ISSN 2347-6893

The productivity of wheat cultivars under salt stress not always linked with their nitrate reductase activity in leaves

Gaber K. Abd El-Baki
Botany and Microbiology Department, Faculty of Science, Minia University, El-Minia, 61519, Egypt.
gaberahmed137@yahoo.com

ABSTRACT

To evaluate the effect of salt stress on four wheat cultivars (Bani suief 1, Bani suief 3, Seds 1 and Seds 6), seeds were cultivated in sand clay soil under normal field conditions. The plants left to grow for 21-days, then treated with different concentrations of NaCl (0.0, 50, 100,150 and 200 mM NaCl) by top irrigation. The plants left till harvest around (100-days from sowing). Fresh and dry matter, spikes weight were also determined. Photosynthetic pigments (chlorophyll a, b) and carotenoides as well as total pigments were measured. The fresh weight of four wheat cultivars ran in order (Bani suief 1> Bani suief 3> Seds 1> Seds 6) and this almost true for dry weight production. The productivity of four wheat cultivars at 200 mM NaCl referred as (spike weight) at harvest time ran in order [Bani suief 1> Seds 6> Seds1> Bani suief 3]. Both chl. a and chl. b were showed slight reduction in most cultivars with increasing salinity. The total pigments showed various responses with different treatments. The activity of NR was increased in both cultivars (Bani suief 3 and seds 1) however, in other two cultivars (Bani suief 1 and seds 6) the activity was decreased with increasing salinity in the soil. The activity of the enzyme at higher salinity levels used ran in order (Seds1> Bani sueif 3> Bani suief 1> Seds6).

Indexing terms/Keywords

Dry weight; Fresh weight; Harvest index; Nitrate reductase; pigments; Wheat.

Academic Discipline And Sub-Disciplines

Biology

SUBJECT CLASSIFICATION

Physiology

TYPE (METHOD/APPROACH)

Stress Physiology

Council for Innovative Research

Peer Review Research Publishing System

Journal: Journal of Advances in Biology

Vol. 6, No. 1

editorsjab@gmail.com , editor@cirjab.com

ISSN 2347-6893

INTRODUCTION

Wheat is a major staple food crop for more than one third of the world population **[1]**. New sources of salinity tolerance are needed for crops grown on salt-affected land. The physiology of plant responses to salinity and their relation to salinity resistance have been much studied and frequently reviewed in recent years **[2, 3]**. Since plants vary widely in their nutrient requirements and in their ability to absorb specific nutrients. The effect of salinity on plant nutrition differs markedly among species **[4]**.Water availability is one of the main environmental factor limiting photosynthesis and growth **[5]**. Sodium chloride salinity can cause osmotic stress and specific salt toxicity by excessive accumulation of salt in plant tissues, including adverse secondary effects on nitrogen metabolism parameters, such as nitrogen uptake and NRA **[6, 7]**. Nitrogen uptake rates in plants have been found to decrease with high concentrations of NaCl salinity **[8].** Nitrate reductase is considered to be a limiting factor for growth, development and protein production in plants, because its activity changes directly and affect plant growth **[9, 10]**.This first enzyme of nitrate assimilation is well known to be influenced by external conditions, such as low temperature, salinity or osmotic stress **[11]**. In higher plants, NR is an oligomeric and NAD(P) H-dependent complex containing FAD, heme (cytochrome b557) and Mo-protein prosthetic group **[12]**. Increasing the salt tolerance of crops will also allow the more effective use of poor quality irrigation water. Ability to grow high return crops such as wheat on salt-affected land will boost farm incomes and support changed farm management practices to address salinization.

The present paper reports the effect of NaCl stress on NR activity in four wheat cultivars which were chosen for their difference in sensitivity to NaCl salinity.

MATERIAL AND METHODS

Four wheat cultivars, (Seds1, Seds6, Bani suief 1 and Bani suief 3) were selected and brought from seds agriculture research center Bani Suief, governorate, Egypt. The seeds were cultivated in sand clay soil under normal field conditions. The plants left to grow for 21days, then treated with different concentrations of NaCl (0.0, 50, 100,150 and 200 mM NaCl) by top irrigation. The plants lefts to grow after salt treatment for 79 days. Photosynthetic pigment (chlorophyll a, b) and carotenoides as well as total pigments were determined using spectrophotometric method recommended by **[13]**. The plants reached to maturity after around 100 days from sowing. The spikes were separated and weighted, to determine the dry matter, the freshly harvested organs (shoots and roots) were dried in an aerated oven at 105 ^{0}C for 24 hours.

NR-activity

Leaves (1 g fresh weight) were ground with mortar and pestle in liquid nitrogen. Two ml extraction buffer (100 mM Hepes-KOH pH 7.6; 20 mM $MgCl_2$, 10µM FAD, 5 mM DTT, 1 mM Pefabloc, 0.2 mM PMSF, 1% polyvinyl Pyrrolidone (PVP) and 0.05 % Casein) were added to the still frozen powder and grinding continued until thawing the suspension was then centrifuged for 12 min. (4^{0}C , 12000 rpm) and the supernatant was removed and kept on ice. The reaction medium contained (total volume 1 ml) of 50 mM HEPES pH 7.6, 10µM FAD, 1 mM DTT, 5 mM KNO_3, 0.2 mM NADH and either 20 mM $MgCl_2$ or 20 mM EDTA. The reaction was started by addition of 100µl extract and terminated after 5 min by addition of 125µl zinc acetate solution (0.5M) after a short centrifugation (4C^0, 5min, 12000 rpm), 10µl PMS was added to 950µl of the supernatant in order to oxidize excess NADH. After 20 min in the dark, formed nitrite was measured colorimetrically by adding 750µl of 1% sulfanilamide in 3M HCl, and 750µl of 0.02% N-naphthyl-ethylene diamine hydrochloride, and absorption was determined at 546 nm. For each series, blank and a nitrite standard (20µM KNO_2) was included.

The data of all experiments were subjected to one way analysis of variance and means were compared using the least significant difference test (L.S.D.) using statistical program (Sta. Base. Exe.) on computer.

RESULTS

The first step in this study was to asses if there was significant variation in salinity tolerance and to compare the productivity of wheat cultivars under salt stress. Salinity tolerance was expressed as the percent biomass production in saline versus control treatments. The fresh weight of four wheat cultivars ran in order (Bani suief 1 > Bani suief 3> Seds 1> Seds 6) and this almost true for dry weight production with increasing salinity (Tables 1&2)**.** The production of fresh and dry matter in Bani suief 1 (the most tolerant cultivar is 303%, 257%) respectively which is higher than seds 6 (the most sensitive cultivar) under 200 mM NaCl.

The productivity of four wheat cultivars referred as (spike weight) at harvest time ran in order [Bani suief 1 (66.4%) > Seds 6 (40.9 %)> Seds1 (39.8%)> Bani suief 3 (29.75%)]. This findings calculated as (spike weight at 200 mM NaCl/spike weighty for reference control) (Table 3). The harvest index in four wheat cultivars which means dry weight of spikes divided by dry weight of shoots were showing various responses. In cultivar Bani suief 1 the harvest index was increased with increasing salinity in the soil and reached about (115.1% of control plants) at 200 mM NaCl, however in cultivar Bani suief 3 the trend is true till 150 mM NaCl which reached around (109%) and then decreased to (81% of control plants) at 200 mM NaCl. In cultivar Seds 1 the harvest index increased in moderate salinity and decreased at higher salt used while cultivar Seds 6 was more or less unchanged.

Generally photosynthetic activity was suppressed under salt stress and was severely suppressed in salt sensitive species such as crops. Both chl. a and chl. b showed slight decrease with increasing salinity in most cultivars, however cultivar seds 6 exhibited a marked and progressive decrease in both chlorophylls at higher salinity levels used (200 mM NaCl). The carotenoids was increased with increasing salinity in all wheat cultivars, the trend ran in order (Bani suief 1 (313%)> Bani suief 3 (216.1%)> Seds 1 (175%)> Seds 6 (154.2%) of control plants (Table 4). The total pigments showed various

responses with salt teratments, both Bani suief 1, Bani suief 3 and Seds 1 were more or less unchanged however, Seds6 showed dramatic decrease in pigment contents (73.5%) of control plants at 200 mM NaCl used.

Nitrate reductase activity the first enzyme in nitrate assimilation pathway was influenced by a variety of environmental factors. Reports on the effects of salinity on NR activity in plants have frequently been contradictory. The activity of NR was increased with increasing salinity in both cultivars (Bani suief 3 and Seds 1), however the activity of NR in other two cultivars (Bani suief 1 and seds 6) were decreased. The activity of the enzyme at higher salinity levels used ran in order (Seds1>Bani sueif 3>Bani suief 1> Seds6) [141.1%, 85.7%, 83.6%, 73.6%] respectively of control plants (Table 5).

DISCUSSION AND CONCLUSION

Salinity is considered to be one of the major factors that limit crop productivity in arid and semi-arid countries. Almost 1000 million ha of cultivated lands are affected by high salinity **[14]**. The physiology of plant responses to salinity and their relation to resistance have been much studied and frequently reviewed in recent years **[2,3]**. Soil salinity is becoming more problematic due to the increase in irrigation around the world. Salt water in the root zone induces osmotic changes and interferes with nutrient uptake **[15].**

According to the data in (Tables 1&2), the fresh weight of four wheat cultivars was decreased with increasing salinity in the soil, this reduction ran in order (Bani suief 1> Bani suief3> Seds 1> Seds6). The reduction in fresh weight in cultivars Bani suief 1 (the most tolerant cultivar) was much higher than the reduction in dry weight, (62.7% and 75% of control plants respectively). However, the reduction in fresh and dry matter in cultivar sed6 (the most sensitive cultivar) was (39.4% and 43.7%) of control plants respectively under high salt used. Therefore the behavior of plants is not only controlled by the plant itself but also the environmental conditions could control the direction of genotypes under stress conditions. Many studies have shown that the plant growth were affected either negatively or positively by variation in salinity doses and types of plant studied, **[16]** working with maize and **[17]** working with *Orzyza sativa* supported our results see (Tables 1&2). Salinity reduced plant growth in all cultivars, **[18]** stated that suppression of plant growth under saline conditions may either be due to decrease the availability to water or increasing in sodium chloride toxicity associated with increasing salinity. Also, salinity adversely affects plant growth and development **[19]**, provokes disorders in plant nutrition, carbon metabolism, nitrogen metabolism, all such physiological changes well result in a decrease in plant growth and consequently in crop yield **[20]**.

Negative effects of salinity on plant growth had a direct effect on ultimate plant productivity (total plant dry mass accumulation, grain yield). The spikes weight ranked as followings (Bani suief 1> Seds 6> Seds1> Bani suief 3). The last 6one in fresh weight order becomes the second in spike production which greatly confirmed the difference in salt tolerance among the four wheat cultivars in vegetative growth and consequently the crop yield production. The main reason for the reduction in spike weight in response to salinity is mostly attributed to decrease in photosynthesis, nitrogen metabolism, and carbon metabolism **[21]**.

According to HI (harvest index) the plants arranged in the following manner [Bani Suief 1 (114%%) 1> Bani suief 3 (88.1%)> Seds6 (83.1%)> Seds1 (81%)]. The difference in harvest index among the four wheat cultivars can used as an suitable marker for the differences in the crop yield production. Past breeding strategies have been highly successfully increased grain yield by reducing height and increasing HI, alternatives for improving biomass while maintaining this index are urgently required if further genetic gains in yield are to be achieved **[22]**

Therefore the behavior of wheat cultivars seemed to be greatly changed due to specific correlation between the environmental conditions, enzyme activity, stress factor, and consequently the gene expression. It has been necessary to grow plants for several weeks to be confident of obtaining reproducible differences in salinity tolerance between genotypes **[23, 24]**. Accordingly, prediction the salt tolerance of species or cultivars before the crop yield stages should be taken carefully, it seems that plants can greatly changed themselves during the whole plant life cycle.

Salinity drastically affects photosynthesis **[25],** and remarkably decreased both chlorophylls (a & b) in four wheat cultivars with increasing salinity in the soil. A decrease in chlorophyll levels due to salt stress has been reported in several plants, such as tomato **[26],** rice **[27],** and wheat **[28, 29].** However the carotenoids increased in four wheat cultivars and ran in order (Bani suief1>Bani suief3> Seds1> Seds6) which concomitant with fresh weight production and almost with harvest index see (Tables 1&2). We attributed that to the protective effect of carotenoids against photooxidation in plants.

Salt stress decreased chlorophyll content as compared to the non saline conditions. Similarly, the adverse effect of salt stress on chlorophyll content of strawberry has been shown by **[30]**. Furthermore, salt stress inhibits the chlorophyll content in leaves of many crops **[31],** in barley plants, **[32]** reported that chlorophyll a, b and carotenoids decreased significantly in NaCl treated plants in comparison to control. Chlorophyll (a, b) and carotenoids increased significantly in NaCl treated plants **[33]**. Our results are in agreement with **[29]** for wheat and **[34]** for cucumber.

Salt accumulated in the plants may inactivate plant enzymes and disrupt osmotic adjustment at the level of cytosol and vacuoles **[35]**. Although the nitrate reductase enzyme itself represents a very small proportion of leaf protein **[36],** the activity of the enzyme plays a pivotal role in the supply of nitrogen, growth of plants, especially in cereals **[37]** and, is a measure of the habitat-dependent nitrate utilization **[38].** Salinity provoked either a decrease in NRA **[39, 40]** or an increase in NRA **[41]** or no change **[42]**.

The data obtained in both cultivars (Bani suief 3 and Seds 1) showed a pronounced increase in NRA however, the other two cultivars (Bani suief 1 and Seds 6) exhibited a marked decreased with increasing salt in the growing medium. The activity of NR in four wheat cultivars under 200 mM NaCl ran in order [Seds1 (141%)> Bani suief 3 (85.7%)> Bani suief 1 (83.6)> Seds6 (73.6%)] compared with reference control.

Both cultivars (Seds 1 and Bani suief 3) have a total activity (141% and 85.7%) of control respectively at 200 mM NaCl but produced less fresh weight and spikes weight compared with other two cultivars. This means that, the activity of NR is not always linked with the productivity (Spike weight, HI, FW) of plants in those two cultivars. However the other two cultivars (Bani suief 1 and Seds6) which have lower nitrate reductase activity (83.6% &73.6%) of control respectively at higher salt used 200 mM NaCl but superiors in producing (FW, HI, Spike weight) especially cultivar Bani suief 1. This confirms our results which stated that NRA is not always correlated with the productivity in these four wheat cultivars.

The reduced nitrate reductase activity in the leaves of salt-stressed plants may be attributed to salinity inhibited nitrate transport to the shoot, which in turn is due to interference with nitrate uptake and xylem loading **[43].** Our observation in both cultivars Bani suief 1 and Seds 6 was consistent also with **[44]** whom stated that, NR in plants growing under NaCl stress can be under strong substrate limitation. Accordingly the main reason for inhibition of NR activity under NaCl stress is rather substrate deficiency than specific salt toxicity.

The fast NRA decrease in the leaves by the external supply of NaCl may be related to osmotic changes following NaCl addition to the medium. In fact, NRA is inhibited by osmotic effects of NaCl treatment in cashew **[45]**. The reduction of the maximum extractable NRA in the leaves could be due to a lower NR protein content. Indeed, NO_3^- regulates NR transcription, translation and activation in higher plants **[46]**. It has been reported that NaCl reduced NO_3^- fluxes from roots to leaves and impaired the NRA in leaves **[47, 48]**.

The decrease in NO_3^- concentrations by NaCl treatment may result from a disruption of root membrane integrity **[49]**, an inhibition of NO_3^- uptake **[50]** and low NO_3^- loading into root xylem **[40]**. As Cl^- ions inhibit NO_3^- uptake or be attributed to competition between Cl^- and NO_3^- for uptake by NO_3^- transporters (Deane-Drummond, 1986)[51], and/or an inactivation of NO_3^- transporters by toxic effects of salt ions **[52]**. These results suggest that NR is regulated by NO_3^- availability in the leaves under NaCl stress. It is well known that, induction of NR requires nitrate in the cells **[53]**. So the observed increase in NRA in other cultivars (Bani suief 3 and Seds 1) may be due to genetic improvement in nitrate uptake by these cultivars in original research centers. This property may improve NR expression and activity there, as salt induced increase in NR capacity of roots was also described for wheat **[54]**.

In Conclusion, NaCl salinity differently affected fresh and dry matter, slightly change the pattern of production of spikes and harvest index in four wheat cultivars comparing with biomass production.The production of photosynthetic pigments was concomitant with biomass production. NRA exhibited various responses in wheat cultivars with increasing salinity, and poorly linked with the spike production in most cultivars, in confirmatory our results showed that NRA is not always correlated with the productivity four wheat cultivars. The activity of NRA in both cultivars (Bani Suief 3 and Seds 6) were in concomitant with fresh production at higher salt used 200 mM NaCl. Determination and identifying the tolerant wheat cultivars to salinity that give minimum depression in yield when grown in saline soils may be an efficient tool in resolving the salinity problem to some extent, also further studies by using new techniques should be carried out to reach to realistic results.

ACKNOWLEDGMENTS

I would like to thank Prof. Dr. M.A.K. Shaddad Prof. of plant physiology, Faculty of Science, Assuit University for his valuable advices and revising the manuscript and Dr. Mostafa D. for helping in statistical analysis.

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ISSN 2347-6893

Table 1. Fresh and dry weight (gm) of wheat Bani suief 1 and Bani suief 3 plants growing for 100 days from sowing and treated with different conc. of NaCl. Data means of 3 replications ±SD.

treatment	Shoot		Root	
	Fresh	Dry	Fresh	Dry
		Bani suief 1		
0.0	14.5±1.2	4.8±0.6	3.1±0.6	1.1±0.34
50	15.1±1.5	4.6±0.4	2.7±0.32	0.77±0.28
100	12.6±3.8	4.0±1.2	1.65±0.21	0.49± 0.1
150	9.7±2.6	3.2±0.8	1.46±0.6	0.37±.0.1
200 mM NaCl	9.1±2.4	3.6±0.83	0.8±0.0	0.25±0.01
LSD at 5%	**4.003**	**1.28**	**0.68**	**0.33**
		Bani suief 3		
0.0	15.7±1.6	6.03±0.9	3.0±1.1	0.88±0.31
50	13.2±1.1	4.6±0.45	1.56±0.5	0.58±0.1
100	8.2±1.1	2.9±0.16	0.98±0.4	0.32±0.1
150	7.7±1.5	3.03±.5	0.68±0.12	0.22±.04
200 mM NaCl	4.6±1.6	1.9±0.7	0.32±0.18	0.11±0.05
LSD at 5%	**2.55**	**1.097**	**0.87**	**0.27**

Table 2. Fresh and dry weight (gm) of wheat Seds1 and Seds6 plants growing for 100 days from sowing and treated with different conc. of NaCl. Data means of 3 replications ±SD.

treatment	Shoot		Root	
	Fresh	Dry	Fresh	Dry
		Seds-1		
0.0	8.7±1.5	3.6±0.68	1.6± 0.0	0.67±0.07
50	8.7±0.28	3.5±0..15	1.3±0.30	0.48±0.11
100	8.0±1.6	3.0±0.55	0.69±0.06	0.31±.0.02
150	5.7±0.25	2.5±0.34	0.51±0.13	0.18±.0.05
200 mM NaCl	3.2±0.40	1.7±0.15	0.41±0.06	0.14±0.01
LSD at 5%	**1.84**	**0.78**	**0.28**	**0.11**
		Seds-6		
0.0	7.6±1.1	3.2±0.75	0.36±0.06	0.12±0.01
50	6.2±0.55	2.6±0.36	0.72±0.34	0.22±0.10
100	5.6±0.66	2.4±0.41	0.63±0.17	0.2±0.06
150	4.4±0.96	1.9±0.28	0.28±0.07	0.09±.04
200 mM NaCl	3.0±0.6	1.4±0.20	0.26±0.02	0.07±0.01
LSD at 5%	**1.42**	**0.81**	**0.32**	**0.108**

ISSN 2347-6893

Table 3. Spikes weight (gm) and harvest index (HI) for four wheat cultivars growing for 100 days from sowing and treated with different conc. of NaCl. Data means of 3 replications ±SD.

Cultivar name	**Bani suief 1**		**Bani suief 3**	
Treatment	**Spike Wt.**	**HI**	**Spike Wt.**	**HI**
0.0	14.3±0.77	2.85±0.35	11.1±0.37	2.01±0.38
50	11.5±0.37	2.5±0.28	10.03±0.9	2.18±0.20
100	10.1±0.07	2.66±0.79	6.9±0.25	2.4±0.22
150	9.7±1.20	3.1±0.59	6.6±0.49	2.2±0.41
200 mM NaCl	9.5±1.00	3.24±1.3	3.1±0.73	1.77±0.50
LSD at 5%	**1.43**	**1.32**	**1.077**	0.696
	Seds 1		**Seds 6**	
0.0	10.3 ±1.2	2.96±0.67	8.8±3.01	2.95±1.6
50	10.5±0.52	2.94±0.03	7.6±1.18	2.97±0.63
100	9.13±0.81	3.13±0.80	7.1±1.12	2.97±0.78
150	6.6± 0.32	2.66±0.44	5.5±0.55	2.92±0.65
200 mM NaCl	4.1±0.25	2.46±0.19	3.6±0.56	2.68±0.71
LSD at 5%	**1.27**	**1.069**	**2.84**	**1.737**

ISSN 2347-6893

Table 4. pigment contents (mg /g Dw) for four wheat cultivars growing for 100 days from sowing and treated with different conc. of NaCl. Data means of 3 replications ±SD.

Cultivar name		**Bani suief 1**		
Pigment contents	Ch a	Ch b	Carot.	Total pigments
0.0	8.4±0.98	5.35±0.63	0.22±0.25	13.97±1.3
50	7.6±0.71	5.1±0.21	0.46±0.26	13.11±0.65
100	8.35±1.34	5.8±4.75	0.97±0.60	15.12±3.2
150	5.53±0.1	4.75±0.77	0.69±0.24	11±0.61
200 mM NaCl	7.7±0.29	5.3±0.14	0.09±0.02	13.1±0.11
LSD at 5%	**2.11**	**1.89**	**0.86**	**4.16**
		Bani suief 3		
0.0	6.3±0.4	4.2±0.09	0.31±0.12	10.8±0.25
50	6.7±.07	4.6±0.32	0.33±0.13	11.7±0.32
100	6.6±0.24	4.5±0.24	0.77±0.26	11.92±0.23
150	6.2±0.24	4.49±0.50	0.48±0.24	11.1±0.55
200 mM NaCl	5.66±0.43	4.7±0.75	0.67±0.02	11.2±0.64
LSD at 5%	**0.73**	**1.498**	**0.466**	**1.132**
		Seds 1		
0.0	7.54±1.3	5.27±0.6	0.41±0.36	13.2±1.5
50	6.46±0.69	4.19±0.03	0.56±0.48	11.2±0.24
100	6.8±0.8	4.38±0.48	0.46±0.31	11.7±1.36
150	5.8±1.2	4.17±0.58	0.52±0.70	10.52±2.5
200 mM NaCl	5.74±0.21	4.01±0.11	0.72±0.22	10.47±0.11
LSD at 5%	**2.66**	**1.22**	**1.15**	**3.8**
		Seds 6		
0.0	11.13±0.11	6.35±0.97	0.83±0.75	18.31±0.12
50	8.51±1.47	5.48±1.1	0.55±0.42	14.54±2.2
100	8.49±0.41	5.18±0.21	1.16±0.37	14.83±0.57
150	7.98±0.41	4.89±0.16	0.98±0.83	13.86±0.25
200 mM NaCl	8.29±0.85	3.9±0.01	1.28±0.14	13.46±1.1
LSD at 5%	**2.07**	**1.788**	**1.46**	**2.91**

ISSN 2347-6893

Table 5. NR activity (µmol g^{-1} FW h^{-1}) of four wheat cultivars growing for 100 days from sowing and treated with different conc. of NaCl. Data means of 3 replications ±SD.

Treatment	Shoot NR			
	Bani suief1	Banisuief3	Seds1	Seds6
0.0	6.1±0.02	4.9 ± 0.82	2.58±0.03	3.79±1.29
50	5.4±0.98	5.3±1.02	4.15±0.60	3.52±1.25
100	4.5±0.81	5.24±1.1	4.97±0.16	3.49±0.96
150	5.3±1.10	6.2±0.19	3.04±0.67	2.86±0.20
200 mM NaCl	5.1±0.73	4.24±0.26	3.64±1.34	2.79±0.06
LSD at 5%	**2.03**	**2.01**	**1.87**	**2.35**

Author' biography with Photo

Name: Gaber khallaf Abd El- Baki Ahmed

Date of Birth: Jan13, 1968

Nationality: Egyptian

Occupation: Associate professor, Dept. of Botany, Faculty of Science, Minia Univ, El-Minia, Egypt.

Adress: Dept. of Botany and Microbiology, Faculty of, Science, Minia University, El-Minia,61519, Egypt.

Education: B.Sc. in Botany, M.Sc. in Botany (Physiology),

Ph.D. Botany (Plant Physiology), 2000

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