37

Egypt. J. Bot. **Vol. 58**, No, 3, pp. 445 - 461 (2018)

The Effect of Seed Presoaking with KNO_3 on Seed Germination, Proline, Protein Pattern, ß-Amylase and Mineral Composition of Two Faba Bean Cultivars Treated with NaCl

G.K. Abd El-Baki [(1)], M.A.K. Shaddad [(2)], D. Mostafa [(1)#] and Al-Shimaa Rafat [(1)]

[(1)]Botany and Microbiology Department, Faculty of Science, Minia University, El-Minia, Egypt; [(2)]Botany Department, Faculty of Science, Assiut University, Assiut, Egypt.

SEEDS of two broad bean cultivars (*Vicia faba*, cv. Nobaria 3 and cv. Sakha 3) were obtained from Mallawi Agriculture Research Center, El Minia Governorate, Egypt. Different salinization levels (0, 40, 80, 120 and 160mM NaCl) were used and both cultivars were presoaked in either water (Reference control) or potassium nitrate (3mM), then left for germination for five days. Plants transported to the field and further growth for 65 days from sowing. The final germination percentage the two broad bean cultivars of *Vicia faba* c were decreased by increasing salinity in the rooting medium. Seed presoaking in KNO_3 resulted in remarkable increase in final germination percentage especially in cultivar Sakha 3. Both cultivars accumulated proline in both different organs reached 3 folds higher than absolute control, Seed presoaking in KNO_3 had a significant increase in proline contents especially in shoots regardless the salinity levels used. Mineral composition the plants of the two broad bean cultivars showed various responses with increasing salinity. Seed presoaking in KNO_3 resulted in remarkable decrease in Na and increased K contents in tested plants. One band of β-amylase were detected under different concentrations of NaCl combined with KNO_3. The intensity of the bands varied between various treatments. Exposure of Sakha 3 and Nobaria 3 cultivars to different concentrations of NaCl salinity and those presoaked in KNO_3 (3mM) were produced marked changes in their protein pattern.

Keywords: Proline, Protein pattern, Faba bean cultivars, β -amylase, Mineral compositions.

Introduction

Soil salinity is one of the most important abiotic factors influencing the growth, development and yields of crops (Chaparzadeh et al., 2004) and causes its considerable losses in crops, (Simiroff & Cumbes, 1989). Salinity is regarded as one of the major and increasing problems in agricultural system in Egypt that affects approximately 7% of the world's total land area. More than 800 million hectares of land around the world are affected by salinity, (Munns, 2005), which results in billions of dollars in crop production losses. The most important problem facing the economic crops production in arid regions is high concentration of ions especially NaCl that is present either in soil or water (Moeinrad, 2008). It is a major problem adversely affecting growth and development of crop plants and results in low agricultural production (Garg & Gupta, 1997; Akramghaderi, et al., 2002 and Hussain et al., 2016).

Seed priming was defined as pre-sowing treatments in water or in an osmotic solution that allows seed to imbibe water to proceed to the first stage of germination (McDonald, 1999). It has been successfully demonstrated that, seed priming improve germination in seeds of many crops, particularly seeds of vegetables and small seeded grasses, increases speed and uniformity of germination and seedling emergence in the range of stress conditions, such as salinity, drought and temperature (Demir Kaya et al., 2006; Habibi & Abdoli, 2013 and Chunthaburee et al., 2014). It has been reported that priming increased enzymatic antioxidants such as glutathione and ascorbate (Hus & Sung, 1997). The depressive effects of salinity stress on germination can be alleviated by various seed priming treatments (Ashraf & Rauf, 2001 and Basra et al., 2006). Priming allows some of the metabolic processes necessary for germination to proceed before germination is completed (Ghobadi et al., 2012).

#Corresponding author email: moustafadoaa@yahoo.com
DOI: 10.21608/ejbo.2018.3423.1166
Edited by: Prof. Dr. Wedad Kasim, Faculty of Science, Tanta University, Tanta, Egypt.

Potassium (K) is an essential nutrient that affects most of the biochemical and physiological processes that influence plant growth and metabolism. It also contributes to the survival of plants exposed to various biotic and abiotic stresses (Kant & Kafkafi, 2002). Among the main nutrients required for plants, nitrogen (N) had significant effect on growth and development and also its direct relationship with plant growth and yield of crops has been proven (Rahmati, 2012 and Sorkhi & Fateh, 2014). The aim of this study was to evaluate priming with KNO_3 results in enhancement of seed germination, mineral composition, proline, β-amylase and protein pattern in two cultivars of broad bean (*Vicia faba*), cv. Sakha 3 and cv. Nobaria 3, under a range of osmotic potentials due to NaCl.

Materials and Methods

Seeds of two broad bean cultivars (*Vicia faba*, cv. Nobaria 3 and cv. Sakha 3) were obtained from Mallwi Agriculture Research Center, El Minia Governorate, Egypt. They were selected for uniformity by choosing those of equal size and with the same color. The selected seeds were washed with distilled water, surface sterilized with 1% sodium hypochlorite solution for about 2min and thoroughly washed again with distilled water. Different salinization levels (0, 40, 80, 120 and 160mM NaCl) were used and both cultivars were presoaked in either water (Reference control) or potassium nitrate (3mM). The germination experiments were performed as described by Maftoun & Sepaskhah (1978). Six seeds were placed on absorbent pads in petri dishes to which 15ml of the experimental solution was added. Seeds were considered to be germinated after the radical emerged from the testa. To evaluate the interactive effect of potassium nitrate (KNO_3) and salt stress on germination, seeds were presoaked in a solution of potassium nitrate (3mM) for 4h, then left to dry at room temperature. Some seeds were presoaked in H_2O and used as control. The percentage of seed germination was followed daily until the control gives constant germination percentage for two successive days. A preliminary experiments were carried out to evaluate the optimum concentration of potassium nitrate and the optimum time for seed soaking.

For further plant growth, the seeds were divided into two groups, the first group was soaked with distilled water, while the second group was soaked with 3mM KNO_3 for 4h, then dried at room temperature (25°C). Five uniform air dried faba bean seeds were sown along a centre row in each pot at 30mm depth in plastic pots, each was filled with about 4kg mixed clay-sand soil (3:1, v:v) in order to reduce compaction and improve drainage. Plants were left to grow for 3 weeks in open field and then treated with different concentrations of NaCl (0.0, 40, 80, 120 and 160mM) by top irrigation, then left to grow further for 65 days after sowing. Plant samples (three replicates for each) were collected after 65 days from sowing for measurement of free proline according to Bates et al. (1973), some minerals, sodium and potassium according to Williams & Twine (1960). Calcium and magnesium recommended by Schwarzenbach & Biedermann (1948). The electrophoresis of protein profiles was carried out in vertical polyacrylamide gels, using the slab gel apparatus "SE 600, vertical slab gel". Polyacrylamide gel electrophoresis was carried out according to Laemmli (1970) with 7.5% acrylamide for isozymes analysis and (12 % acrylamide + 1.0 % SDS) for protein analysis.

β- amylase were separated on non-denaturizing polyacrylamide gels (7.5%) at 100V for 2h at 4°C. Gels were then soaked in substrate (2% soluble starch) for 30min at 27°C and incubated in 0.025% acidified iodine solution for 5min (Shuster & Gifford, 1962).

Scanalytic Inc. Data were obtained by Total Lab version 1.10 electrophoresis data system program (Scanalytics Inc.). The molecular weights of protein bands were determined against the protein marker 20-175kDa.

The data of all experiments were subjected to one-way analysis variance and means were compared using the least significant difference test (L.S.D.) using statistical program (Sta. Base. Exe.) on computer (Steel & Torrie 1960).

Results

The final germination percentage of two broad bean cultivars *Vicia faba* cv. (Sakha 3 and Nobaria 3) were decreased by increasing salinity in the rooting medium. In cultivar Sakha 3 it remained more or less unchanged till 80mM NaCl and then decreased. At higher levels of salinity 120 and 160mM NaCl this cultivar exhibited a dramatic reduction in final germination percentage. Seed

presoaking in KNO_3 resulted in remarkable increase in final germination percentage especially in cultivar Sakha 3 which reached to 112.5% at 80mM (NaCl+KNO_3) compared with absolute control (0.0 NaCl and 0.0 KNO_3). However in cultivar Nobaria 3, seed presoaking had an enhanced effect on final germination percentage at almost all treatments except at 120mM (NaCl+KNO_3) (Table 1).

In shoots of cultivars Sakha 3, plants accumulated proline under NaCl salinity, this accumulation was pronounced at all salinity levels used however, In roots, the higher salinity levels enhanced proline accumulation which reached around two folds at 160mM NaCl as compared with absolute control. Seed presoaking in KNO_3 resulted in a progressive increase in proline contents in both organs as compared with reference control. On other side, in cultivar Nobaria 3 shoots, the amount of proline accumulated under different NaCl concentrations reached to 3 folds higher than absolute control, but in roots the accumulation was vanishingly small at lower and moderate salinity level used. Seed presoaking in KNO_3 had a significant increase in proline contents especially in shoots regardless the salinity levels used (Fig. 1).

The data obtained in Table 2 demonstrated that, there was an increase in sodium contents in either Sakha 3 shoots or roots with increasing salinity levels in the culture media, this accumulation was significant at most salinization levels. Presoaking treatment with KNO_3 retarded the accumulation of sodium at least at lower salinity levels in shoots and most levels in roots.

The potassium contents in both shoots and roots of cultivar Sakha 3 showed various responses in respect to salinity stress, it was increased in shoots and decreased in roots in most cases with increasing NaCl in the medium, compared with reference control (Table 2). Seed presoaking in KNO_3 significantly enhanced potassium accumulation in shoots especially at higher salinity levels used (80 and120mM) and decreased it in roots. Calcium contents in both shoots and roots of Sakha 3 cultivar had non-significant increase with increasing salinity levels. Seed presoaking in KNO_3 resulted in considerable increase of calcium contents especially in shoots. Magnesium contents in both shoots and roots of Sakha 3 plants were higher than absolute control. This increase was obvious at lower and moderate levels of salinity. Seed presoaking in KNO_3 resulted in significant accumulation of magnesium in shoots and higher levels of salinity in roots, which reached more than 1.5 folds higher than absolute control.

TABLE 1. Seed germination percentage in two faba bean cultivars (*Vicia faba* cv. Sakha 3 & Nobaria 3) after 5 days (Presoaked in KNO_3 and treated with different concentrations of NaCl).

		% of seed germination									
	NaCl (mM)	Sakha 3					Nobaria 3				
		1st day	2nd day	3rd day	4th day	5th day	1st day	2nd day	3rd day	4th day	5th day
Absolute control	0	0.00	61.11	83.33	88.89	88.89	0.00	61.11	88.89	88.89	88.89
Reference control	40	0.00	50.00	72.22	83.33	88.89	0.00	27.78*	72.22	94.44	94.44
	80	0.00	38.89	66.67	77.78	88.89	0.00	16.67**	44.44**	88.89	88.89
	120	0.00	11.11**	38.89**	66.67	72.22	0.00	0.00**	16.67**	77.78	83.33
	160	0.00	0.00**	11.11**	50.00*	72.22	0.00	0.00**	11.11**	50.00*	66.67
KNO_3 (3mM)	0	0.00	27.78*	72.22	77.78	77.78	0.00	44.44	83.33	94.44	94.44
	40	0.00	50.00	77.78	83.33	94.44	0.00	5.56**	66.67	94.44	94.44
	80	0.00	27.78*	55.56	83.33	100.00	0.00	0.00**	22.22**	88.89	88.89
	120	0.00	5.55**	22.22**	83.33	88.89	0.00	11.11**	22.22**	61.11	77.78
	160	0.00	5.55**	11.11**	66.67	72.22	0.00	0.00**	5.56**	38.89*	77.78
LSD at 5%		0.00	1.63	1.52	1.66	1.54	0.00	1.23	1.56	1.88	1.70
LSD at 1%		0.00	2.38	2.23	2.44	2.26	0.00	1.80	2.29	2.76	2.50

TABLE 2. Minerals (mg/g dry matter) of faba bean plants (*Vicia faba* cv. Sakha 3) after 65 days from sowing (presoaked in KNO_3 and treated with different concentrations of NaCl).

	NaCl (mM)	Root							
		Sodium	%	Potassium	%	Calcium	%	Magnesium	%
Absolute control	0	141.00	100	19.65	100	5.70	100	3.06	100
Reference	40	134.00	95.04	12.89**	65.60	5.55	97.37	4.32	141.18
	80	155.00	109.93	21.51	109.47	6.15	107.89	4.23	138.24
	120	153.00	108.51	16.24	82.65	7.65	134.21	4.32	141.18
	160	181.00	128.37	17.72	90.18	6.75	118.42	3.96	129.41
KNO_3 (3mM)	0	129.00	91.49	9.98**	50.79	7.20	126.32	5.13	167.65
	40	83.50*	59.22	3.35**	17.05	4.80	84.21	2.70	88.24
	80	115.00	81.56	8.67**	44.12	4.95	86.84	2.43	79.41
	120	148.50	105.32	10.47**	53.28	9.30	163.16	5.13	167.65
	160	137.50	97.52	10.22**	52.01	5.85	102.63	3.87	126.47
LSD at 5%		4.4		2.03		2.91		0.25	
LSD at 1%		6.8		3.21		3.96		0.39	

	NaCl (mM)	Shoot							
		Sodium	%	Potassium	%	Calcium	%	Magnesium	%
Absolute control	0	81.00	100	13.32	100	6.45	100	1.80	100
Reference	40	108.00	133.33	16.30	122.37	7.65	118.60	2.70	150.00
	80	89.00	109.88	13.26	99.55	6.30	97.67	1.98	110.00
	120	109.50	135.19	14.13	106.08	7.05	109.30	2.34	130.00
	160	91.00	112.35	15.06	113.06	5.55	86.05	1.35	75.00
KNO_3 (3mM)	0	59.00	72.84	9.60	72.07	9.00	139.53	3.42*	190.00
	40	82.50	101.85	6.69**	50.23	8.70	134.88	3.42*	190.00
	80	110.50	136.42	2.60**	19.52	7.65	118.60	2.70	150.00
	120	108.00	133.33	16.73	125.60	9.00	139.53	3.15*	175.00
	160	106.00	130.86	17.23	129.35	5.55	86.05	1.35	75.00
LSD at 5%		6.2		0.4		0.3		0.1	
LSD at 1%		9.5		0.6		0.5		0.19	

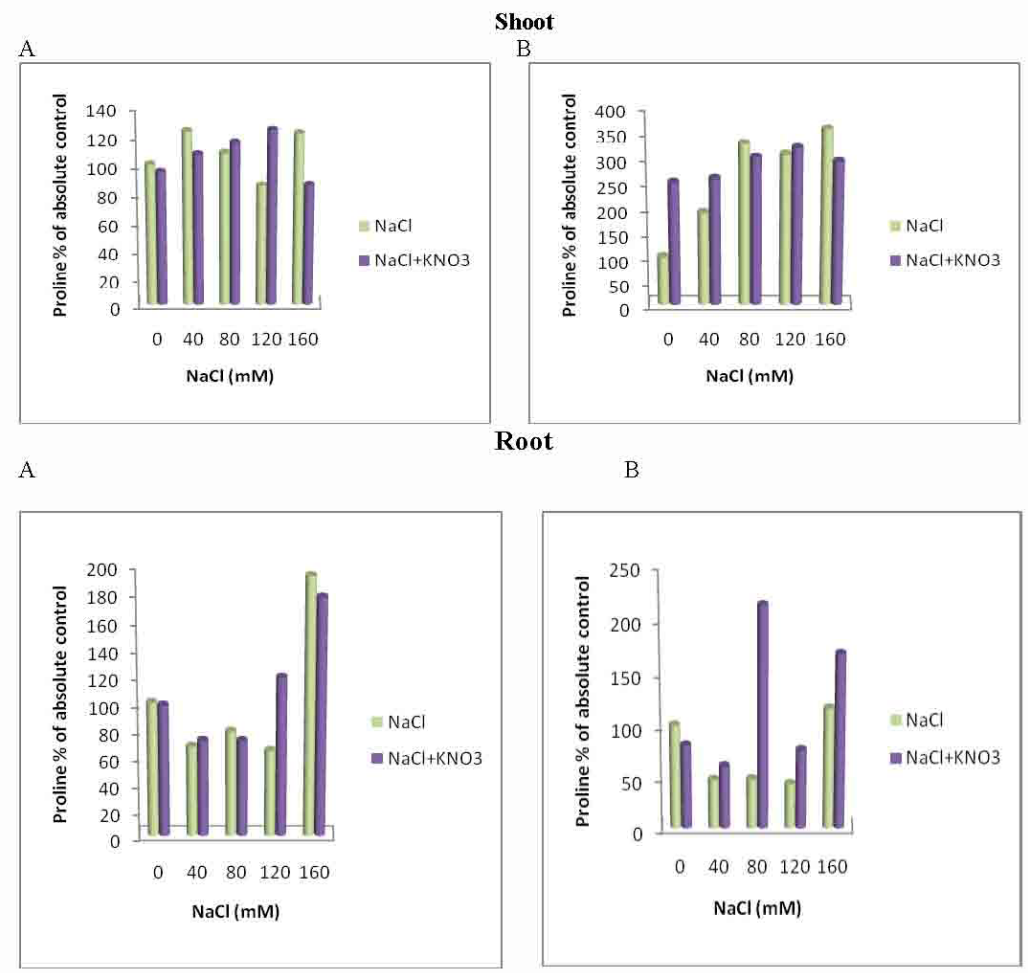

Fig. 1. Proline (mg/g dry matter)) in both shoots and roots of *Vicia faba* cv. Sakha 3 (A) and cv. Nobaria 3 (B), presoaked in KNO_3 and treated with different concentrations of NaCl. Data means of 3 replications and presented as percentage of absolute control.

The data obtained in Table 3 showed that sodium accumulated in both shoots and roots of cultivar Nobaria 3, which reached two folds higher compared with absolute control. Seed presoaking in KNO_3 resulted in significant accumulation of sodium in shoots and reduction of it in roots at almost salinity levels used. Potassium contents in both shoots and roots of Nobaria 3 cultivar showed various responses with increasing salinity in the culture medium. In shoots, it was slightly increased with increasing salinity but still lower than absolute control, however in roots, it was increased gradually especially at moderate and higher salinity used which reached (111.24% and 109.35%) over absolute control. Seed presoaking in KNO_3 decreased potassium contents in shoots and increased it in roots.

Calcium contents in shoots was increased with increasing NaCl in the culture medium, this was pronounced at moderate levels of salinity (80, 120mM NaCl), however in roots, the calcium contents decreased compared with absolute control (0.0mM NaCl). Seed presoaking in KNO_3 resulted in progressive accumulation of calcium in roots and lower levels in shoots compared with reference control. Magnesium contents in both shoots and roots of cultivar Nobaria 3 were reduced in most cases as compared with absolute control. Seed presoaking in KNO_3 decreased magnesium contents in both organs shoots and roots regardless the salinity levels used.

One band of β-amylase were detected in both cultivars, under different concentrations of NaCl combined with potassium nitrate. The intensity of the bands varied between treatments due to changes in total enzyme activity. In Sakha 3 cultivar, the intensity of the band decreased with increasing concentration of NaCl in the soil. On

the other hand, combined treatment of NaCl with potassium nitrate, the band intensity was more or less unchanged. In Nobaria 3 cultivar, the intensity of the band more or less changed with increasing salinity. The band most intensive at 80mM NaCl when mixed with potassium nitrate (Fig. 2).

TABLE 3. Minerals (mg/g dry matter) of faba bean plants (*Vicia faba* cv. Nobaria 3) after 65 days from sowing (Presoaked in KNO_3 and treated with different concentrations of NaCl).

	NaCl (mM)	Root							
		Sodium	%	Potassium	%	Calcium	%	Magnesium	%
Absolute control	0	129.00	100	13.26	100	6.45	100	8.82	100
Reference	40	149.50*	115.89	10.22*	77.07	6.75	104.65	8.10	91.84
	80	140.00	108.53	13.07	98.57	5.70	88.37	6.84	77.55
	120	154.00*	119.38	14.75	111.24	6.30	97.67	7.83	88.78
	160	129.00	100.00	14.50	109.35	5.85	90.70	4.59**	52.04
KNO_3 (3mM)	0	112.50	87.21	13.08	98.64	8.55	132.56	7.38	83.67
	40	100.00*	77.52	11.40	85.97	8.25	127.91	6.03*	68.37
	80	107.50*	83.33	11.03*	83.18	7.50	116.28	4.59**	52.04
	120	136.50	105.81	13.01	98.11	8.10	125.58	5.76*	65.31
	160	115.00	89.15	16.80**	126.70	7.80	120.93	5.04**	57.14
LSD at 5%		19.5		2.16		2.21		2.04	
LSD at 1%		29.74		3.29		3.36		3.11	

	NaCl (mM)	Shoot							
		Sodium	%	Potassium	%	Calcium	%	Magnesium	%
Absolute control	0	57.00	100	15.00	100	7.20	100	2.61	100
Reference	40	57.50	100.88	13.20	88.00	6.90	95.83	2.61	100.00
	80	98.50**	172.81	11.83*	78.87	8.10	112.50	3.06	117.24
	120	109.50**	192.11	13.94	92.93	8.70	120.83	2.07	79.31
	160	94.00**	164.91	14.13	94.20	6.00	83.33	2.07	79.31
KNO_3 (3mM)	0	67.00	117.54	14.87	99.13	7.80	108.33	1.98	75.86
	40	51.50	90.35	11.53*	76.87	7.20	100.00	2.43	93.10
	80	74.00	129.82	8.80**	58.67	5.85	81.25	1.89	72.41
	120	79.00*	138.60	7.81**	52.07	6.45	89.58	1.98	75.86
	160	80.50*	141.23	5.82**	38.80	6.45	89.58	2.07	79.31
LSD at 5%		18.69		2.68		2.32		0.40	
LSD at 1%		28.52		4.08		3.53		0.49	

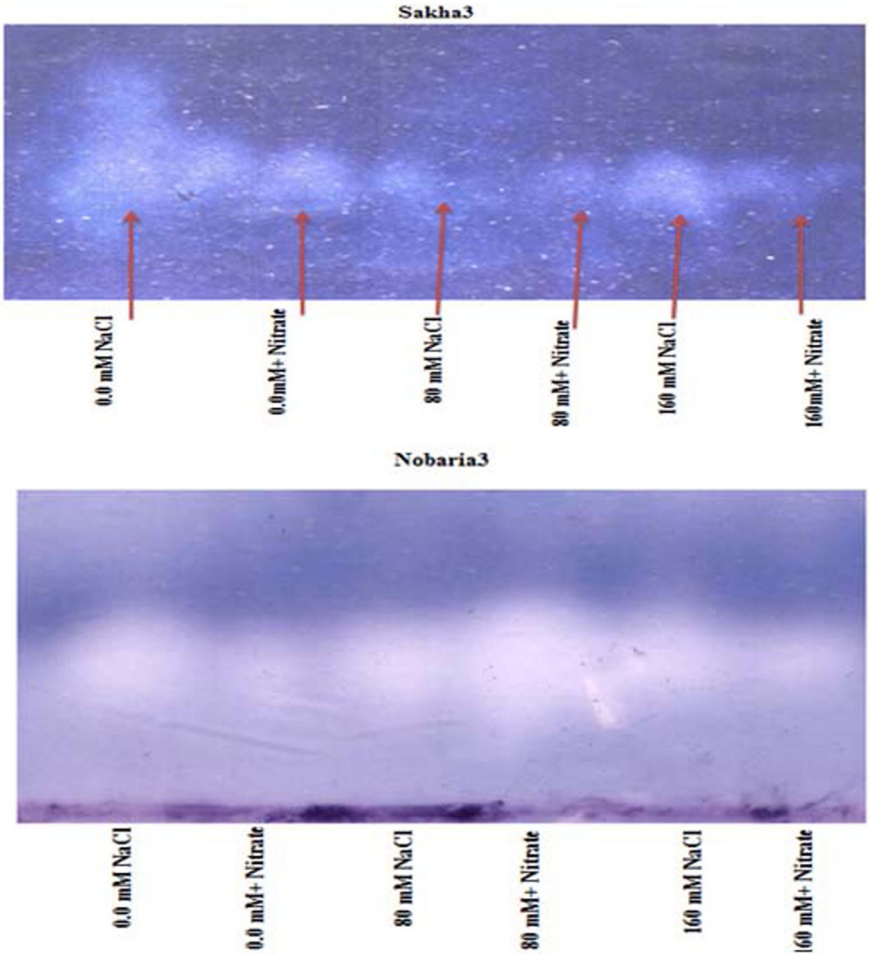

Fig. 2. Changes in β-amylase activity in two *Vicia faba* cv. (Sakha 3 and Nobaria 3) as presoaked in KNO_3 and treated with different concentrations of NaCl. (A) Changes in β-amylase in cv. Sakha 3 and (B) Changes in β-amylase in cv. Nobaria 3. Lane 1 (Control), Lane 2 (Control + KNO_3), Lane 3 (80mM NaCl), Lane 4 (80mM NaCl + KNO_3), Lane 5 (160mM NaCl) and Lane 6 (160mM NaCl + KNO_3).

Exposure of Sakha 3 and Nobaria 3 cultivars to different concentrations of NaCl salinity and others presoaked in KNO_3 (3mM) produced marked changes in their protein pattern (Tables 4, 5 and Fig. 3, 4).

In Sakha 3 cultivar, the results reveled that, the band numbers from (7:10) were detected under different concentration of NaCl, while the protein pattern as a result of seed presoaking in potassium nitrate displayed (12-13 protein bands). Four protein bands at MW (26.69, 35.69, 50.177 and 67.898KDa) were common bands for different concentration of NaCl and presoaked with potassium nitrate. On the other hand, there were de nove synthesis of three bands of MW (106.04, 127.429 and 134.16KDa) not detected in control. Under mid and higher salinity and presoaked with potassium nitrate there were de nove synthesis of three bands with MW (43.47, 78.36 and 92.91KDa) detected. Some bands were considered as markers to special treatments, for example (107.23KDa) which considered as a marker for combined effect of 80mM NaCl and potassium nitrate. In this respect, 2 protein bands at MW (17.389 and 23.4KDa) were induced under combined treatments with 160mM NaCl and potassium nitrate, while, the 91.67KDa protein was newly expressed under combined treatment of control (0.0mM NaCl) and potassium nitrate only. Under high salt conc. combined with potassium nitrate there were a specific band appeared at molecular weight 20.34KDa. While 44.063KDa protein band was also appeared under control and presoaked with potassium nitrate (Table 4 and Fig. 3).

TABLE 4. Electrophoretic profile of protein extracted from the leaves of faba bean (*Vicia faba* cv. Sakha 3) treated with (0.0, 80 and 160mM NaCl) or soaked with KNO_3 (3mM).

MW	c	c+n	80	8o+n	160	160+n	Frequency	Polymorphism
134.168	-	+	+	+	+	+	0.833	Polymorphic
127.429	-	+	+	+	+	+	0.833	Polymorphic
107.238	-	-	-	+	-	-	0.167	Unique
106.044	-	+	+	-	+	+	0.667	Polymorphic
92.913	-	-	+	+	+	+	0.667	Polymorphic
91.673	-	+	-	-	-	-	0.167	Unique
78.367	-	-	+	+	+	+	0.667	Polymorphic
67.898	+	+	+	+	+	+	1.000	Monomorphic
50.177	+	+	+	+	+	+	1.000	Monomorphic
44.063	+	+	-	-	-	-	0.333	Polymorphic
43.474	-	-	+	+	+	+	0.667	Polymorphic
35.695	+	+	+	+	-	+	0.833	Polymorphic
26.796	+	+	+	+	+	+	1.000	Monomorphic
24.444	+	+	+	+	-	-	0.667	Polymorphic
23.478	-	-	-	-	-	+	0.167	Unique
20.617	+	+	+	+	-	-	0.667	Polymorphic
20.342	-	-	-	-	+	+	0.333	Polymorphic
17.823	-	+	+	+	-	-	0.500	Polymorphic
17.389	-	-	-	-	-	+	0.167	Unique
Total	7	12	13	13	10	13		

TABLE 5. Electrophoretic profile of protein extracted from the leaves of faba bean (*Vicia faba* cv. Nobaria 3) treated with (0.0, 80 and 160mM NaCl) or presoaked with KNO_3 (3mM).

MW	c	c+n	80	80+n	160	160+n	Frequency	Polymorphism
131.687	-	-	-	-	-	+	0.167	Unique
130.106	-	+	+	+	+	-	0.667	Polymorphic
128.543	+	-	-	-	-	-	0.167	Unique
120.425	+	-	-	-	+	+	0.500	Polymorphic
118.979	-	+	+	+	-	-	0.500	Polymorphic
105.951	-	-	-	-	+	+	0.333	Polymorphic
104.679	+	+	+	+	-	-	0.667	Polymorphic
90.991	-	-	+	+	+	+	0.667	Polymorphic
89.899	+	+	-	-	-	-	0.333	Polymorphic
76.094	-	-	-	-	-	+	0.167	Unique
74.999	+	+	+	+	+	-	0.833	Polymorphic
58.901	-	-	-	+	+	+	0.500	Polymorphic
58.194	-	-	+	-	-	-	0.167	Unique
57.357	+	+	-	-	-	-	0.333	Polymorphic
41.694	+	+	+	+	+	+	1.000	Monomorphic
37.129	+	+	+	+	+	+	1.000	Monomorphic
34.868	-	-	-	-	+	+	0.333	Polymorphic
34.449	-	-	-	+	-	-	0.167	Unique
29.872	-	+	+	+	+	+	0.833	Polymorphic
27.318	+	+	+	+	+	+	1.000	Monomorphic
23.746	-	-	-	-	+	+	0.333	Polymorphic
21.715	-	+	+	+	+	+	0.833	Polymorphic
19.384	-	+	+	+	+	+	0.833	Polymorphic
17.942	-	-	-	-	-	+	0.167	Unique
15.785	+	+	+	+	+	+	1.000	Monomorphic
Total	10	13	13	14	15	16		

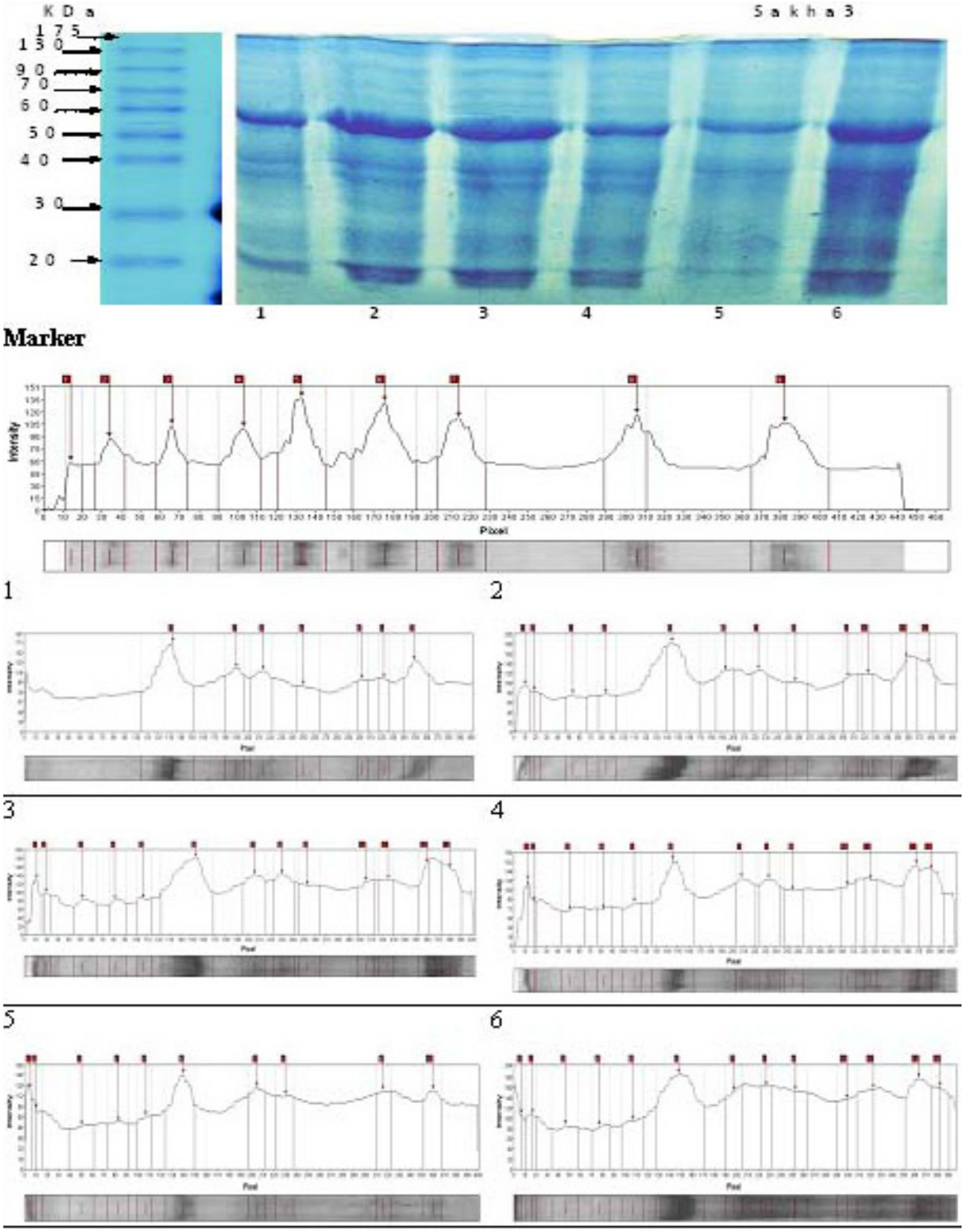

Fig. 3. Polyacrylamide gel electrophoresis of protein profile separated from leaves of *Vicia faba* cv. Sakha 3 treated with (0.0, 80 and 160mM NaCl) or presoaked with KNO_3 (3mM). Lane 1 (Control), Lane 2 (Control+KNO_3), Lane 3 (80mM), Lane 4 (80+KNO_3), Lane 5 (160mM) and Lane 6 (160+ KNO_3).

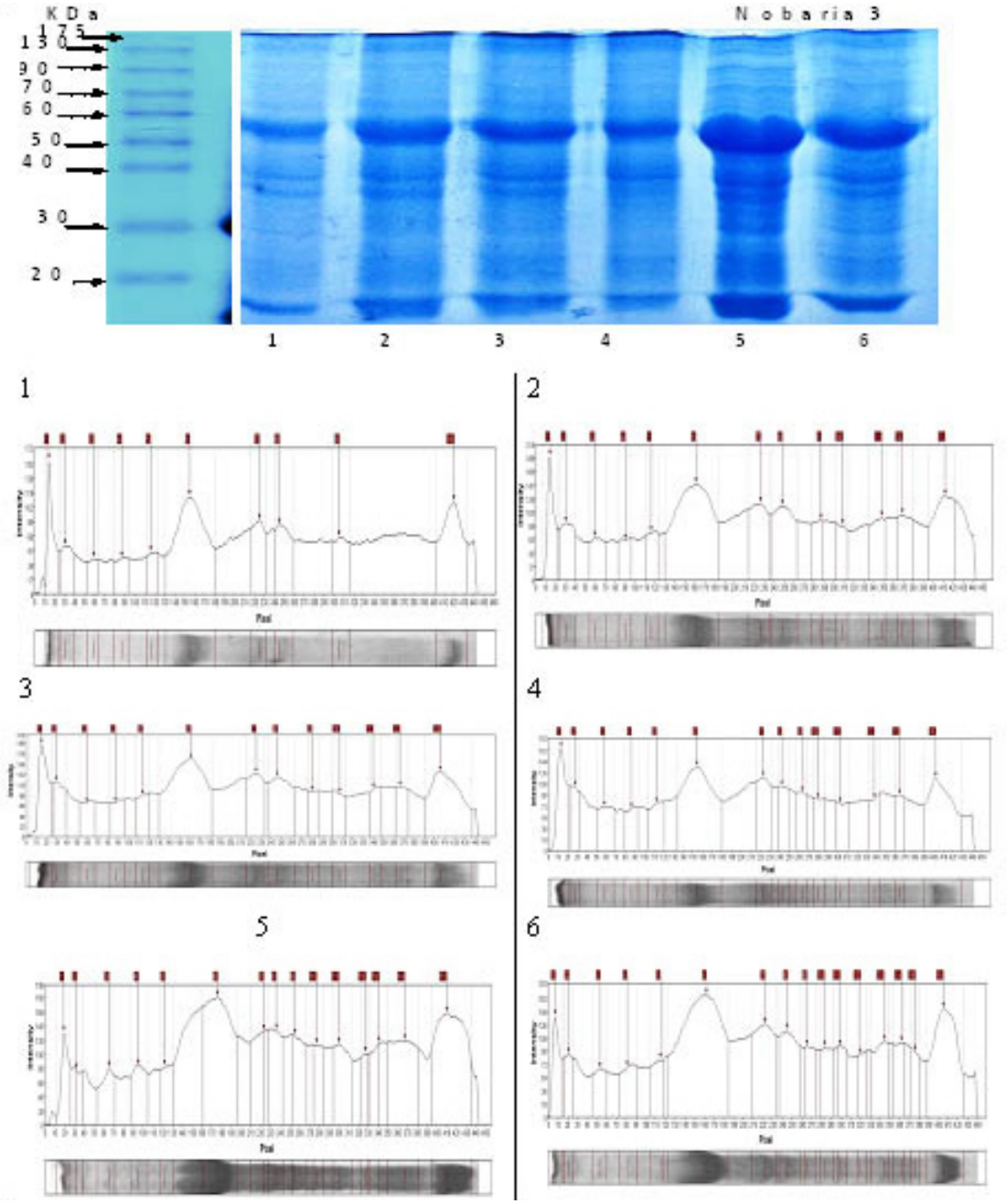

Fig. 4. Polyacrylamide gel electrophoresis of protein profile separated from leaves of *Vicia faba* cv. Nobaria 3 treated with (0.0, 80 and 160mM NaCl) or presoaked with KNO_3 (3mM). Lane 1 (Control), Lane 2 (Control+KNO_3), Lane 3 (80mM), Lane 4 (80+KNO_3), Lane 5 (160mM) and Lane 6 (160+ KNO_3).

In Nobaria 3 cultivar, the results reveled also that, the number of bands from (10:15) were detected under different concentrations of NaCl. Either the combined effect of both salt concentrations or salinity only induced the synthesis of new proteins and suppressed the expression of others. The results showed marked changes in protein patterns as a result of presoaking in potassium nitrate which displayed (13-16 protein bands). Four protein bands at MW (15.78, 27.31, 37.129 and 41.69KDa) are common bands under different concentration of NaCl and presoaking in potassium nitrate. As mentioned above, the two bands (58.9 and 90.99KDa) were commonly induced under mid and higher salinity and soaked with potassium nitrate. While, the (58.19KDa) protein bands was newly expressed under 80mM NaCl alone and also (34.44KDa) appeared with the

combined treatment of presoaking in 80mM NaCl + KNO_3. In this respect bands of 17.94, 76.09 and 131.6KDa are specific protein bands for combined effect of 160mM NaCl and KNO_3. Under high concentration of salinity and combined with KNO_3 there were a specific bands detected at molecular weight (23.74, 34.86 and 105.95KDa). While (57.35 and 89.89KDa) protein bands were detected either with control or combined with potassium nitrate. As mentioned above (128.54KDa) was unique band under control. In addition to these newly synthesized proteins, there were de nove synthesis of three bands of MW (19.38, 21.7 and 29.87KDa) not detected under control. Two bands at (74.99 and 130. 118.97KDa) protein bands were suppressed under 160mM NaCl only and 160mM NaCl +KNO_3) (Table 5 and Fig. 4).

Discussion

The final germination percentage of two broad bean cultivars *Vicia faba* cv. (Sakha 3 and Nobaria 3) were decreased by increasing salinity in the rooting medium. Salt and osmotic stresses as well as increased availability of toxic ions are responsible for either inhibition or delayed seed germination and seedling establishment (Farooq et al., 2015 and Hussain et al., 2016).

Seed Presoaking in KNO_3 resulted in a remarkable increase in final germination percentage especially in cultivar Sakha 3. Seed presoaking increased seed vigor, improved each of germination, seedling growth, reduction of seedling germination time and stand establishment which resulted in higher grain yield (Basra et al., 2005; Ghobadi et al., 2012; Bhati-Kushwaha et al., 2013 and Abou-Zeid & Moustafa, 2014). Aquaporins which responsible for water entry during imbibitions might reduced their expression in the presence of salt (Boursiac et al., 2005) also affects mobilization of starch by reducing amylase activity (Voigt et al., 2009) and lipid storage breakdown through a reduction in the activity of glyoxysomal cycle enzymes (Ben Miled-Daoud & Cherif, 1992). Seed presoaking in KNO_3 might be responsible for reactivation of aquaporins and activate amylase activity which responsible for embryo development.

Proline accumulation in response to abiotic stresses has been mentioned by a number of authors as an adaptive mechanism concerned with stress tolerance, and it is generally reported that proline is acting as a compatible solute in osmotic regulation (Gama etal., 2007 and Hameda, 2011). The two broad bean cultivars (Sakha 3 and Nobaria 3) accumulated proline differentially in both organs shoots and roots with elevating salinity in the culture medium. The Arial parts of two broad bean cultivars accumulated proline more than their roots even under presoaked conditions. The accumulation of proline was caused either by both the activation of its biosynthesis and inactivation and/or its degradation (Kapoor & Srivateva, 2010; Hameda, 2011 and Sadia et al., 2015). The compatible solutes may help to maintain the relatively high water content necessary for growth and cellular function which was concomitant with the present results.

Considerable mineral variations in the tissues of the experimental plants were induced by salt stress. The changes in the contents of mineral composition could be a mechanism for osmotic adjustment (Flowers & Yeo, 1986).

The contents of Na^+ in both shoots and roots of the two broad bean cultivars generally increased by increasing salinity in the soil. In this respect some authors reported that the concentrations of Na^+ and Cl^- have been shown to increase in chickpea on exposure to salt with tissue Cl^- concentrations generally exceeding those of Na^+ (Flowers et al., 2010). In Salt stressed sunflower, Ashraf, (2004), Munns (2002) and Rahnama et al. (2011) found that, salinity accumulated Na^+ and Cl^- coupled with decreasing N, P, K^+, Ca^{+2} and Mg^{+2} and also reported that plants growing under saline conditions suffered from ionic imbalance, nutrient deficiency, and specific ion toxicity. Seed presoaking in KNO_3 resulted in major cases in an increase in Na^+ contents in shoots and decreased it in roots in both broad bean cultivars. In this manner, Jabeen & Ahmed (2011) working with sunflower and safflower leaves under salinity, found that foliar application of KNO_3 alleviate the toxicity of Na^+ by decreasing the chances of its accumulation in plant parts.

The potassium contents in both broad bean cultivars showed various responses even between the two plant organs (shoot and root) with increasing salinity. Tammam (2003) reported that the sodium content in both roots and shoots of *Vicia fava* plants increased with increasing salinity, whereas potassium and calcium were decreased.

Also, Mohsen et al. (2014) worked with *Vicia faba* (Misr 2) using ascorbic acid and salinity found that, potassium content decreased in both organs of the tested plants and the application of ascorbic acid slightly affected the mineral compositions of both organs (Cuin et al., 2010). The alteration in distribution and accumulation of mono and divalent cations in the different organs of salt-stressed plants may be an indication of the role of these cations in regulating the physiological activities of the plants. The confusing trend in K^+ accumulation especially in shoots and roots of cultivars (Sakha 3 and Nobaria 3), respectively due to salt stress, means that this element participate in osmotic adjustment phenomenon.

Calcium contents in the main organs of stressed plants showed variable responses, a marked increase in calcium content in shoots of both broad bean cultivars could be an important marker for the degree of salt tolerance in plants; calcium ions are required in maintaining membrane integrity and transport of other ions (Salisbury & Ross, 1992). Hamada & El–Enany (1994) using *Vicia fava* and pea plants found that, in *Vicia fava*, calcium concentration in shoots, and potassium and calcium contents of roots increased with increasing salinity, while in pea plants, the contents of potassium and calcium were almost unaffected by salinity. Salinity induced an increase in the content of these ions in pea roots (Jaleel et al., 2007 and Shoresh et al., 2011) .

Magnesium contents in both tested cultivars showed variable alterations. The reduction in Ca^{+2} and Mg^{2+} uptake under salt stress conditions might be due to the suppressive effect of Na^+ and K^+ on these cations or due to reduced transport of Ca^{2+} and Mg^{2+} ions. In addition, salinity has an antagonistic effect on the uptake of Ca^{+2} and Mg^{+2} which caused by displacing Ca^{+2} in membranes of wheat root cells (Asik et al., 2009). Salinity priming also increased the ability to absorb K^+, Mg^{2+} and Ca^{2+} in cumin (*Cuminum cyminum* L) and reduced Na^+ uptake (Shoor et al., 2014). In cultivar Sakha 3, seed presoaking in KNO_3 enhanced the accumulation of Mg^{+2} in shoots and at higher levels of salinity in roots.

β-amylase in both broad bean cultivars showed various responses in band intensity either with NaCl or presoaked with potassium nitrate. β-amylase expressed during stress has been shown to play a major role in transitory breakdown of starch (Scheidig et al., 2002 and Bať ková et al., 2008). Presoaking improved the quality of aged seeds by increasing enzymes activity such as amylases and antioxidant enzymes (Seiadat et al., 2012 and Ansari et al., 2013). Naik & Devaraj (2016) using 10-day old seedlings of Niger (*Guizotia abyssinica Cass*.) under 500mM NaCl in combination with $CaCl_2$ for evaluating abiotic stress response found that both enzymes β-amylase and acid phosphatase were exhibited a moderate increase relative to controls. Mobilization of starch during stress appears to be a common mechanism to protect cellular integrity. β-amylases have been shown to contribute to turgidity and osmotic adjustment (Kotting et al., 2010). Similar results have been reported in cucumber under water stress (Todaka et al., 2000) and *Triticum aestivum* under salt stress (Chiraz et al., 2013).

Exposure of two broad bean cultivars (Sakha 3 and Nobaria 3) to different NaCl salinity levels and others soaked in potassium nitrate produced marked changes in their protein pattern, three types of alterations were observed:

I- The Synthesis of certain proteins had been increased significantly.

II- Specific synthesis of certain other proteins were markedly observed in both treatments.

III- Synthesis of a set specific proteins was induced de novo in plant soaked in potassium nitrate in both cultivars.

In the same context, salinity induced a considerable variation in the protein pattern in *Triticum aestivum* L, these variability have been reflected in the novel expression of some polypeptides, the absence of other and the over expression of a third class of polypeptides (Barakat, 2003). In *Nicotiana tabacum* (tobacco) cells growing in salt stress conditions (Cokuysal et al., 2006) showed a distinct changes in their pattern of accumulation of total RNA and poly (A)+ RNA for the synthesis of salt stressed specific proteins. Tammam (2003) found that, salt treatment of broad bean seedling resulted in the disappearance of five polypeptides, while the peptides with low molecular mass increased in their intensity on the gel. Also Khedr et al. (2003) on *Pancratium maritimim* L, Bahrman et al. (2003) on wheat, Chourey et al. (2003) on rice, Muayed et al. (2012) on *Citrus sinensis* L and Abd El-Baki & Mosta

(2014) on broad bean treated with *Trichoderma harizianum*, they concluded that, osmotic stresses were capable to induce several major stress proteins. They also declared that the enhancement of these proteins correlated with stress tolerance in the various plant species. They also suggested a protective role of these proteins under desired osmotic stress. Therefore, it can be concluded that the new proteins which appeared in plants grown under salinity stress alone or combined with KNO_3 and disappeared in untreated plants (control), might play a vital role in induction of a special system enabling plants to resist stress and increase their ability to survive under these extreme conditions.

Conclusion

From the previous discussion it can be concluded that, seed presoaking alleviate the effect of salt stress on two broad bean cultivars to some extent as far as the parameters used but still need further invistigaqtions.

References

Abd El-Baki, G.K. and Mostafa, D. (2014) The potentiality of *Trichoderma harzianum* in alleviation the adverse effects of salinity in faba bean plants. *Acta Biol. Hung.* **65**(4), 451–468.

Abou-Zeid, H.M. and Moustafa, Y. (2014) Physiological and cytogenetic response of wheat and barley to silver nanopriming treatment. *Int. J. Appl. Biol. Pharm. Technol.* **5**, 265–278.

Akramghaderi, F., Farzanehs and Zeinali, A. (2002) Salinity on germination and seedling growth of four cultivars of subterranean clover. *J. Res. Dev*. **57**, 98–103.

Ansari, O., Sharif-Zadeh, F., Moradi, A., Azadi, M.S. and Younesi, E. (2013) Heat shock treatment can improve some seed germination indexes and enzyme activity in primed seeds with gibberellin of mountain rye (*Secale montanum*) under accelerated aging conditions. *Cercet. Agron. Moldova*, **156**(4) s, 21–30.

Ashraf, M. (2004) Some important physiological selection criteria for salt tolerance in plants. *Flora,* **199**, 361–376.

Ashraf, M. and Rauf, H. (2001) Inducing salt tolerate in maize (*Zea mays* L) through seed priming with chloride salts: Ggrowth and ion transport at early growth stages. *Acta. Physiol. Plant*, **23**, 407–414.

Asik, B.B., Turan, M.A., Celik, H. and Katkat, A.V. (2009) Effects of humic substances on plant growth and mineral nutrients uptake of wheat (*Triticum durum* cv. *Salihli*) under conditions of salinity. *Asian J. Crop Sci*. **1**, 87–95.

Bahrman, N., Le Gouis, J., Negroui, L., Amilhat, L., Leory, P., Laine, A. and Jaminon, O. (2003) Differential protein expression assessed by electrophoresis for two wheat varieties grown at four nitrogen levels. *Proteomics,* **4**, 709–719.

Barakat, H. (2003.) Interactive effects of salinity and certain vitamins on gene expression and cell division. *Agric. Biol.* **5**(3), 219–225.

Basra, S.M.A., Afzal, I., Rashid, R.A. and Hameed, A. (2005) Inducing salt tolerance in soybean by seed vigor enhancement techniques. *J. Biotech. Biochem.* **1**, 173–179.

Basra, S.M.A., AfzalI, Anwar, S., Anwar-ul-haq, M.,Shafq, M.and Majeed, K. (2006) Alleviation of salinity stress by seed invigoration techniques in wheat (*Triticum aestivum* L). *Seed Technol.* **28**, 36–46.

Bates, L.W., Waldern, R.P. and Teare, I.D. (1973) Rapid determination of free proline for water stress studies. *Plant and Soil,* **39**, 205–207.

Baťková, P., Pospíšilová, J. and Synková, H. (2008) Production of reactive oxygen species and development of antioxidative systems during *in vitro* growth and *ex vitro* transfer. *Biol. Plant.* **52**, 413–422.

Ben Miled-Daoud, D. and Cherif, A. (1992) Effect du NaCl sur l'utilisation des lipides et les activités enzymatiques glyoxysomales au cours de la germination de deux espèces de Medicago. *Can. J. Bot.* **70**, 876–883.

Bhati-Kushwaha, H., Kaur, A. and Malik, C.P. (2013) The synthesis and role of biogenic nanoparticles in overcoming chilling stresses. *Ind. J. Plant Sci.* 2, 54–62.

Boursiac, Y., Chen, S., Luu, D.T., Sorieul, M., Dries, N. and Maurel, C. (2005) Early effects of salinity on water transport in Arabidopsis roots: Molecular

and cellular features of aquaporin expression. *Plant Physiol.* **139**, 790–805.

Chaparzadeh, N., D'amico, M.L., Khavari-Nejad, R.A., Izzo, R. and Navari-Izzo, F. (2004) Antioxidative responses of *Calandula officinalis* under salinity conditions. *Plant Physiol. Biochem*. **42**, 695–701.

Chiraz, C.H., Afef, H.N., Donia, B. and Houda, G. (2013) Variations in α-, β-amylase and α-glycosidase activities in two genotypes of wheat under NaCl salinity stress. *Afri. J. Agric. Research,* **8**, 2038–2043.

Chourey, K., Ramani, S. and Apte, S.K. (2003) Accumulation of LEA proteins in salt (NaCl) stressed young seedlings of rice (*Oryza sativa* L) cultivar Bura Rata and their degradation during recovery from salinity stress. *J. Plant Physio.* **160**, 1165–1174.

Chunthaburee, S., Sanitchon, J., Pattanagul, W. and Theerakulpisut, P. (2014) Alleviation of salt stress in seedlings of black glutinous rice by seed priming with spermidine and gibberellic acid. *Not. Bot. Horti. Agrobo*. **42**(2), 405–413.

Cokuysal, B., Cakici, H. and Erbas, E. (2006) Effects of salt stress on total RNA and poly (A) + RNA contents in tobacco suspension cultured cells at the different time course. *Biol. Sci.* **9**(1), 22–25.

Cuin, T.A., Parsons, D. and Shabala, S. (2010) Wheat cultivars can be screened for NaCl salinity tolerance by measuring leaf chlorophyll content and shoot sap potassium. *Func. Plant Biol.* **37**, 656–664.

Demir Kaya, M., Okcu, G., Atak, M., Cikili, Y. and Kolsarici, O. (2006) Seed treatment to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L). *J. Eur. Agron*. **24**, 291–295.

Farooq, M., Hussain, M., Abdul Wakeel and Siddique, K.H.M. (2015) Salt stress in maize: Effects, resistance mechanisms, and management. *Agron. Sustain. Dev.* **35**, 461–481.

Flowers, T.J. and Yeo, A.R. (1986) Ion relations of plants under drought and salinity. *Aust. J. Plant Physio.* **13**, 75–91.

Flowers, T.J., Pooran, M.G., Laxmipathi, G.C., Krishnamurthy, L., Srinivasan Samineni, Kadambot, H.M. Siddique, N.C., Turner, V.V., Rajeev, K.V. and Colmer, T.D. (2010) Salt sensitivity in chickpea. *Plant, Cell Environ.* **33**, 490–509.

Gama, P.B., Inanaga, S., Tanaka, K. and Nakazawa, R. (2007) Physiological responses of Common bean (*Phaseolus vulgaris*) seedling to salinity stress. *Afric. J. Biotech.* **6**(2), 79–88.

Ghobadi, M., Shafiei-Abnavi, M., Jalali-Honarmand, S., Ghobadi, M.E. and Mohammadi, G.R. (2012) Does KNO_3 and hydropriming improve wheat (*Triticum aestivum* L) seeds germination and seedlings growth? *Annals of Biol. Res.* **3**, 3156–3160.

Garg, B.K. and Gupta, I.C. (1997) Plant relations to salinity In: "*Saline Wastelands Environment and Plant Growth*", pp. 79–121. Scientific Publishers, Jodhpur.

Habibi, A. and Abdoli, M., (2013) Influence of salicylic acid pre-treatment on germination, vigor and growth parameters of garden cress (*Lepidium sativum*) seedlings under water potential loss at salinity stress. *Inter. Res. J. Applied Basic Sci.* **4**(6), 1393–1399.

Hamada, A.M. and El-Enany, A.E. (1994) Effect of NaCl salinity on growth, pigment and mineral element contents, and gas exchange of broad bean and pea plants. *Biol. Plant,* **36**(1), 75–81.

Hameda, E.S.A.S. (2011) Influence of salinity (NaCl and Na_2SO_4) treatments on growth development of broad bean (*Vicia faba* L) plant. A*meri. Euras. J. Agric. Envir. Sci.* **10**(4), 600–610.

Hus, J.L. and Sung, J.M. (1997) Antioxidant role of glutatnione associated with accelerated agina and hydration of triploid watermelon seeds. *Physiol. Plant,* **100**, 967–974.

Hussain, M.I., Lyra, DA., Farooq, M., Nikoloudakis, N. and Khalid, N. (2016) Salt and drought stresses in safflower: A review. *Agron. Sustain. Dev.* **36**(1), 4–13.

Jabeen, N. and Ahmad, R. (2011) Foliar application of potassium nitrate affects the growth and nitrate reductase activity in sunflower and safflower leaves under salinity. *Not. Bot. Horti. Agrobo.* **39**(2),172–178.

Jaleel, C.A., Gopi, R., Manivannan, P. and Panneerselvam, R. (2007) Response of antioxidant defense system of *Catharanthus roseus* (L) G. Don. to paclobutrazol treatment under salinity. *Acta Physiol. Plantarum.* **29**, 205–209.

Kant, S. and Kafkafi, U. (2002) Potassium and abiotic stresses in plants. In: "*Potassium for Sustainable Crop Production*", Pasricha, N.S., Bansal, S.K., (Ed.), pp. 233–251. Potash Institute of India; Gurgaon, India.

Kapoor, K. and Srivastava, A. (2010) Assesment of salinity tolerance of *Vigana mungo* var. Pu—19 using *ex-vitro* and *in vitro*. Metho. *Asian J. Biotech.* **2**(2), 73–85.

Khedr, A.A., Abbas, M.A., Abdel Wahid, A.A., Quick, W.P. and Abogadallah, G.M. (2003) Proline induces the expression of salt-stress-responsive proteins and may improve the adaptation of *Pancratium maritimum* L to salt stress. *J. Exp. Bot.* **54**, 2553–2562.

Kotting, O., Kossmann, J., Zeeman, S.C. and Lloyd, J.R. (2010) Regulation of starch metabolism: The age of enlightenment. *Current Opinion in Plant Biology*, **13**, 321–329.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, **227**(5259), 680–685.

Maftoun, M. and Sepaskhah, A.R. (1978) Effect of temperature and osmotic potential on germination of sunflower and safflower and on hormone treatment sunflower seeds. *Can J. Plant. Sci.* **58**, 295–301.

McDonald, M.B. (1999) Seed deterioration: Physiology, repair and assessment. *Seed Sci. Technol.* **27**, 177–237.

Moeinrad, H. (2008) The relationship between some physiological traits and salt tolerance in pistachio genotypes. *Desert,* **13**, 129–136.

Mohsen, A.A., Ebrahim, M.K.H. and Ghoraba, W.F.S. (2014) Response of salt stressed *Vicia faba* plant to application of ascorbic acid on the growth and some metabolites. *Iranian J. Plant Physiol.* **4**(2), 957–976.

Muayed, F., Abbas, A., Jasim, M. and Al-Taha, H.A. (2012) Effect of exogenous proline on protein pattern changes in *Citrus sinensis* (L) Osbeck under *in vitro* salt stress. *Advances in Agriculture & Botanics. Inte. J. Bioflux Soci.* **4**, 36–41.

Munns, R. (2002) Comparative physiology of salt and water stress. *Plant Cell Environ.* **25**, 239–250.

Munns, R. (2005) Genes and salt tolerance: Bringing them together. *New Phytol*. **167**, 645–663.

Naik, H.N.K. and Devaraj, V.R. (2016) Effect of salinity stress on antioxidant defense system of Niger (*Guizotia abyssinica* Cass.). *American J. Plant Sci.* **7**, 980–990.

Rahmati, H. (2012) Effect of plant density and nitrogen rates on morphological characteristics grain maize. *J. of Basic and Applied Scien. Research,* **2**(5), 4680–4683.

Rahnama, A., Poustini, K., Tavakkol-Afshari, R., Ahmadi, A. and Alizadeh, H. (2011) Growth properties and Ion distribution in different tissues of bread wheat genotypes (*Triticum aestivum* L) differing in salt tolerance. *J. Agron. Crop Sci.* **197**, 21–30.

Sadia, C., Said, H., Belgat, H., Bodour, L., Gharoucha, H. and Baka, M. (2015) Interactive effects of potassium and sodium on the growth and nodulating capacity of pea (*Pisum sativum* L) Var: Merveile de Kelvedon under salt stress. *Agric. Biol. J. North Amer.* **6**(1), 34–46.

Salisbury, F.B. and Ross, C.W. (1992) "*Plant Physiology*". 4th ed. by Wads Worth Publishing Co. Inc. California.

Scheidig, A., Fro¨hlich, A., Schulze, S. and Lloyd, J.R. and Kossmann, J. (2002) Down regulation of a chloroplast- targeted beta-amylase leads to starch-excess phenotype in leaves. *Plant J.* **30**, 581–591.

Schwarzenbach, G. and Biedermann, W. (1948) Komplexone, X. Erdalkalikomplexe vono, 0,6-Dioxyazofarbstoffen. *Helv. Chim. Acta.* **31**, 678–687.

Seiadat, S.A., Moosavi, A. and Sharafizadeh, M. (2012) Effect of seed priming on antioxidant activity and germination characteristics of maize seeds under different aging treatments. *Res. J. Seed Scienc.* **5**(2), 51–62.

Shoor, M., Maryam, A., Javad, R. and Mojtaba, V. (2014) The effect of salinity priming on germination andgrowth stage of cumin (*Cuminum cyminum* L). *Res. J. Agric.Environ. Manag.* **3**(7), 340–352.

Shoresh, M., Spivak, M. and Bernstein, N. (2011) Involvement of calcium mediated effects on ROS metabolism in the regulation of growth improvement under salinity. *Free Radical Biol. Medicine,* **51**, 1221–1234.

Shuster, L. and Gifford, R.H. (1962) Assay of α/β amylases. *Arch Biochem Biophys.* **194**, 534–540.

Simiroff, N. and Cumbes, Q.J. (1989) Hydroxyl radical scavenging activity of compatible solutes. *Phytoche*. **28**, 1057–1060.

Sorkhi, F. and Fateh, M. (2014) Effect of nitrogen fertilizer on yield component of maize. *Inter. J. Biosci.* **5**(6), 16–20.

Steel, R.G.D. and Torrie, J.H. (1960) "*Principles and Procedures of Statistics, with Special Reference to the Biological Sciences*". McGraw-Hill Book Company, New York.

Tammam, A. A. (2003) Response of *Vicia fava* plants to the interactive effect of sodium chloride salinity and salicylic acid treatment. *Acta-Agron. Hungarica,* **51**(3), 239–248.

Todaka, D., Matsushima, H. and Morohashi, Y. (2000) Water stress enhances β-amylase activity in cucumber cotyledon. *J. Exp. Bot.* **51**, 739–745.

Voigt, E., Almeida, T., Chagas, R., Ponte, L.F., Viegas, R.A. and Silveira, J.A. (2009) Source-sink regulation of cotyledonary reserve mobilization during cashew (*Anacardium occidentale*) seedling establishment under NaCl salinity. *J. Plant Physiol.* **166**, 80–89.

Williams, V. and Twine, S. (1960) Flame photometric method for sodium, potassium and calcium. In: "*Modern Methods of Plant Analysis*", Peach, K. and Tracey, M.V. (Ed.), pp. 3–5, Vol. V. Springer-Verlag, Berlin.

(Received 4/ 4 /2018;
accepted 23/ 5 /2018)

تاثيرالنقع بنترات البوتاسيوم على الأنبات و البرولين و التفريد الكهربى للبروتين و بيتا اميلز والعناصر المعدنية لصنفين من الفول البلدي تحت ثاتير الملوحة

جابر خلاف[1]، محمد عبده خضر شداد[2]، دعاء مصطفي محمود[1] و الشيماء رأفت[1]
[1] قسم النبات والميكروبيولوجى– كلية العلوم – جامعة المنيا – المنيا – مصر و [2] قسم النبات والميكروبيولوجى– كلية العلوم – جامعة أسيوط – أسيوط – مصر.

أجريت هذه الدراسة لتقييم التأثيرات المختلفة من الملوحة (0، 40، 80، 120، 160مللي مول) من كلوريد الصوديوم على الإنبات و النمو وبعض الأنشطه الفسيولوجيه لصنفين من أصناف الفول البلدي (صنف سخا 3، صنف نوباريه 3) بعد نقعهما في الماء كمرجع أو في نترات البوتاسيوم تركيز 3 مللي مول لمدة 4 ساعات و قد أسفرت الدراسة عن النتائج التالية.

1- عند معالجة كلا" الصنفين بتركيزات مختلفة من كلوريد الصوديوم تناقصت النسبة المئوية النهائية للإنبات فى كلا الصنفين وذلك بعد خمسة أيام، وقد أظهرت التركيزات العالية من الملوحة (120، 160مللى مول من كلوريد الصوديوم) نقصا" ملحوظا" فى النسبة المئوية النهائية للإنبات فى الصنف نوبارية 3. عند نقع البذور فى 3مللى مول من نترات البوتاسيوم أدى ذلك لتحسن ملحوظ فى النسب المئوية النهائية للإنبات خصوصا" لصنف سخا 3 مقارنة بالكنترول المطلق.

2- أبدى كلا الصنفين تراكما" واضحا" للبرولين في المجموع الخضري والجذري بزيادة الملوحة في الوسط وعند معالجة النباتات بنترات البوتاسيوم فإن تركيز البرولين إزاد إلى ثلاثة أضعافه مقارنة بالكنترول.

3- اما بالنسبه تراكم العناصر في كلا الصنفين قد اظهر إستجابات مختلفه بين المجموع الخضري والجذري حتى في نفس الصنف مع زيادة الملوحه. وقد اسفر النقع في نترات البوتاسيوم عن مزيد من النقص في نسبة البوتاسيوم إلى الصوديوم في كلا من المجموع الخضري والجذري لصنفين مقارنة بالكنترول المرجعي.

٤- عند تعرض كلا الصنفين لمعالجات مختلفه من كلوريد الصوديوم أو نقعهما في نترات البوتاسيوم 3مللى مول أظهر ذلك تغييرا" واضحا" في بيتا اميلز و التفريد الكهربي للبروتين. وقد تم ملاحظه بناء نوعي لبروتينات بعينها عند المعالجة بكلا" من (كلوريد الصوديوم ، نترات البوتاسيوم) ولكن كميه البروتينات المتفرده أكثر وضوحا" عند النقع في نترات البوتاسيوم وبناء بروتينات جديدة تم إستحثاثها في النباتات المنقوعه في نترات البوتاسيوم في كلا الصنفين.

ومما سبق يمكن القول بان البروتينات المستحدثه تحت تأثير الملوحه أو النقع في نترات البوتاسيوم مقارنة بتلك في الكنترول ربما تلعب دورا" هاما" في إستحساس نظام خاص يُمكن النباتات من مقاومة الملوحه وزيادة مقدرتها للتعايش تحت تلك الظروف القاسية. و من الممكن الجزم بأن نقع البذور في 3مللي مول من نترات البوتاسيوم يمكن ان يخفف لحد ما من التأثير السلبي للملوحه لكلا الصنفين. و طبقا لما تم دراسته من عوامل فإن الدور الذي تلعبه نترات البوتاسيوم لتخفيف الإجهاد الملحي معقد للغايه.