1-1 Behavior Cultivars

The data obtained from combined analysis of variance (table 2) showed that, there were significant differences in all growth traits of soybean cultivars. Giza 21 cultivar recorded the highest mean values for plant height, number of branches, number of nodules /plant, fresh and dry weights / plant at 75 DAS. Whereas Giza 35, and Crawford soybean cultivars ranked in the second and third order respectively. On other hand Clark soybean cultivar exhibited the lowest mean values for all growth traits.

Table 2: Varietal differences on growth characters of soybean at 75 DAS.

(Combined analysis over the two seasons)

Treatments	Plant height (cm)	No. of Branches	No. of Nodules/plant	Fresh and dry Weights gm / plant	
				Fresh	Dry
Clark	52.08	3.63	5.13	69.20	17.23
Crowford	58.04	3.79	5.08	79.43	20.13
Giza 35	56.96	4.88	6.71	83.82	20.88
Giza 21	58.21	5.13	7.58	95.95	23.97
L.S.D	1.61	0.28	0.46	5.63	2.13

From the previous results we noticed that Giza 21 soybean cultivar was the best cultivar for all growth traits compared with other cultivars. These finding could be partially attributed to increment in the number of nodules / plant under calcareous soil which fixing atmospheric nitrogen that reflected for improving plant height, No. of branches, fresh and dry weights / plant (El-Samahy, 2000 and Mohammed, 1994). In This respect, Crowford soybean cultivar was superior than Clark for all growth traits (Salama and Ghonema, 1990; Sharief and El-Bially, 1992; Sharief and Salama, 1996; and Amara, 1998).

1-2Effect of foliar Application on all cultivars:

Data presented in Table 3 represent the data of the effect of different foliar application treatments on plant height, No. of branches, No. of nodules/ plant, fresh and dry weights / plant for different soybean cultivars at 75 DAS. All growth characters significantly increased with all foliar application treatments (Zn, Mn and Fe) as compared with the control. The highest increments percentages values for plant height, fresh and dry weights /plant were achieved by ZnSO₄ 0.1% which reached 28.08 % , 7.13 % 9.73% respectively as compared with the control. Meanwhile Mn and Fe foliar application recorded the second and third order for all growth traits receptively.

Table 3: Effect of micronutrients foliar application on over all means of growth characters of all soybean cultivars grown under calcareous soil at 75 DAS.(Combined analysis over the two seasons)

Treatments	Plant height (cm)	No. of Branches/ plant	No. of Nodules /plant	Fresh and Dry Weights gm / plant	
				Fresh	Dry
Control	50.00	2.46	2.83	78.93	19.62
ZnSO ₄ 0.1%	64.04	6.33	9.54	84.56	21.53
MnSO ₄ 1%	57.46	4.75	7.13	82.62	20.54
Fe SO ₄ 0.5%	53.79	3.88	5.00	82.29	20.52
L.S.D	2.62	0.40	0.50	2.01	0.52

Concerning the stimulatory effect of micronutrients on plant growth it is clearly established that, Zinc has an important role in protein synthesis and the synthesis of the growth controlling compound such as indole acetic acid, (Boawn *et al.*, 1957). In addition, manganese participates in O₂ – evolving system of photosynthesis and has a role in the production of chlorophyll (Robertson and Lucas, 1976). Iron has an essential role for the maintenance of chlorophyll in plants and for RNA metabolism of chloroplasts. Also Iron considered to be an essential constituent of leg hemoglobin present in the nodules of leguminous plants and is essential in the process of a symbiotic N₂-fixation (Taffin, 1970). These findings were in harmony with that obtained by Amara 1998, on soybean plant sallam and Khafaga (1999) on fodder beet plant.

Effect of interaction for cultivars by foliar application:

The interaction between foliar application treatments on different soybean cultivars had significant effect on all growth traits as shown in Table (4). Generally, ZnSO₄ 0.1% as a foliar application treatment, on Giza 21 cultivar achieved the highest mean values for plant height, No. of branches, no of nodules /plant fresh and dry weights / plant. Whereas, FeSO₄ 0.5% foliar application on with the same cultivar recorded the second order for fresh and dry weights/ plant. However, ZnSO₄ 0.1% on with Giza 35 interaction marked the 2nd mean values for plant height, No. of branches and No. of nodules/plant.

Table 4: Effect of interaction between different foliar application and soybean cultivars on *j* growth traits at 75 DAS. (Combined analysis of two seasons)

Treatments		Plant height (cm)	No. of Branches/ plant	No. of Nodules /plant	Fresh and Dry Weights gm / plant	
Foliar application	Variety				Fresh	Dry
Control	Clark	48.17	2.17	2.66	65.22	16.57
	Crowford	52.76	2.17	2.16	75.18	18.61
	Giza 35	48.33	2.50	3.00	84.01	21.21
	Giza 21	50.83	3.00	3.50	91.32	22.07
ZnSO ₄ 0.1%	Clark	57.00	5.00	7.50	73.00	17.30
	Crowford	63.33	5.17	8.00	78.221	19.84
	Giza 35	65.67	7.00	10.33	86.03	21.22
	Giza 21	70.17	8.17	12.33	101.00	27.78
MnSO ₄ 1%	Clark	52.50	4.17	6.00	70.55	18.03
	Crowford	59.50	4.17	6.16	83.34	21.55
	Giza 35	59.33	5.50	7.83	80.21	20.05
	Giza 21	58.50	5.17	8.50	96.36	22.51
Fe SO ₄ 1%	Clark	50.67	3.17	4.33	68.04	17.03
	Crowford	56.67	3.67	4.00	81.00	20.50
	Giza 35	54.50	4.50	5.66	85.01	21.03
	Giza 21	53.33	4.17	6.00	95.11	23.53
L.S.D		3.22	0.56	0.93	3.03	1.02

Calcareous soil is known to be low in their natural fertility (El-Hamdi, 1990); for example, it has a short supply of nitrogen (El-Kased *et al.*, 1993). Mineral nutrition has played an important role in establishing soybean due to the problem of nodules formation on plant roots (Abd El-Gawad and El-Batal, 1995). The growth of soybean plants was found to be enhanced by the foliar application of micronutrients such as Zn, Mn and Fe especially on 21 cultivars (Ali and Maria, 1992).

Yield and yield components:

Behaviour cultivars

The differences among cultivars performances on yield and yield components were presented in table (5). It was obvious that Giza 21 cultivar recorded the highest significant mean values for No. of pods / plant, seed weight / plant, seed yield kg / fed , straw yield ton/fed, oil % and oil yield kg / fed which reached 51.06, 29.70 gm, 1089 kg/fed 2.56 ton/ fed , 19.62 % and 214 kg / fed respectively. Meanwhile Giza 35 was better than Giza 21 cultivar for 100- seed weight. Generally Giza 35 and Crawford ranked in the second and third order for yield and its components.

Table 5: Varietal differences on yield and yield components of soybean at harvesting day. (Combined analysis of two seasons)

Treatments Variety	Plant height (cm)	No. of pods/ plant	100-seed weight (gm)	Seed weight gm/ plant	Seed yield Kg/fed	Straw yield ton/fed	Oil %	Oil yield kg/fed
Clark	85.58	38.88	11.15	22.72	832.9	1.532	18.46	154.5
Crawford	101.5	49.08	12.50	24.64	907.1	1.934	18.93	172.2
Giza 35	86.22	44.60	13.94	26.83	983.9	1.143	19.11	188.7
Giza 21	100.5	51.06	13.29	29.70	1089	2.560	19.62	214.1
L.S.D	1.49	2.80	0.22	0.67	23.91	0.09	0.17	5.74

Soybean cultivars under investigation differed under slight saline water irrigation and calcarious soil for its. These differences could be due to the ability of cultivars to reduce NaCl accumulation in their cells that enables the cultivar to thrive well under saline and calcarious soil conditions which reflected on yield and its components (El-Samahy, 2000). Also El-Borai *et al.* (1988); Parres *et al.* (1989); Sharief and Salama, (1996), reported that there were genetic variation in yield and yield components among soybean cultivars. Also Crawford cultivar markedly surpassed Clark cultivar in pods number/plant, seed and straw yield/fed, oil percentage as well as oil yield/fed. They added that, no marked effect between cultivars concerning 100- seed weight. Mohammed, (1994) mentioned that Giza 21 cultivar was superior than Crawford and Clark cultivars in pods number, 100-seed weight, seed oil and straw yield/fed.

Effect of foliar application

Data presented in table (6) revealed that all micronutrients (Zn, Mn and Fe) achieved the highest significant mean values for yield and its components, as compared with the control. However ZnSO₄ 0.1% as a foliar

application surpassed the other micronutrients treatments for the same characters. The percentages of magnitude increments for 100-seed weight, seed yield/fed, straw yield/fed and oil yield /fed reached 14.8%, 23.7%, 55.0% and 31.9% as compared with the control respectively. Meanwhile, $MnSO_4$ 1.0% ranked next in this regard. However, $FeSO_4$ 0.5% recorded the 3rd order for yield and its components.

Table 6: Effect of micronutrients foliar application on yield and yield components of soybean cultivars growing under calcareous soil at harvesting day. (Combined analysis of two seasons)

Treatments foliar	Plant height (cm)	No. of pods/plant	100-seed weight (gm)	Seed weight gm/ plant	Seed yield Kg/fed	Straw yield ton/fed	Oil %	Oil yield kg/fed
Control	85.63	40.00	11.81	22.83	836.9	1.511	18.43	154.6
ZnSO ₄ 0.1%	97.96	52.17	13.56	28.13	1035.0	2.342	19.62	203.9
MnSO ₄ 1%	95.63	46.67	12.98	27.31	1001.0	2.152	19.13	191.9
Fe SO ₄ 1%	94.55	44.79	12.54	25.63	939.6	2.011	18.94	179.1
L.S.D	2.13	2.34	0.33	0.97	35.14	0.18	0.23	8.93

It is obvious from the data on table (6) also, that foliar application with micronutrients had no significant effect on seed oil content (Kamel *et al.*, 1983, and Heenen and Campbell, 1980). Whereas, the above factors had a different significant effect on oil yield / fed, which attributed manly to the different increments in seed yield / fed among clutivars (Kamel *et al.*, 1983, and Sureshumar *et al.*, 1977). On the other hand, several investigators have proved the significant role of some sprayed micronutrients for correcting the adverse effect of salinity and calcareous soil for improving salt tolerance of some field crops which reflect on improving yield and its components (El-Bagouri *et al.*, 1983; Shukla and Nukhi, 1985; and Dahdoh, 1986).

Effect of inter action:-

As for the effect of microelement among cultivars, as foliar application treatments on yield and yield components of soybean plant are shown in table (7). Generally the highest mean values for plant height, seed weight / plant, seed yield /fed, straw yield/ fed, oil % and oil yield /fed, were obtained by ZnSO₄ 0.1% foliar application on Giza 21 cultivar. With the same interaction the Percentage of increment in seed yield /fed and oil yield /fed reached 21.6% and 30.0% respectively compared with the control. On the other hand, the best mean value for No. of pods/plant achieved by ZnSO₄ 0.1% on 35 cultivar. Meanwhile, 100- seed weight surpassed with MnSO₄ 1% foliar application on the same cultivar. Also, spraying microelement MnSO₄ 1% on Giza 21 recorded the second order.

Concerning the effect of foliar application and soybean cultivars yield and its compounds, there were increments by the application of micronutients such as (Zn, Mn and Fe) on Giza 21 cultivar compared with the other cultivars. These findings were in harmony with that obtained by (Ali, and Maria, 1992) on soybean plant. Also this results may be attributed to ability of Giza 21 with ZnSO₄ 0.1% to adapted under saline and calcareous soil of

Maryut experimental station than other cultivars with the other microelement. In addition, the efficiency of the application of some trace elements such as ZnSO₄, MnSO₄ and FeSO₄ may be with a corrective and/or compensative effect on mineral balance (Misra, 1964).

Table 7: Effect of interaction between different foliar application and soybean cultivars on Yield and yield components at harvesting day. (Combined analysis of two seasons)

Treatments Inter action		Plant height (cm)	No. of pods/ plant	100-seed weight (gm)	Seed weight gm/ plant	Seed yield Kg/fed	Straw yield ton/fed	Oil %	Oil yield kg/fed
Foliar application	Varity								
Control	Clark	75.0	39.0	10.42	19.70	722.5	0.9733	18.15	131.2
	Crowford	100	44.0	11.60	21.21	777.7	1.713	18.03	140.2
	Giza 35	72.7	30.8	12.98	24.31	891.2	1.398	18.63	166.0
	Giza 21	94.8	46.2	12.23	26.08	956.3	1.958	18.90	180.8
ZnSO ₄ 0.1%	Clark	90.8	40.8	11.97	25.37	930.0	1.963	18.92	178.0
	Crowford	103.8	53.7	13.43	26.94	1003	2.120	19.72	197.7
	Giza 35	93	59.7	14.38	28.51	1046	2.337	19.61	205.1
	Giza 21	104.2	54.5	14.47	31.70	1163	2.948	20.25	235.1
MnSO ₄ 1%	Clark	89	38.8	11.25	24.36	893.3	1.693	18.57	165.8
	Crowford	102	51.5	12.77	26.43	969.3	1.973	19.18	185.8
	Giza 35	89	45.2	14.50	27.27	999.7	2.118	19.00	189.9
	Giza 21	102.5	51.2	13.42	31.18	1143	2.825	19.78	226.1
Fe SO ₄ 1%	Clark	87.5	36.8	10.98	21.43	785.8	1.498	18.20	143.0
	Crowford	100	47.2	12.22	23.98	879.2	1.930	18.78	165.1
	Giza 35	90.2	42.8	13.90	27.25	999.2	2.106	19.22	193.8
	Giza 21	100.5	52.4	13.05	29.85	1094	2.509	19.56	214.4
L.S.D		2.98	5.59	0.43	1.33	47.82	0.18	0.34	11.40

SDS Protein banding patterns:

The effect of micronutrients (Zn, Mn and Fe) foliar application in addition of water as a control, and the protein banding patterns of the succeeded four soybean cultivars grown under Maryut conditions were studied using SDS polyacrylamide gel (Fig. 1 and Table 8). Results revealed a genetic variability among different protein subunits among different studied cultivars, under different micronutrients treatments.

Table 8: Genetic variation in the protein banding patterns of the four studied soybean cultivars treated with micronutrients (Zn, Mn and Fe) and water as control, as detected by SDS – PAGE.

Aprox molecular weight (KD)	Zn				Fe				H ₂ O				Mn			
	Gz 21	Gz 35	Graw-ford	Clark	Gz 21	Gz 35	Graw-ford	Clark	Gz 21	Gz 35	Graw-ford	Clark	Gz 21	Gz 35	Graw-ford	Clark
110.00	+2	+2	+2	+1	-	-	+1	-	+1	+1	-	-	+2	+2	+2	+2
95.0	+1	+1	+1	+1	-	-	-	+1	+1	+1	-	-	+1	+1	+1	+1
82.0	+2	+2	+2	+1	+1	-	+1	+1	+1	+1	-	-	+2	+2	+2	+2
76.0	+4	+4	+4	+3	+3	+3	+4	+3	+3	+3	+3	+2	+4	+4	+3	+3
72.0	+4	+4	+4	+3	+3	+3	+4	+3	+3	+3	+3	+2	+4	+4	+3	+3
69.0	+2	+2	+1	+1	+2	+2	+3	+3	+2	+2	+1	+1	+2	+2	+2	+2
68.0	+2	+2	+2	+1	+2	+2	+3	+3	+2	+2	+1	+1	+2	+2	+2	+2
60.0	+1	+1	+1	+1	+1	+1	+1	+2	+2	+2	+1	+1	+1	+1	+1	+1
52.0	+3	+2	+2	+2	+1	+2	+2	+2	+2	+2	+1	+1	+2	+2	+2	+2
51.0	+4	+4	+4	+3	+2	+2	+2	+1	+2	+2	+1	+1	+4	+4	+3	+3
47.0	+4	+4	+3	+3	+3	+3	+3	+3	+2	+3	+2	+2	+3	+3	+3	+2
46.0	+2	+2	+2	+2	-	-	+1	+1	+2	+2	+1	+1	+2	+2	+2	+2
42.0	+1	+1	+1	+1	-	-	-	+2	+2	+1	+1	+1	+1	+1	+1	+1
40.0	+3	+3	+2	+3	-	+1	+2	+2	+1	+1	-	-	+2	+2	-2	+2
41.0	+4	+4	+4	+4	+3	+4	+4	+3	+4	+4	+3	+3	+4	+4	+3	+4
37.0	+4	+4	+4	+3	+3	+3	+4	+4	+4	+4	+3	+3	+3	+3	+3	+3
36.0	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2
34.0	+1	+1	+1	+1	-	-	-	+1	+2	+1	+1	+1	+1	+1	+1	+1
32.0	+1	+1	+1	+1	-	-	-	-	+1	+1	-	-	+2	+2	+2	+2
31.0	+3	+3	+2	+2	+1	+1	+2	+2	+1	-	-	-	+2	+2	+2	+2
20.0	+4	+4	+3	+3	+3	+3	+3	+3	+3	+2	+2	+2	+3	+3	+3	+3
18.0	+4	+4	+4	+4	+4	+4	+3	+3	+3	+3	+3	+3	+4	+4	+4	+4
16.0	+4	+3	+2	+2	+2	+2	+2	+1	-	+1	+2	+2	+1	+1	+2	+1
15.0	+3	+3	+3	+2	+1	+1	-	-	+1	+2	-	-	+1	+2	+1	-
10.0	+2	+2	+2	+1	+1	+1	+1	+1	+1	+1	+1	+1	+2	+2	+2	+2

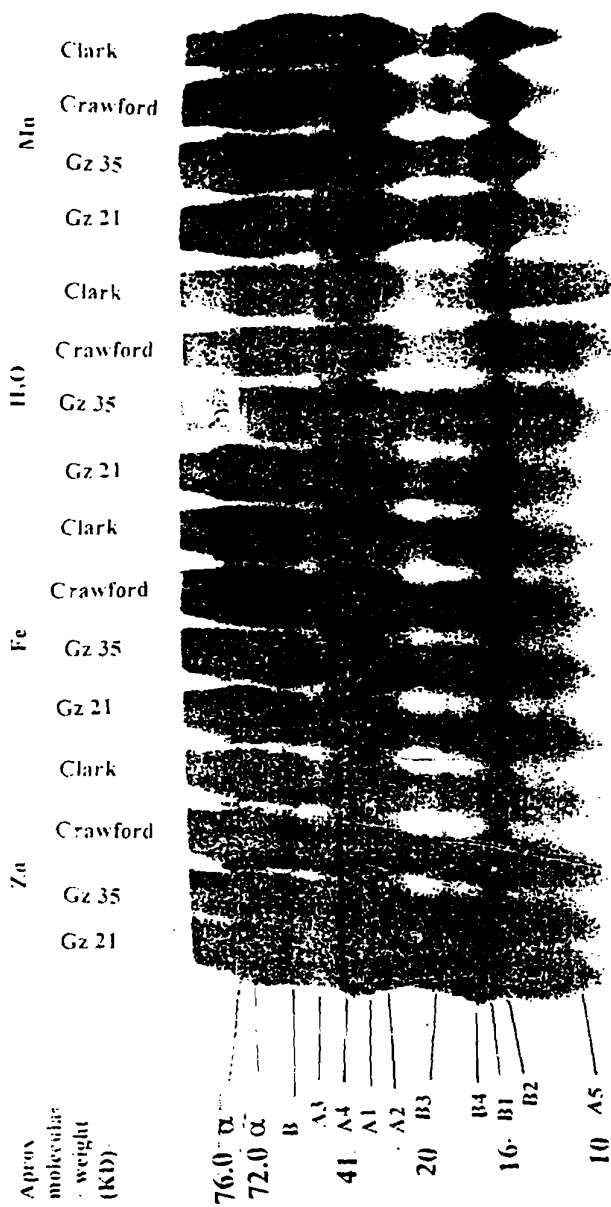
* +, +2, +3 and +4 represent gradual increase in band intensity

Total soybean seed storage proteins consist approximalty 70% of the two main classes of multi-subunit seed storage proteins glycinin (11S) and B-conglycinin, (7S) (Meinke *et al.*, 1981).

B-conglycinin is a complex protein, which three prevalent types of subunits are associated with B-conglycinin and are referred to as α , α' and B (Derbyshire *et al.*, 1976; Fontes *et al.*, 1984). These subunits migrated at molecular weights 76.0, 72.0 and 51 KD respectively. Glycinin is (11S) considered a complex family of five different protein subunits, each one composed of one or more large acidic and small basic polypeptides which are linked by a single disulfide bond (Staswick., 1983). The acidic polypeptide A3, A4, (A1, A2) and A5 migrated at molecular weight 47, 41, 37 and 10 kD respectively (Nielsen *et al.*, 1989; Eweda, 1991). Concerning the basic glycinin subunits B3, (B1, B2) and B4 appeared at molecular weight 20 and approximatly 16-18, respectively

In addition to the two major subunits B-conglycinin and glycinin. of soybean proteins there are other uncharacterized bands. These bands are taken into consideration to evaluate the genetic identity of the different cultivars. These bands could be classified according to their molecular weights into high molecular weight bands (69- 110 KD), medium (23 – 36kD) and low molecular weight bands (13 and 15 KD) (Eweda. 1991).

Fig. (1) SDS-PAGE of total proteins of different soybean cultivars (Giza 21, Giza 35, Clark and Crawford) with micronutrient Zn, Mn and Fe foliar application in addition to water as control.



Results (Fig. 1 and Table 8) showed variability in B-conglycinin (7S) and glycinin (11S), of different soybean cultivars, where Giza 21 and Giza 35 cultivar scored higher values followed by Crawford and finally with Clark cultivars in this regard. Fontes *et al.*, (1984) studied the protein banding patterns of different soybean cultivars reported genetic variability in the α , α and B subunits of B-conglycinin fractions.

In addition, there was a sharp increase in B-subunit of B-conglycinin intensities with micronutrients application especially Zn then Mn treatments. Whereas α , α subunits intensities were less changed. Meanwhile the relative abundance of most subunits of 11S protein (glycinin) was slightly increased by Zn, Mn and Fe treatments. In this concern, (Paek *et al.*, (1997) showed that the amount of 11S protein is slightly stable among the variable protein concentrations of seeds.

Concerning the uncharacterized bands, there was a great variability in the presence of some bands and intensity of all groups of bands (High, medium and low), between different cultivars under control and treated conditions. Also Zn and Mn treatments resulted in increment of intensities of these groups. Also results showed that foliar application of Giza 21 cultivar with Zn resulted in increase intensities of the most subunits of B-conglycinin and glycinin. Zinc application increased seed protein content under normal soils (Chaturvedi and Pathak, 1972) and calcareous soils (Amin, 1978; Gettier *et al.*, 1985). Also manganese increase nitrate reduction amino acids and protein content, Iron in addition play a role in nitrate reduction and protein metabolism. When total seed protein increased B-Subunit of B-conglycinin increased, meanwhile the relative abundance of α and α subunits were little changed (Gayler and Sykes 1985; Holowach *et al.*, 1986). The concentration (intensities) of (11S) protein glycinin remained more or less constant while the total protein concentration increased (Paek *et al.*, 1997). These variation in seed storage protein composition may result from the relative abundance of S-and N- metabolites available to developing soybean seed (Grabau *et al.*, 1986; Thompson and Madison, 1990; Paek *et al.*, 1997).

From the obtained results, it could be concluded that under the conditions of this investigation, enrichment of the soybean phyllosphere could be achieved by applied foliar application of micronutrients (Zn, Mn and Fe), that reflected a considerable increase in the growth, yield and the subunit of the two major storage protein of soybean plants cultivated under calcareous soil.

In addition, the choice of cultivars play an important role in establishing soybean that could be partially due to the problem of noduly formation of plant roots (Abd El-Gawad and El-Batal, 1995). Giza 21 were the more adapted cultivars under Maryuit stress condition then Giza 35 and Crawford cultivars attained the highest nutrition values as well as yield, & protein types such as B conglycinin and glycinin

REFERENCES

- A.O.A.C. (1985). Official Methods of Analysis (9th Ed.). Association of Official Agricultural Chemists, Washington, D.C.
- Abd El-Gawad, M.H. and M.A. El-Batal (1995): Response of different soybean cultivars to rhizobium inoculation and nitrogen application. 1-yield and its attributes. *J. Agric. Sci. Mansoura Univ.*, 20(9): 4013-4019.
- Ali, A. A. and A. M. Maria (1992). Effect of nitrogen sources and mineral supply on nodulation, growth and yield of soybean. *J. Agric. Mansoura Univ.*, 17 (6): 2176-2183.
- Amara, M.A.T. (1998). Soybean response to inoculation with biofertilizer and fertilization with Micro-nutrients in calcareous soil, desert inst. *Bull. Egypt.*, 48(1): 75 – 92.
- Amin, M.A.H. (1978) Micronutrient work in Agric Res. Center. Problems of micronutrients in plant nutrition under Egyptian soil conditions proc. Of First workshop. Egypt.
- Badawy, A. A. S. (1990). Studies on the effect of some growth regulators, macro and micro nutrient on some biochemical constituents of soybean under the condition of reclaimed soils. Ph. D. thesis, Fac. Of Agric. Ain Shams Univ.
- Boawn, L.C.; F. Jr. Viets and C.L. Crawford (1957). Plant utilization of zinc from various types of zinc compounds and fertilizer materials. *Soil Sci.*, 83: 219-223
- Chaturvedi, S.P. and A.N. pathak (1972). Effect of soil and foliar nutrition of some trace elements on the yield and quality of soybean Ind. *J. Agric. Chem.* 5 , 13 (C.F. Field Crop Abst. 28, 2856).
- Coates, J.B; J.S. Medeiros; V.H. Thanh and N.C. Nielsen (1985). Characterization of the subunits of B-Conglycinin. *Archives of biochemistry and Biophysics.*, 243 (1): 184-194.
- Dahdoh, M.S.A. (1986). Response of different crops to zinc application. Ph. D. Thesis Fac. of Agric., Ain Shams Univ.
- Derbyshire, E.; D.B. Wright and d. Boulter (1976). Legume and vicilin, storage proteins of legume seeds *Phytochemistry*, 15: 3-24.
- El-Bagouri, I.H.; M.M. Wassif; M.A. El-Kadi and S.A. Sabet (1983). Response of barley to foliar application of some micronutrients under the conditions of saline water irrigation and highly calcareous soil. *Desert Inst. Bull.*, A.R.E. 14: 1-15.
- El-Borai, M. A.; M.I. Amer and F.M. Hamouda (1988). Effect of inoculation and N-Fertilization on inoculation, N-fixation and yield of two soybean (*Glycine max.*, (L.) Merr.) Cultivars. Proc. 3rd Egyptian conf. Agron., 11: 185 – 192.
- El-Hamdi, Kh. H. (1990). Phosphate fertilization of faba bean grown on calcareous soils. *J. Agric. Sci. Mansoura Univ.*, 15 (9): 1529 – 1536.
- El-Kased, F.A.; A.A. El-Gharabawy and S.Y. Besheit (1993). Yield of sugar beet and quality as affected by nitrogen and phosphorus rates in calcareous soils. *J. agric. Sci. Mansoura Univ.*, 18 (2): 581-287

- El-Samahy , R.S.R (2000). Changes in growth criteria and metabolism in soybean plants grown in calcareous soil consequent to some mineral nutrients rational supply. Msc Fac. Of Science Mansoura University.
- Eweda MA. (1991). Genotypic variation in the electrophoretic banding patterns of seed storage proteins of soybean. Egypt J. Genet & Cytol., 20 : 43 –49.
- Fontes, E.P.B.; M.A. Moreira; C.S. Davies and NC Nielsen (1984). Urea-elicited in relative electrophoretic mobility of certain glycinin and B-Conglycinin subunits. Plant Physiol., 76: 840-842.
- Fukushima, D. (1991). Recent progress of soyben protein foods. Chemistry, technology and nutrition. Food Rev. Int., 7 : 323 – 351.
- Gaber, A.M. (1979). Status of manganese in soils of some new valley extension areas. M. Sc. Thesis, Soil Dept. Fac. Of Agric. Zagazig Univ.
- Gayler, K.R. and G.E. Sykes (1985). Effects of nutritional stress on the storage proteins of soybeans. Plant Physiol., 78 : 582-585.
- Gettier, S.W.; D.C. Martens and T.B. J. Brumack (1985). Timing of foliar manganese application for correction of manganese deficiency in soybean. Agron. J., 77: 627-630.
- Grabau L.J.; DG Blevins and HC Minor (1986). Stem infusion enhanced methionine content of soybean storage protein. Plant Physiol., 82 : 1013-1018.
- Heenan, D.P. and L.G. Campbell (1980). Transport and distribution of manganese in two cultivars of soybean Glycine max , L. Merr. Aust. J. Agric. Res. 3, 943. (C.F.Field Crop Abst. 34, 8251).
- Holowach L.P.; JF. Thompson and J. T. Madison (1986). Studies on the mechanism of regulation of the mRNA level for a soybean storage protein subunit by exogenous L. methionine. Plant Physiol., 80 : 561-567.
- Jackson, M.L. (1958). Soil chemical analysis. Constable and Co., Ltd., London, 38: 325.
- Kamel M.S.; R. Shabana; A. El-firgani; A.A. Abd El-Hafeez and A.A. El-Sayed (1983). Progress in protein and oil developing soybean seeds and productivity under inoculation and foliar application of micronutrients. First conference of Agronomy, 2: 299-308.
- Kitamura, K.; C.S. Davis and N.C. Nielsen (1984). Inheritance of alleles for Cgy1 and Gy4 storage protein genes in soybean. Theor Appl. Genet., 68 : 253-257.
- Masconia, H.J. and F.R. Cox (1985). Effective rates of fertilization for correcting manganese deficiency in soybean. Agron. J., 77(3) : 363-366.
- Meinke, D.w.; J. Chen and R.N. Beachy(1981). Expression storage-protein genes during soybean seed development Planta, 153: 130-139.
- Misra, D.K. (1964). Arid zone research work. Indian Fmg, 14: 18-19.
- Mohammed, S.A.M. (1994). Evaluation of some soybean genotypes and different population densities under potassium and late sowing dates. Ph.D. Thesis Fac. of Agric at Moshtohor-Zagazig Univ.

- Nielsen, N.C.; CD. Dickinson; T.J. Cho; VH Thank; B.J. Scallon; R.L. Fischer; T.L. Sims; G.N. Drews and R.B. Goldberg (1989): Characterization of the glycinin family in soybean *Plant Cell.*, 1: 313 – 328.
- Ogawa, T.; E. Tayama; K. Kitamura and N. Kaizuma (1989). Genetic improvement of seed storage proteins using three variant alleles of 7s globulin subunits in soybean (*Glycine max* L.) *Jpn. J. Breeding.*, 39 : 137-147.
- Paek N.C.; J. Imsande; RC. Shoemaker and R. Shibles (1997): Nutritional control of soybean seed storage protein. *Crop Sci.*, 37 : 498-503.
- Parres, A. Q.; F. P. Gardner and K. J. Boote (1989). Determinate and indeterminate- type soybean cultivars response to pattern, density and planting date, *Crop Sci.*, 29 : 149-157.
- Pazdernik, D.P.; P.H. Graham; C.P. Vance and JH Org (1996). Host variation in traits affecting early nodulation and dinitrogen fixation in soybean *Crop Sci.*, 36 : 1102-1107.
- Pazdernik, D.P.; P.H. Graham and J.H. Orf. (1997). Variation in the pattern of nitrogen accumulation and distribution in soybean *Crop Sci.*, 37 : 1482-1486.
- Richards, H.A. (1954). Diagnosis and improvement of saline and alkaline soils. *Agriculture Handbook*, 60.
- Robertson, L.S. and R.E. Lucas (1976). Essential micronutrients: Manganese. *Mich. Ext. Bull. E.*, 1031-1050.
- Salako, E.A.; L.S. Murphy; P.L.J. Gallagher and R.Hr. Elis (1975). Research show soybeans need zinc. "*fertilizer solutions*" 19 (6) : 96-100.
- Salama, A.M. and M. H. Ghonema (1990). Response of soybean cultivars to *Rhizohium* inoculation, molybdenum and some growth regulators. *Proc. 4th Conf. Agron.*, Cairo, 15 – 16 Sept. vol. II. 161 – 175.
- Sallam, H.A. and H.S. Khafaga (1999). A comparative study between spraying and seed hardening with some nutrients for improving salt tolerance of fodder beet plants. *Annals of Agric. Sc., Moshtohor*, 37 (1): 65-83 (1999).
- Sebastiani, FL.; L.B. Farrell; MA Schuler and RN Beach (1990). Complete sequence of a cDNA of a subunit of B-conglycinin. *Plant Mol. Biol.* 15 : 197 –201.
- Serraj, R. and T.R. Sinclair (1997). Variation among soybean cultivars in Dinitrogen fixation response to drought *Agron J.*, 89 : 963-969.
- Sharief, A.E. and A.M. Salama (1996). Performance of some soybean cultivars under nitrogen and PK fertilizers. *Proc. 7th Conf Agronomy*, 9-10 sept. 409-421.
- Sharief, A.E. and M.E. El-bially (1992). Yield and path analysis of five soybean cultivars under three plant densities. *Egypt. J. Appl. Sci.*, 7 (12) : 518 – 529.
- Shukla, U.C. and A.K. Nukhi (1985). Ameliorative role of zinc on maize growth (*Zea mays* L.) under salt affected soil conditions. *Plant and Soil*, 87: 423-432.
- Staswick, P.E. and N.C. Nielsen (1983). Characterization of a soybean cultivar lacking certain glycinin subunits *Archives of Biochemistry and Biophysics*, 223 (1): 1-8.

- Steel, R.G.D. and J.H. Torrie (1960). "Principle and procedures of statistics" MC-Grew-Hill Book Comp. Inc. New York.
- Sureshumar M.; G. Romanahan; S. Loganathan; and K.K. Krishamoorthy (1977). Effect of maganese on quanlity of soybean seeds . Mysr J. Agric. Sci., 11: 517.
- Taffin, L.O. (1970). Translocation of Iron citrate and phosphorus in xylem exudate of soybean. Plant Physiol, 45: 280-283.
- Thompson, J.F. and JT. Madison (1990). The effect of sulfate and methionine on legume proteins P. 145-158. In H. Rennenberg *et al.* (Ed.) Sulfur nutrition and sulfur assimilation in higher plants. SPB Acadimic publ., Hague, Netherlands.
- Tomkins, J. P. and E. R. Shipe (1997). Environmental adaptation of long-juvenile soybean cultivars and elite strains Agron. J., 89 : 257-262.

تأثير الرش بالعناصر الصغرى على زيادة القدرة التأقلمية والسلوك الوراثى لبعض أصناف نباتات فول الصويا تحت ظروف الأراضي الجيرية الصحراوية
حسين سعيد عبدالنبي خفاجة و ناهد أحمد كامل راشد
قسم الأصول الوراثية النباتية - مركز بحوث الصحراء - المطرية - القاهرة - مصر

- أجريت هذه الدراسة تحت ظروف الأراضي الجيرية بمحطة بحوث مريوط - محافظة الاسكندرية خلال موسمي الزراعة ٢٠٠٠ - ٢٠٠١، لدراسة تأثير الاختلافات الوراثية والرش بالعناصر الصغرى (٠,١% سلفات الزنك - ١% سلفات المنجنيز - ٠,٥% كبريتات الحديدوز) بالإضافة إلى معاملة الكنترول (الماء العادي) على النمو والمحصول ونتاج الزيت وتركيب تحت الوحدات لبروتينات بذور أصناف فول الصويا الرئيسية وكذلك بعض تحت الوحدات الغير معرفة بين الأصناف (Giza 21, Giza) (Crawford, Clark 35). اتبع تصميم القطع المنشقة مرة واحدة في ثلاث مكررات حيث نفذت معاملات الرش في القطع الرئيسية والأصناف في القطع المنشقة وفيما يلي أهم النتائج:
- لدى الرش بالعناصر الصغرى إلى استجابة معنوية لجميع صفات النمو والإنتاجية ونسبة الزيت مقارنة بالكنترول (الرش بالماء العادي) خلال مرحلة النمو الخضري وكذلك عند الحصاد. كما حقق الرش إلى زيادة كثافة تحت الوحدات البروتينية للبذور (الكوجاليسين القوي في الكبريت - الجليسين) مما أدى إلى زيادة القيمة الغذائية لبذور الأصناف تحت الدراسة مقارنة بالكنترول.
 - حقق الرش بكبريتات الزنك أعلى قيم معنوية لصفات النمو والإنتاجية مقارنة بالعناصر الأخرى.
 - وجد أن الاختلافات الوراثية بين الأصناف الأربعة تؤثر على النمو والمحصول ومنتقلته وإنتاج الزيت وكذلك على تحت الوحدات البروتينية المخزونة للبذور. حيث سجل الصنف جيزة ٢١ أعلى قيم معنوية على جميع الصفات تحت الدراسة.
 - أدى التفاعل بين الرش بكبريتات الزنك مع الصنف جيزة ٢١ إلى تسجيل أفضل قيم معنوية للصفات تحت الدراسة وكذلك نسبة الزيت مقارنة بالكنترول.