Ameliorative role of some antioxidant compounds on physiological parameters and antioxidants response of wheat (*Triticum aestivum* L.) seedlings under salinity stress.

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Abstract: Salinity is the most important stresses that reduce growth and yield of wheat plant. In order to study effect of salinity only as well as presence of antioxidants (β-carotene, glutathione, uric acid) individually or in combination of both on growth and some related physiological activities of wheat plant an experiment was conducted completely randomized design with three replications.. The decreased levels of seed germination and growth alterations induced by NaCl were alleviated by various levels of antioxidants. Application of antioxidants led to significant differences between responses of antioxidant defense system either non-enzymatic {glutathione and ascorbate} or enzymatic {catalase (CAT), ascorbate peroxidase (APX), Superoxide dismutase (SOD) and Glutathione reductase (GR)}. Protein profile of *T. aestivum* show variations in the number appearance, disappearance of bands, and variation in the protein content in each band compared to control and to the percentage of each band in the same sample and finally its molecular weight. When treated with NaCl or in combination with antioxidant the organic solutes of wheat seedlings exhibited somewhat variable responses to the salinity levels.
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1. Introduction

A wide range of environmental stresses (such as high and low temperature, drought, salinity, UV stress and pathogen infection) are potentially harmful to the plants (Van Breusegem *et al.*, 2001; Zia *et al.*, 2006; Jamil *et al.*, 2010; Osakabe *et al.*, 2011).

Salinity reduced quality and seed yield of crop plant (Francois *et al.*, 2000 and Rawson *et al.*, 2002). Bread wheat is one of the most important crop plants in the world and the main food for people of arid and semi-arid area (Jing and Chang, 2003 and Sadat Noori and Harati, 2005).

Salinity stress limits plant growth by adversely affecting various physiological and biochemical processes like photosynthesis, antioxidant phenomena and nitrogen metabolism **(**Ashraf, 2004; Misra *et al.*, 2006 and Mehr *et al.*, 2012**)**. Much of the injury to plants exposed to stress is connected with oxidative damage at the cellular level **(**Foyer and Noctor, 2003**)**. Germination percentage also significantly decreased as the level of salinity of the medium increased **(**Gulzar *et al.*, 2001 and Mauromicale and Licandro, 2002**)**.

Seed germination is a major limiting factor for establishment plants under saline conditions **(**AL-Karaki, 2001**)**.Seedlings are the most vulnerable stage in the life cycle of plants and germination determines when and where seedling growth begins **(**Lianes *et al.*, 2005**).** Seed germination is an important and critical development phase in the life cycle of plants and is highly responsive to existing environment **(**Besma and Mounir, 2010**)**, especially in saline environment **(**Ali and Abbas, 2003 and Ali *et al.*, 2009**)**. Salt stress affects germination percentage, germination rate, and seedling growth in different ways depending on plant species (Meloni *et al.*, 2008 and Ríos – Gómez *et al.*, 2010**)**.

Antioxidants seem to be involved in salt, osmotic, drought and oxidative responses in plants (Munns and Tester, 2008 and Maevskaya and Nikolaeva 2013). These days, regarding the invention of new methods and technology in physiology, biochemistry science, it seems that negative effects of environmental stresses would be decreased.

In recent years there has been an increase interest in studying the role of antioxidant in stress related process of plants (Zadeh *et al.*, 2007). Furthermore, exogenous application of antioxidant has been shown to protect against various stress conditions such as drought and salinity (Zhang li xin *et al.*, 2011; Ejaz *et al.*, 2012 and Demiralay *et al.*, 2013). Antioxidans are also potent reactive oxygen species scavengers and inhibitors of lipid peroxidation (Bakshi Hamid *et al.*, 2009; Rai *et al.*, 2009b and Petacci *et al.*, 2010 and). Among the different groups of naturally occurring antioxidant from plants, carotenoids, uric acid are the most important (Han *et al.,*2004; Yagi and Al-Abdulkareem,2006; Krishnaiah *et al.*,2011; Tuna *et al.*,2013).

A number of studies indicated that the degree of oxidative cellular damage in plants exposed to abiotic stress is controlled by the capacity of antioxidant systems (Ali, 2000; Mittler, 2002; Omami *et al*., 2006 and Gao *et al*., 2008). However, the response of antioxidants treated grains to salinity on wheat plants was poorly investigated. Thus, the present work was conducted to study the response of antioxidants treated grains to various levels of salinity. The interactive effect of salinity and grains presoaking in antioxidants (β-carotene, glutathione and uric acid) on growth and the chemical composition of the test plant *Triticum aestivum* L were considered in the current study.

2. Material and Method

2.1. Plant materials

Grains of wheat (*Triticum aestivum L.*) were obtained from Horticultural Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

Antioxidants (β-carotene, glutathione and uric acid) were obtained from Sigma-Aldrich Company, Egypt.

2.2. Experimental design

A homogenous lot of grains of wheat plant were selected for uniformity of size, shape and viability. Before germinating, the grains were surface sterilized by soaking for 3 minutes in 2.5 % sodium hypochlorite solution, after which they washed several times with distilled water. The sterilized grains were presoaked in distilled water (control) and different concentrations of antioxidants (1, 5 and 10 mM) for 12 hours. Thereafter the grains were allowed to drain for one hour. The grains were transferred to sterile Petri-dishes containing two sheets of Whatman No.1 filter paper moisten with 15 ml of different concentrations of NaCl solutions (0, 50, 100, 150, 200 and 250 mM). Each Petri-dishe contained 20 grains and each treatment was replicated 3 times. The grains were allowed to germinate at 25°C in the darkness and 2 ml of each NaCl solutions was added to each Petri dish on the third day of the germination. At the end of the experimental period (7 days), the germination percentage, seedlings fresh and dry matter, some metabolites and some enzymes activities were recorded in addition to protein patterns.

2.3. SDS-PAGE analysis

2.3.1. Protein extraction

The extraction was carried out according to Polar (1976). Fresh seedlings (3: 1 buffer volume: fresh weight) were homogenized in ice cold 250mM Tris-sucrose buffer (pH 7.2) in a chilled pestle. The homogenate was filtered through cheesecloth and centrifuged at 12,500 r.p.m for 20 min at 4C°. The supernatant was used for electrophoretic analysis.

2.3.2. Samples preparation

Protein extract (400 µl) were added to l00 µl SDS 10% and 25 µl p-mercaptoethanol (P-ME), the mixture was heated in boiling bath for 4 min and was cooled down to the room temperature then Coomassie blue (2 drops) was added. The samples were kept in deep freezer until used.

2.3.3. Protein Markers

Five standard molecular weight marker proteins were obtained from Sigma-Aldrich (protein with high molecular weight) as follows; Catalase 240.000Da, albumin bovine 67.000Da, albumin egg 45.000Da, chemotrypsinogen A 25.000Da and cytochrome C 12.400Da.

2.3.4. Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) was used for determining the molecular weight of the extracted proteins according to Raymond and Weintraub (1959) and Laemmli (1970). Electrophoresis was carried out at 150 volt/ hours.

2.4. *Enzyme activity assay*

For extraction of SOD, CAT, GR and APX, samples of plant tissues (0.5g) were homogenized in ice cold 0.1 M phosphate buffer (pH=7.5) containing 0.5 mM EDTA. Each homogenate was centrifuged at 4°C for 15 min at 15000g. The supernatant was used for enzyme activity assay **(**Esfandiari *et al*., 2007**)**.

SOD activity was estimated according to Sen Gupta *et al*. (1993), CAT activity was measured according to Aebi (1984), APX activity was measured according to Yoshimura *et al*. (2000) and GR activity was assayed according to Sairam *et al*. (2002).

2.4. Determination of glutathione

Glutathione was extracted by grinding 0.5g of plant tissues in 1% picric acid (w/v) under cold condition. After centrifugation at 10,000g for 10 min, the supernatant was collected immediately for assay. Glutathione was estimated according to Anderson (1985).

2.6. Estimation of ascorbic acid

The total ascorbic acid content was estimated using Folin phenol reagent according to Jagota and Dani (1982)**.**

2.7. Statistical analysis

The experimental design was a random complete block, with three replications. The data were analyzed by the STATGRAPHICS (Statistical Graphics Corporation, Princeton USA) statistical package by the t-test and ANOVA functions to assess significant differences among means.

3. Results

The data represented in Figure (1) revealed a gradual decrease in the percentage germination of *Triticum aestivum* grains germination in response to the

increase in the concentration NaCl. The inhibitory effect was more obvious at the highest level of salinity.

The final germination percentage of *Triticum aestivum* plants increased significantly with increasing concentration of antioxidants (β-carotene, glutathione and uric acid) when treated with NaCl compared with corresponding control. Generally, the final percentage of germination alternation induced by NaCl was alleviated by various levels of antioxidants.

Figure (2) showed that fresh-dry matter of *Triticum aestivum* seedlings were markedly decreased significantly with increasing NaCl levels. Fresh-dry matters of *Triticum aestivum* seedling were considerably increased with increased β-carotene concentrations. On the other hand, fresh-dry matter of *Triticum aestivum* seedlings were considerably decreased with increasing glutathione and uric acid concentrations when compared to control.

Generally antioxidants treated grains were alleviated the adverse effects of NaCl on the growth of *Triticum aestivum* seedling when compared with the corresponding treatments of NaCl.

Figure (3) represented glutathione and ascorbic acid contents of *Triticum aestivum* which were significantly decreased with rising salinization levels. Treatment application with different concentration of antioxidant increased the contents of glutathione and ascorbic acid in seedling of *Triticum aestivum* plant. Soaking of grains in different concentrations of antioxidants then treated with NaCl increased the contents of glutathione and ascorbic acid up to 150 mM of NaCl above that decreased but higher than corresponding NaCl.

Results in Figure (4) showed that, the activity of enzymes APX, SOD and CAT in wheat seedling significantly decreased with increasing NaCl levels. However, the activity of GR significantly increased with increasing NaCl levels.

Grains presoaked in β-carotene and treated with NaCl significantly decreased the activity of APX and GR with increasing NaCl level when compared with corresponding NaCl. Conversely CAT and SOD activity significantly increased with increasing NaCl levels.

Treatment with different concentrations of glutathione then treated with different levels of NaCl caused increase in the activity of CAT, SOD and GR, although APX activity decreased with rising salinity level.

Presoaking of grains in uric acid followed by treatment with different concentrations of NaCl increased the activity of APX, CAT and SOD with increasing NaCl levels. Conversely GR activity decreased with increasing NaCl level.

Table (1) and Figure (5) showed the electrophoretic pattern of *T. aestivum* seedlings protein, which presoaked in antioxidants for 12 hour before its treatment with water. The band with R_f 0.556 and molecular weight (MW) 79.7kDa appeared in the presoaked grains in 1mM and 5 mM β-carotene, 1mM glutathione, 1 mM, 5mM and 10mM uric acid.

The % content of protein in these bands increased by 5.31, 52.39, 58.21, 53.64, 64.85 and 79.18 % in 1mM and 5mM β-carotene, 1mM glutathione, 1, 5 and 10 mM uric acid respectively compared to the proteins content in the control band with the same R_f and MW.

The bands with R_f 0.641 and R_f 0.678 appeared as response to the 10mM and 1mM uric acid treatment, respectively. The 1mM β-carotene treatment lead to appear a new band with R_f 0.682 (MW 38.69kDa), and the band with R_f 0.711 (MW 32.23kDa) appear in the presoaked grains in 1 and 5 mM glutathione and with 1, 5 and 10mM uric acid.

Band identified by R_f 0.740 and MW 22.85 kDa showed decrease in its percent content due to the presoaking treatment of the germinated grains by 1and 5 mM β-carotene, but disappeared in the treatment by 10 mM β-carotene On the other hand, presoaked in 1, 5 and 10 mM glutathione and 1,5 and 10mM uric acid show increasing in the protein content of the same mentioned band compared to the untreated control this by 0.93,25.05,9.17,43.42,36.77 and 38.62% respectively.

The protein band has R_f with value 0.822appear only for treatment with 10 mM β-carotene. The band with R_f 0.923 (MW 11.92kDa) decreased its protein content according to the treatment with all tested concentrations of β-carotene. In contrast observations an increasing occurred in those pretreated with 1, 5 and 10 mM glutathione by 41.63, 39.11 and 57.06% respectively and uric acid by 156.80, 105.15 and 163.41% respectively.

Table (2) and Figure (6) showed the electrophoretic proteins pattern of *T. aestivum* seedlings in response to different concentrations of antioxidant with application of 50mM NaCl. Only one band appeared in the protein profile of the germinate seed in 50mM salt with R_f 0.988 and MW 10.63 kDa. The seedling of the presoaked grains in β-Carotene exhibited the same profile. The mentioned band appeared in the presoaked grains in glutathione and uric acid. The band with R_f 0.309 (MW 245.09 kDa) appeared in the protein profile of the presoaked grains in 1 and 10mM uric acid, while the band with R_f 0. 801 (MW 19.58 kDa) appeared for 1, 5 and 10mM uric acid treatment., the band with R_f value 0.858 (MW 17.09kDa) appeared only for treatment with 5mM glutathione. The last band with R_f value 0.988 (MW 10.63 kDa) showed significant increase in % content of protein for all treatment while it decreased with treatment by 10mM β-carotene by 12% and not totally appeared with 10mM uric acid.

The electrophoretic protein pattern of *T. aestivum* seedlings pretreated with the antioxidants and salinized with 100mM NaCl represented in Table (3) and Figure (7). This treatments leads to enhance the appearance of 16 bands in all the treatments.

The band with R_f 0.285 (MW 247.78 kDa) only appeared in the protein profile of the presoaked grains in 10 mM β-Carotene, also the band with R_f 0.336 (MW 230.10 kDa) only appeared in the protein profile of grains treated with 100mM saline solution without any antioxidant treatment.

A newly band with R_f 0.364 (MW 215.04 kDa) appeared in protein profile of the presoaked grains in 5mM β-carotene.

The bands with R_f 0.380 (MW 162.45 kDa), R_f 0.431 (MW 132.44 kDa) are only appeared in protein profile of seedling presoaked in 10mM uric acid and 10mM glutathione while the three bands with R_f 0.465 (MW 117.84kDa), R_f 0.475 (MW 104.36 kDa) and R_f 0.494 (MW 98.089 kDa) appeared only as a result of treatment with 5mM uric acid.

The band with R_f 0.563 (MW 77.03 kDa) only appeared as treatment with 5 and 10mM glutathione, on the other hand bands with R_f 0.585 (MW 52.733kDa), 0.609 (MW 48.09kDa) and 0.730 (MW 29.64 kDa), only appeared in protein profile of seedling presoaked in 10 and 5mM β-carotene and 10mM glutathione, respectively.

The protein band with R_f 0.412 (MW 142.40 kDa) appeared in seedling presoaked in 1mM β-carotene compared to untreated grains and it's percent content decreased by 34.75 %..

The band with R_f 0.659 (MW 42.26 kDa) appeared in the protein profile of untreated with 100 mM NaCl and pretreated grains with, 10mM β-carotene, 1,5 and 10mM glutathione and 5and 10 mM uric acid. The percentage content of protein increased with pretreatment with 1,5 and 10 mM glutathione by 6.58, 3.43 and 55.69 % respectively while it decreased in 10mM β-carotene and 5 and 10mM uric acid by 20.08, 13.24 and 51.41% respectively.

Protein band with R_f 0.778 (MW 21.98 kDa) only appeared when treated with 1mM β-carotene and 10mM glutathione. The last band that identified by R_f 0.939 (MW 11.35kDa) showed decrease in % content with all pretreatment with all antioxidant.

Table (4) and Figure (8) represented the protein profile of seedling treated with 150mM NaCl and presoaked in the different antioxidants. Protein band has Rf value 0.542 (MW 80.53kDa) only appeared as a result of presoaking of grains with 5mM glutathione and 1and 10 mM uric acid.

The percent content of the band identified by Rf 0.705 and MW 34.89 kDa increased with increasing the concentrations of (1, 5 and 10 mM) glutathione and (1, 5 and 10 mM) uric acid, while % content of the band with Rf 0.795 (MW 20.12kDa) decreased with pretreatment with1,5 and 10 mM β-carotene by 17.07,29.23 and 34.38%, respectively.

The bands with Rf 0.842 (MW 17.82 kDa) and Rf 0.877 (MW 16.45 kDa) are newly appeared when compared with untreated seedling the first band appeared as a result of presoaking in 5 and 10 mM uric acid while the second band appeared due to presoaking of grains in 1,5 and 10mM glutathione and 1 mM uric acid.

The band with Rf 0.915 (MW 12.72 kDa) are appeared at all treatments of 150mM and antioxidant. The percent content of protein bands appeared increased with increasing the concentration of glutathione and uric acid by 24.75, 58.35, 27.45, 14.13, 0.01 and 6.84% respectively while it decreased with all β-carotene levels 1.5 and10mM by 15.78, 29.64 and 21.83% respectively.

The protein profile of *T. aestivum* seedling either presoaked in antioxidants or treated with 200mM NaCl shown in Table (5) and Figure (9).

Protein bands with R_f values 0.585 (MW 52.73kDa) and 0.695 (MW 35.83 kDa) are newly appeared within protein profile of grains presoaked in in 1,5 and 10 mM β-carotene.

In the other hand the bands with R_f 0.593 (MW 49.01 kDa) and 0.785 (MW 20.55 kDa) appeared only as response to treatment with 200mM saline solution.

The bands with R_f 0.958 (MW11.21 kDa) appeared in all treatments with 200mM NaCl and antioxidants.The percent content of protein in the last band that has R_f value 0.958 (MW 11.21 kDa) increased by 70% when grains presoaked in 10mM β-carotene, while it decreased with all the other antioxidant treatments.

Table (6) and Figur (10) represented the electrophoretic protein pattern of *T. aestivum* seedling when presoaked in antioxidant for 12 hours and treated with 250mM NaCl.

The first two bands are newly appeared comparing with untreated grains, the first with R_f 0.271 (MW 345.60 kDa) appeared as a result of pretreatment with 10mM uric acid, and the second one with R_f 0.613 (MW 46.88 kDa) appeared when grains presoaked in 1 and 10 mM β-carotene.

The band with R_f 0.636 (MW 44.72 kDa) showed increase in its percent content due to presoaking in 5mM β-carotene by 74.23 %.

The protein band with R_f 0.761 showed increase in protein percent when treated with 5mM β-carotene by 4.68 %, while the protein percent decreased when grains pretreated with 1 and 10mM

Table (1): Electrophoretic protein pattern of *Triticum aestivum* Seedlings protein for grains presoaked in different concentration of antioxidants and distilled water as untreated control.

MW	Rf	Control		Control															
		Control		β-Carotene						Glutathione						Uric acid					
				1mM		5mM		10mM		1mM		5mM		10mM		1mM		5mM		10mM	
		% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %
79715.19	0.556	100	14.2	105.31	12.6	152.39	23.29			158.21	15.12					153.64	8.37	164.85	12.05	179.48	11.2
43204.31	0.641																			0	7.16
41287.76	0.678															0	11.4				
38684.57	0.682			0	29																
32230.09	0.711									0	15.59	0	11.5			0	15.5	0	13.37	0	7.4
22847.57	0.740	100	45.5	77.09	29.6	76.12	37.28			100.93	30.9	125.05	44.6	109.17	43.98	143.42	25	136.77	32.04	138.62	27.7
18060.61	0.822							0	43.2												
11924.67	0.923	100	40.3	84.66	28.8	90.95	39.43	91.28	56.8	141.63	38.39	139.11	43.9	157.06	56.02	256.8	39.7	205.15	42.54	263.41	46.6

Table (2): Electrophoretic protein pattern of *Triticum aestivum* Seedlings in response to different concentration of antioxidants and 50mM NaCl.

MW	Rf	Control		50mM																	
				β-Carotene						Glutathione						Uric acid					
				1mM		5mM		10mM		1mM		5mM		10mM		1mM		5mM		10mM	
		% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %
245088.24	0.309															0	20.42			0	39.52
19584.67	0.801															0	24.99	0	42.4	0	60.48
17087.31	0.858											0	49.53								
10632.64	0.988	100	100	187.55	100	143.6	100	88	100	119.675	100	151.35	50.47	259.65	100	154.29	54.59	115.77	57.6		

Table (3): Electrophoretic protein pattern of *Triticum aestivum* Seedlings in response to different concentration of antioxidants and 100 mM NaCl.

MW	Rf	Control		100mM																	
				β-Carotene						Glutathione						Uric acid					
				1mM		5mM		10mM		1mM		5mM		10mM		1mM		5mM		10mM	
		% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %
247782.7	0.285							0	26												
230104	0.336	100	15.39																		
215040.1	0.364					0	29.23														

11350.18	21980.36	29636.57	42261.29	48090.85	52733.2	77027.44	98085.66	104357	117837	132436.2	142395.6	162452.4
0.939	0.778	0.73	0.659	0.609	0.585	0.563	0.494	0.475	0.465	0.431	0.412	0.38
100			100								100	
50.12			18.68								15.81	
74.09	0										85.88	
59.17	19.19										21.64	
64.24				0								
44.19				26.58								
84.68			79.92		0							
44.5			15.6		13.9							
48.44			106.58									
54.9			45.1									
65.89			103.43			0						
51.27			30			18.73						
44.69	0	0	155.69			0				0		
20.49	11.15	6.82	26.6			22.92				12.02		
37.95												
100												
43.35			86.76				0	0	0			
36.2			27				11.1	7.96	17.8			
44.34			48.59									0
56.6			23.1									20.3

Table (4): Electrophoretic protein pattern of *Triticum aestivum* Seedlings in response to different concetration of antioxidants and 150mM NaCl.

MW	Rf	Control		150mM																	
				β-Carotene						Glutathione						Uric acid					
				1mM		5mM		10mM		1mM		5mM		10mM		1mM		5mM		10mM	
		% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %
80534.12	0.542											0	17.3			0	8.94			0	20.6
65759.22	0.578																				
34886.16	0.705	100	17.13							206.9	24	207.87	21.4	216.26	22.4	185.53	16.7	206.18	33.32	218.51	18.2
20117.15	0.795	100	36.07	82.93	43.2	70.77	43.7	65.62	39.3												
17818.4	0.842																	0	22.54	0	36.9
16451.01	0.877									0	36.6	0	16.9	0	41.7	0	46.4				
12714.61	0.915	100	46.8	84.22	56.9	70.36	56.3	78.17	60.7	124.75	39.5	158.35	44.4	127.45	36	114.13	28	100.01	44.14	106.84	24.3

Table (5): Electrophoretic protein pattern of *Triticum aestivum* Seedlings in response to different concentration of antioxidants and 200mM NaCl.

MW	Rf	Control		200mM																	
				β-Carotene						Glutathione						Uric acid					
				1mM		5mM		10mM		1mM		5mM		10mM		1mM		5mM		10mM	
		% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %
52733.2	0.585			0	34.77	0	27.76	0	27.3												
49011.7	0.593	100	24.06																		
35827.5	0.695			0	32.15	0	39.88	0	36.22												
20554.8	0.785	100	30.18																		
11213.6	0.958	100	45.76	55.01	33.09	63.41	32.36	170	36.48	27.39	100	35	100	39.76	100	47.6	100	41.4	100	29.9	100

Table (6): Electrophoretic protein pattern of *Triticum aestivum* Seedlings in response to different concentration of antioxidants and 250mM NaCl.

MW	Rf	Control		250mM																	
				β-Carotene						Glutathione						Uric acid					
				1mM		5mM		10mM		1mM		5mM		10mM		1mM		5mM		10mM	
		% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %	% Content	Band %
345595.6	0.271																			0	20.4
46884.53	0.613			0	36.1			0	34.23												
44719.9	0.636	100	18.7			174.23	27.02														
22502.97	0.761	100	38.5	98.58	34.5	104.68	33.36	78.14	24.2							62.13	25.8	52.78		54.14	18.1
15074.47	0.892															0	30.9	0	50.68		
13951	0902																			0	17.5
11325.56	0.945	100	42.8	75.79	29.5	111.99	39.62	120.93	41.57	91.58	100	87.04	100			93.93	43.3	80.36	30.99		
10998.78	0.973													0	100					0	44.1

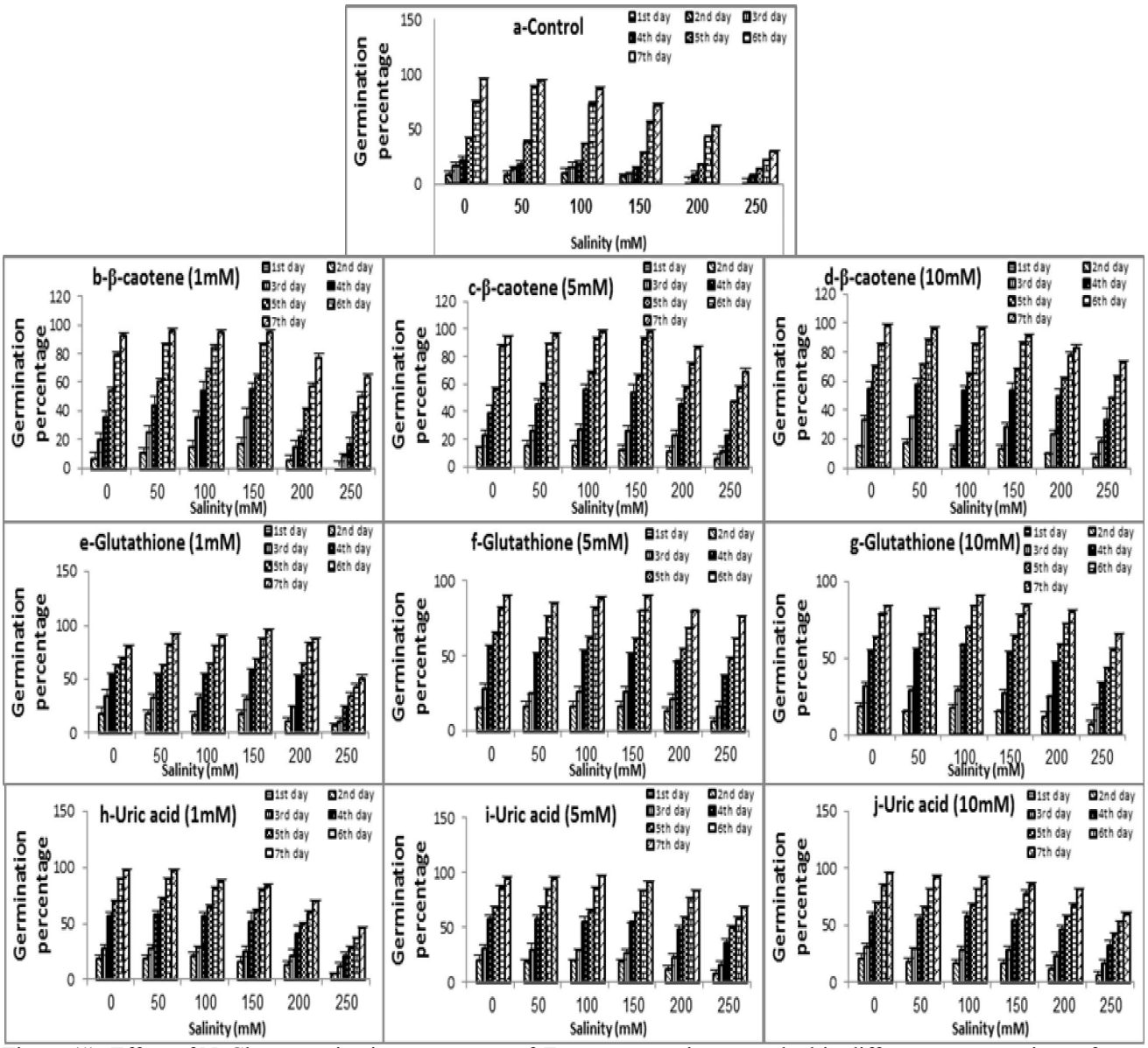
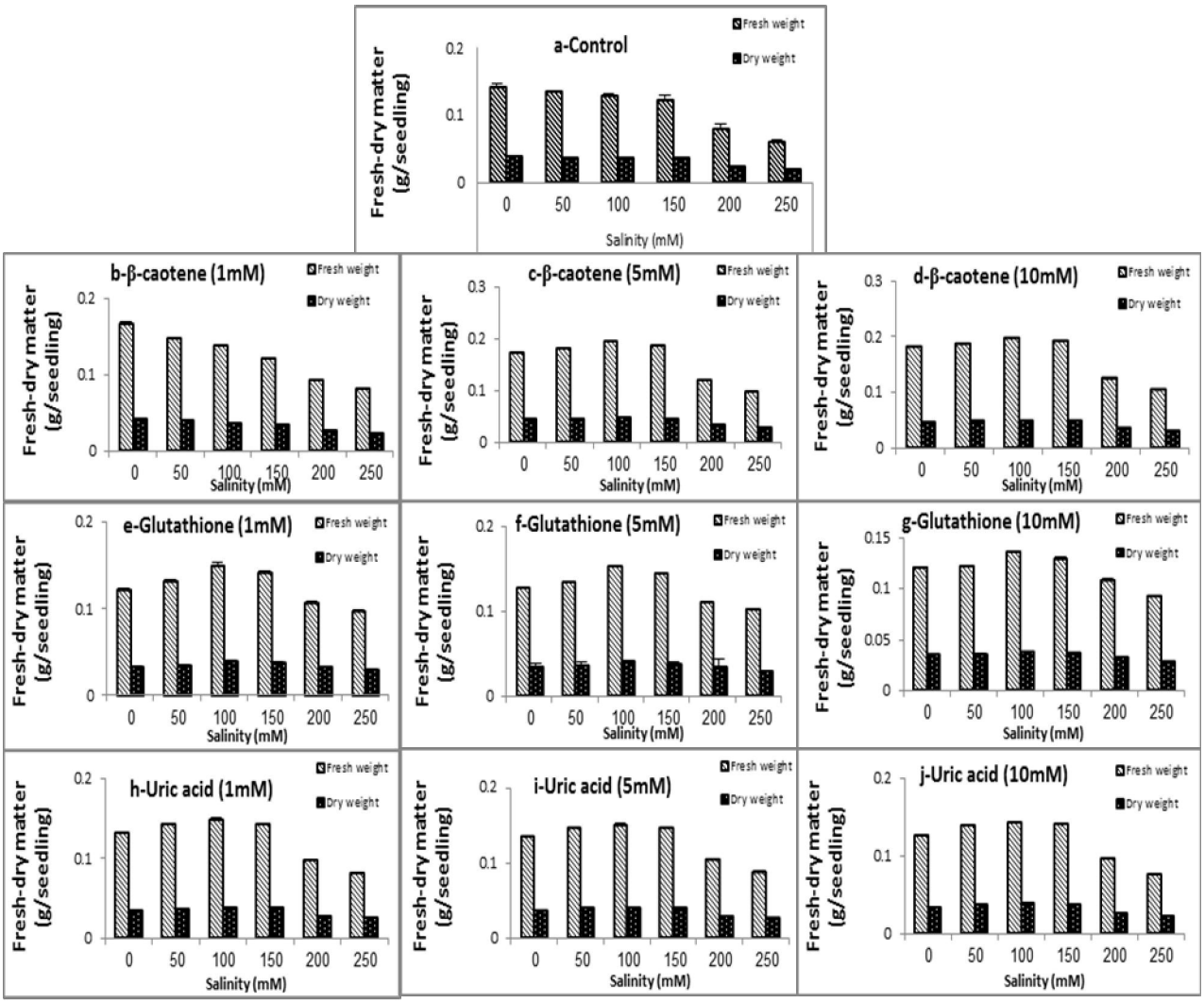
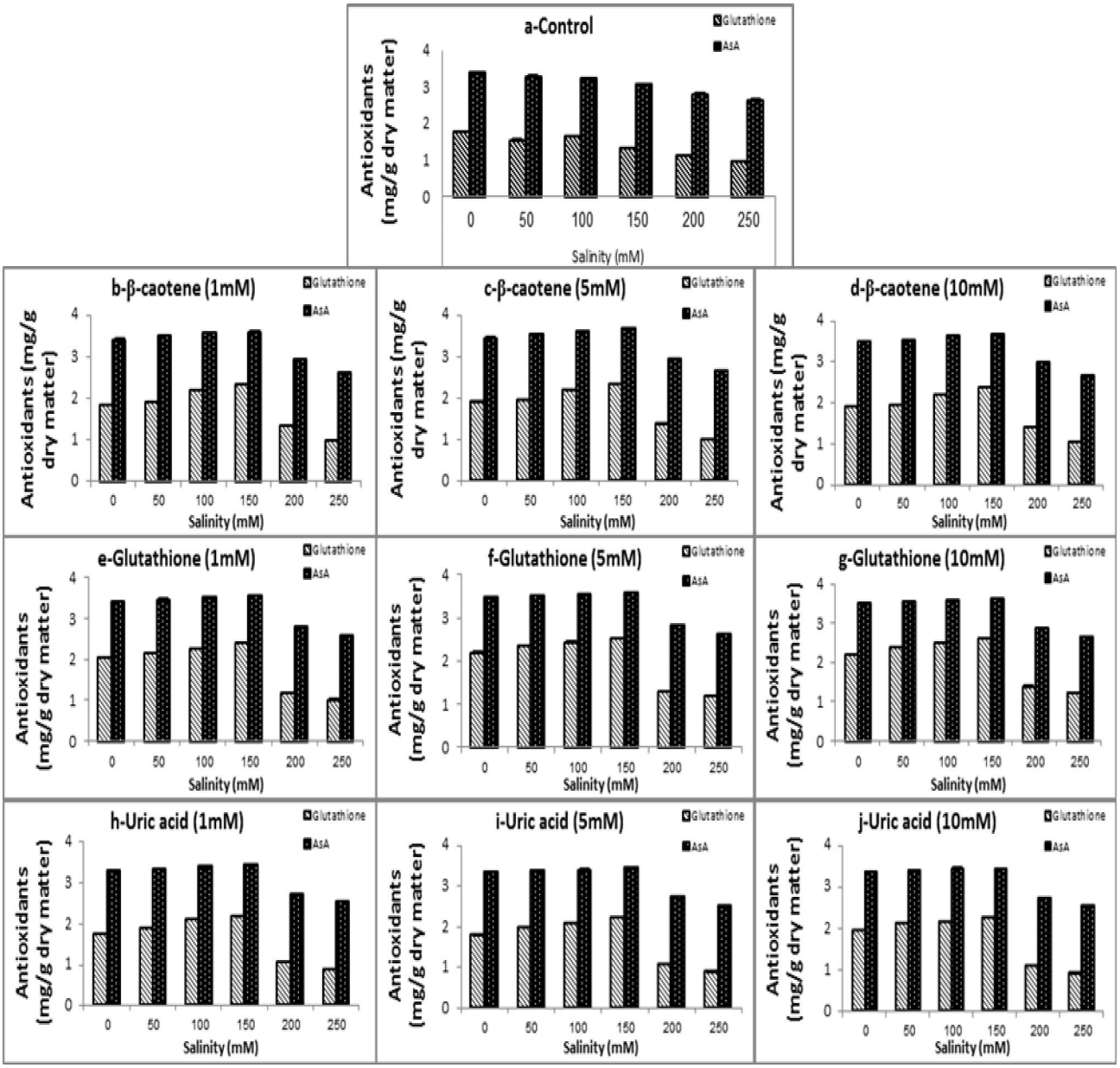


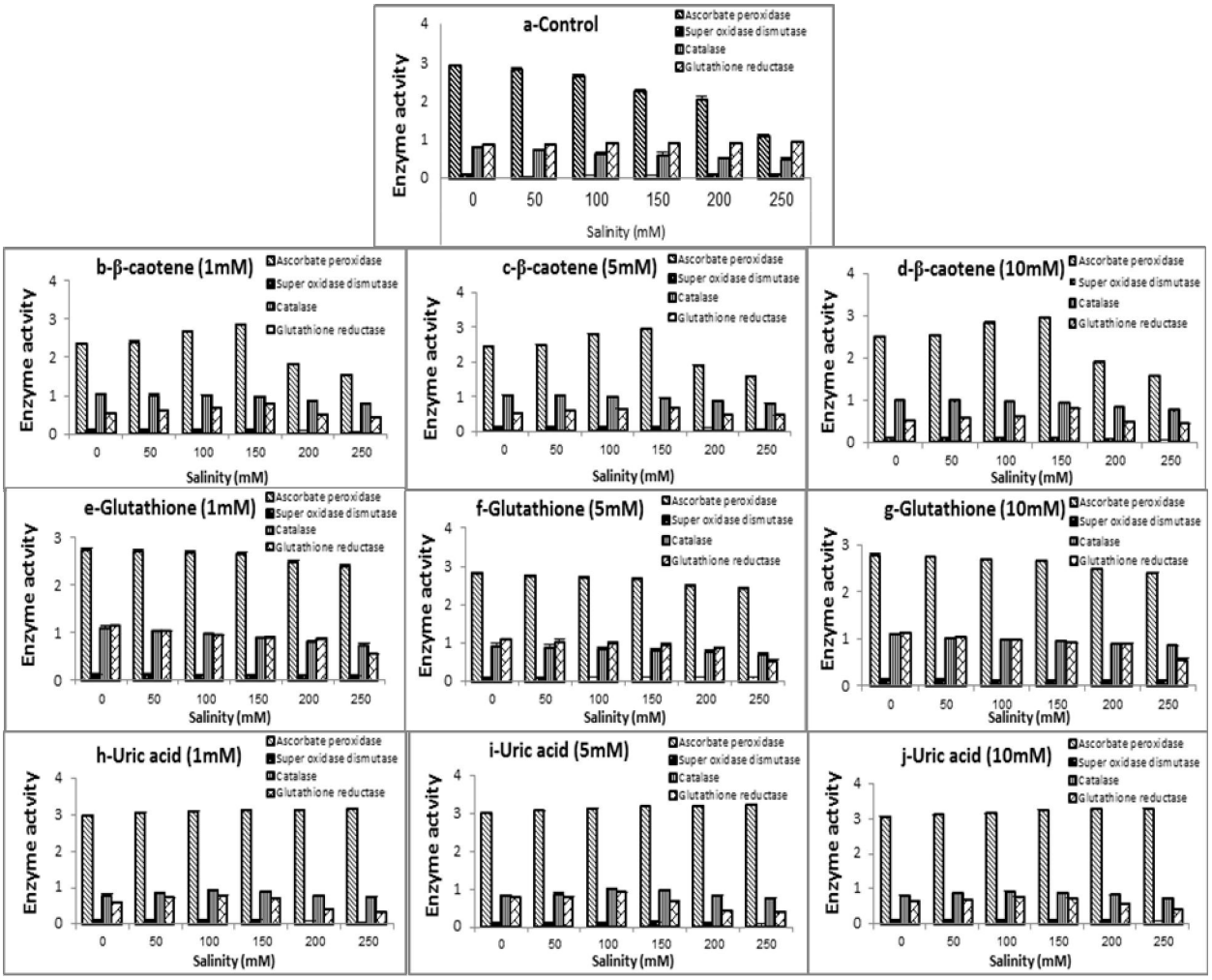
Figure (1): Effect of NaCl on germination percentage of *T. aestivum* grains presoaked in different concentrations of antioxidants (a) control, (b) β-carotene (1mM), (c) β-carotene (5mM), (d) β-carotene (10mM), (e) glutathione (1mM), (f) glutathione (5mM), (g) glutathione (10mM), (h) uric acid (1mM), (i) uric acid (5mM) and (j) uric acid (10mM). Data are the mean of three replicates and error bars represent the standard errors of the means.



Figure(2): Effect of NaCl on fresh-dry matter of *T. aestivum* grains presoaked in different concentrations of antioxidants (a) control, (b) β-carotene (1mM), (c) β-carotene (5mM), (d) β-carotene (10mM), (e) glutathione (1mM), (f) glutathione (5mM), (g) glutathione (10mM), (h) uric acid (1mM), (i) uric acid (5mM) and (j) uric acid (10mM). Data are the mean of three replicates and error bars represent the standard errors of the means.



Figure(3): Effect of NaCl on glutathione and ascorbate of *T. aestivum* grains presoaked in different concentrations of antioxidants (a) control, (b) β-carotene (1mM), (c) β-carotene (5mM), (d) β-carotene (10mM), (e) glutathione (1mM), (f) glutathione (5mM), (g) glutathione (10mM), (h) uric acid (1mM), (i) uric acid (5mM) and (j) uric acid (10mM). Data are the mean of three replicates and error bars represent the standard errors of the means.



Figure(4): Effect of NaCl on enzyme activity of *T. aestivum* grains presoaked in different concentrations of antioxidants (a) control, (b) β-carotene (1mM), (c) β-carotene (5mM), (d) β-carotene (10mM), (e) glutathione (1mM), (f) glutathione (5mM), (g) glutathione (10mM), (h) uric acid (1mM), (i) uric acid (5mM) and (j) uric acid (10mM). Data are the mean of three replicates and error bars represent the standard errors of the means.

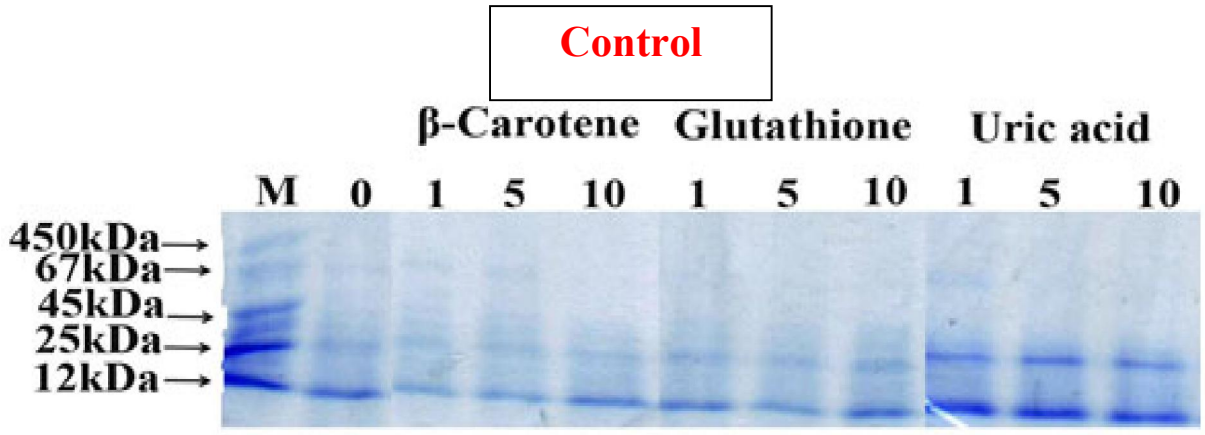


Figure (5): Electrophoretic protein pattern of *Triticum aestivum* Seedlings protein for grains presoaked in different concentration of antioxidants and distilled water as untreated control.

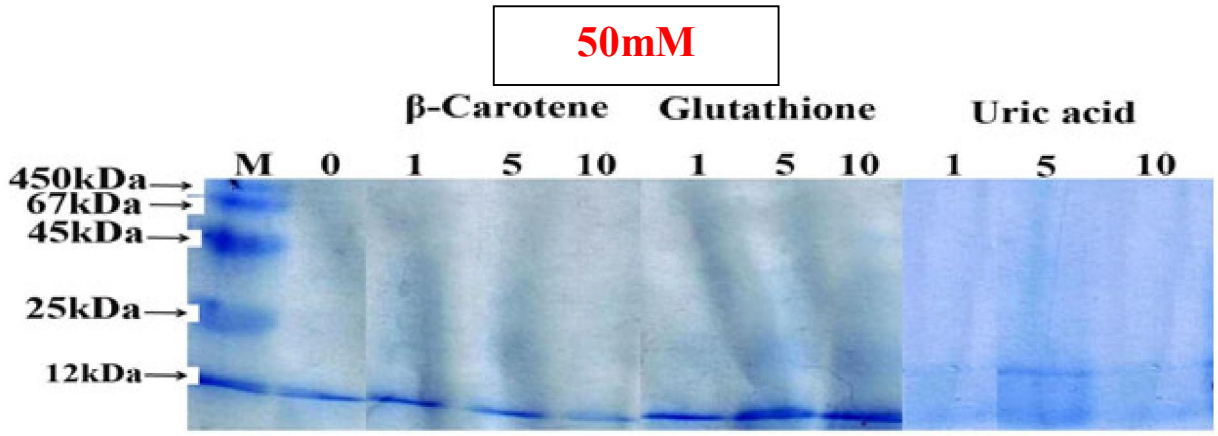


Figure (6): Electrophoretic protein pattern of *Triticum aestivum* Seedlings in response to different concentration of antioxidants and 50mM NaCl.

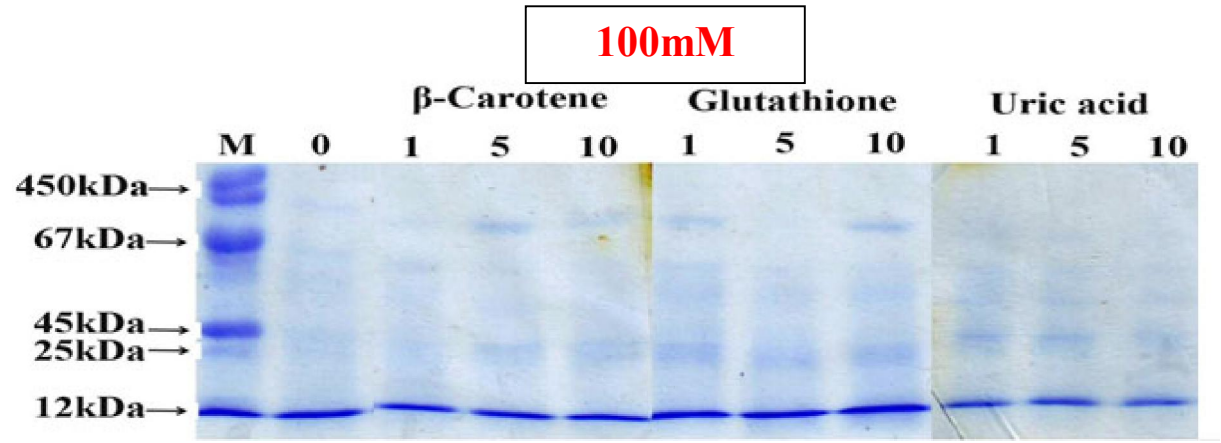


Figure (7): Electrophoretic protein pattern of *Triticum aestivum* Seedlings in response to different concentration of antioxidants and 100 mM NaCl.

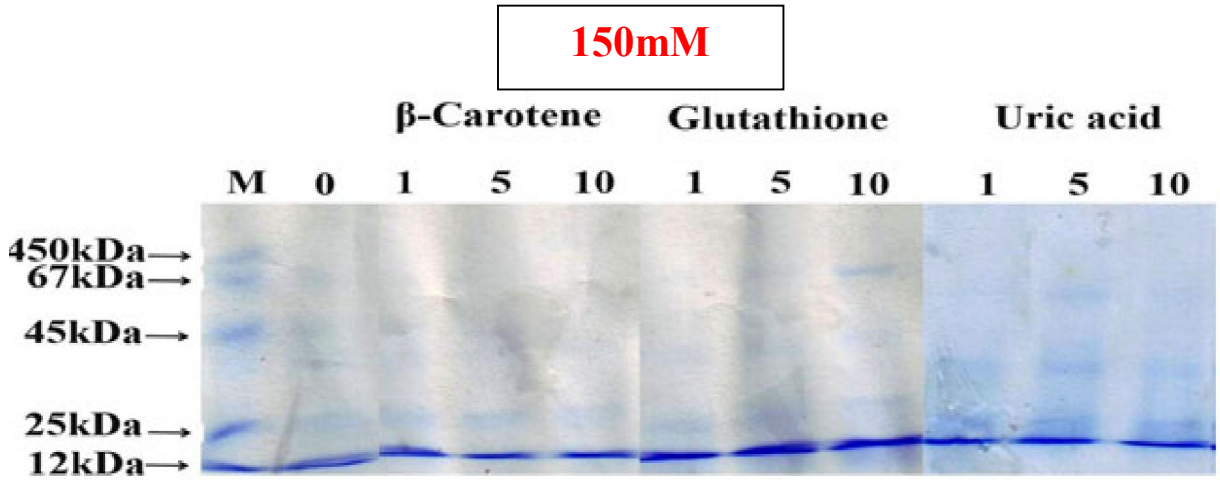


Figure (8): Electrophoretic protein pattern of *Triticum aestivum* Seedlings in response to different concentration of antioxidants and 150mM NaCl.

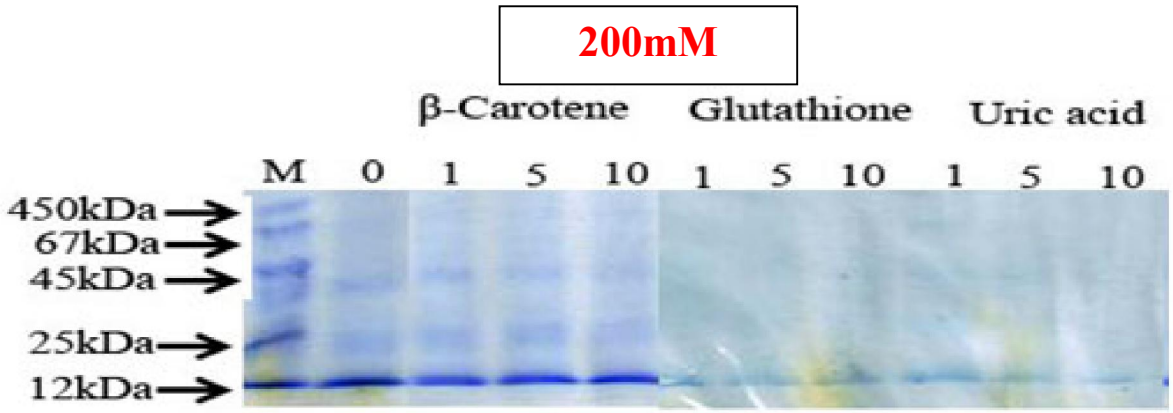


Figure (9): Electrophoretic protein pattern of *Triticum aestivum* Seedlings in response to different concentration of antioxidants and 200mM NaCl.

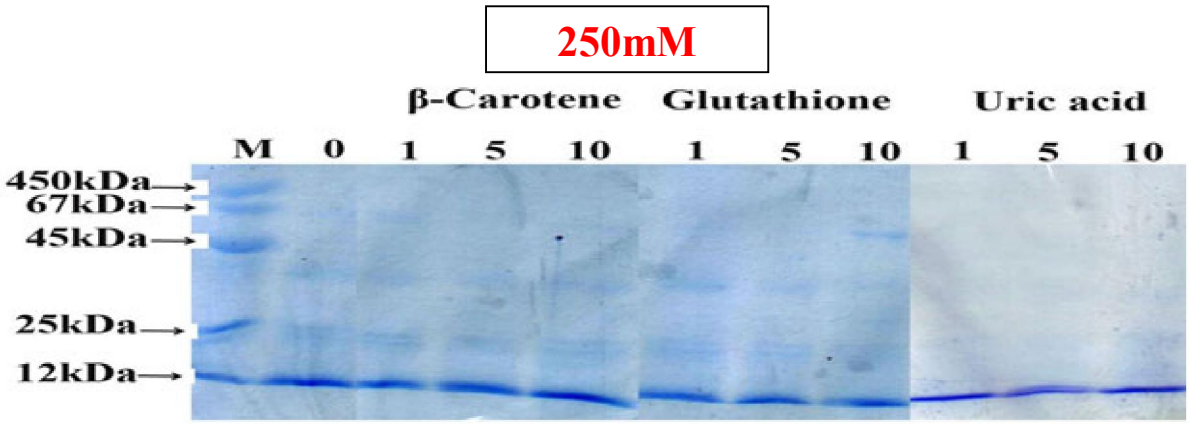


Figure (10): Electrophoretic protein pattern of *Triticum aestivum* Seedlings in response to different concentration of antioxidants and 250mM NaCl.

β-carotene, 1,5 and 10mM uric acid, thus by 1.42, 21.86, 37.87, 47.22 and 45.86 % respectively.

Band with R_f 0.892 (MW 15.08 kDa) only appeared with presoaking in 1and 5 mM uric acid and that with R_f 0.902 (MW 13.95 kDa) is due to presoaking in 10 mM uric acid.

Percent content of the band R_f 0.945 (MW 11.33 kDa) increased as a result of pretreatment of grains with 5 and 10mM β-carotene by 11.99 and 20.93%. On the other hand, it decreased due to pretreatment with, 1mM β-carotene, 1and 5 mM glutathione, 1and 5 mM uric acid by, 24.21, 8.42, 12.96, 6.07 and 19.64 % respectively.

Protein band with R_f value 0.973 and Molecular weight 11.00 kDa appeared only as a result of presoaking in 10mM glutathione and 10mM uric acid.

4. Discussion

Most environmental stresses are thought to result in production of reactive oxygen species (ROS) in plants causing oxidative stress (Karuppanapandian *et al*., 2011, Baek and Skinner, 2012 and Sharma *et al*., 2012). To ameliorate the harmful effects of salinity on plant growth, seeds presoaked in certain exogenous protectant such as osmoprotectants (proline, glycinebetaine, trehalose, etc.) (Hoque *et al*., 2007 and Nounjan *et al*., 2012), plant hormone (gibberellic acids, jasmonic acids, brassinosterioids, salicylic acid, etc.) (Hayat and Ahmad 2011,Hossain *et al*., 2011, Poór *et al*., 2011, Iqbal *et al*. 2012 and Yusuf *et al*. 2012), antioxidants (ascorbic acid, glutathione, tocopherol, etc.) (Ahmad *et al*., 2010a, Azzedine *et al*., 2011, Rawia *et al*., 2011 and Ahmad *et al*., 2012), signaling molecules (nitric oxide, hydrogen peroxide, etc.), polyamines (spermidine, spermine, putrescine) (Ali *et al*., 2009 and Ioannidis *et al*., 2012), trace elements (selenium, silicon, etc.) (Hasanuzzaman *et al*., 2011a, b) have been found effective in mitigating the salt induced damage in plant.

The data was clearly demonstrated that, NaCl was significantly inhibiting the germination percentage at all salinity levels. The adverse effect of NaCl has been attributed to changes in osmotic potential resulting from reduced water (Moosavi *et al*., 2013). The application of antioxidants stimulates grains

germination of wheat plant under NaCl salinity is in accordance with the result obtained by (Hernandez *et al*., 1995 and Hemmat, 2007).

Antioxidants led to a marked increase in seedling growth (Fresh-dry matter) this is in accordance with results obtained by Verma and Mishra (2005) and Yagi and Al-Abdulkareem (2006). Presoaking in β-carotene had significantly increased growth criteria this due to that carotenoids being antioxidants have the potential to detoxify the plants from the ill effects of stress (Verma and Mishra, 2005).

The role played by grains presoaking in antioxidants (β-carotene, glutathione and uric acid) on the biosynthesis of some cellular component in the salinized seedlings was also followed in the current studies.

A correlation between antioxidants capacity and NaCl tolerance has been demonstrated in several plant species (Benavides *et al*., 2000; Mandhania *et al*., 2006 and Sklodowska *et al*., 2009).

The present investigation was therefore undertaken to study the effect of NaCl on ascorbate and glutathione contents. The results showed that presoaking of grains in antioxidants (β-carotene, glutathione and uric acid) significantly increased the content of glutathione and ascorbic acid under saline conditions when compared with control. These results are in accordance with the result obtained by (Koca *et al*., 2007; Nagesh and Devaraj, 2008 and Chen *et al*., 2012; Akladious and Abbas, 2013).

Antioxidative enzymes are the first response mechanism against environmental stresses. As such, their activity profiles are important in the evaluation of tolerance mechanisms. The results showed significantly decrease in enzymes APX, SOD and CAT in wheat seedling with increasing NaCl while activity of GR significantly increased.

Grains presoaked in B-carotene and treated with NaCl significantly decreased the activity of APX and GR with increasing NaCl level when compared with corresponding NaCl. Conversely CAT and SOD activity significantly increased with increasing NaCl levels.

Treatment with different concentrations of glutathione then treated with different levels of NaCl caused increase in the activity of CAT, SOD and GR, although APX activity decreased with increasing salinity level.

Presoaking of grains in uric acid followed by treatment with different concentrations of NaCl increased the activity of APX, CAT and SOD with increasing NaCl levels. Conversely GR activity decreased with increasing NaCl level.

The results showed decrease in enzymes activity is the same as that obtained by Hai-Hua *et al*. (2002) Li *et al*.(2008), Azooz *et al*.(2009) and Amirjani (2010).

On the other hand the increases in enzymes activities are in accordance with (Vega *et al*., 2003; Shi *et al*., 2007; Zhao *et al*., 2008 and Sheokand *et al*., 2010).

Application of NaCl stress or different antioxidants (B-carotene, glutathione and uric acid) either individually or in combination caused changes in the number appearance and disappearance of bands and variation in the protein content in each band compared to control and to the percentage of each band in the same sample and finally its molecular weight.

Under stress conditions total protein synthesis usually decreases in plant cells (Khattab, 2001; Vabulas *et al*., 2010 and Surendar *et al*., 2013), but some proteins are that specifically respond to stress (stress-induced proteins) are induced in many plants (Hashimoto *et al*., 2004;Bassuony *et al*., 2008;Patil, 2011and Ekmekci and Karaman,2012). Although both the expression and function of such proteins is nuclear, it is suggested that there is a relationship between some forms of plant stress adaptation and the expression of stressed induced proteins.

One possible explanation for completely disappearance of some proteins under salt stress is the gene(s) responsible for certain proteins and had been completely suppressed as a result of stress. Therefore, the developed tissues had lost their ability to synthesis these proteins. It is also possible that the genes had not been completely suppressed, but inhibited as the result of stress, and complete recovery of inhibition was not achieved. This may apply to protein that stained less density stress (El-Obeidy *et al*., 2001and Mohamed,2005)It may also be possible to breed stress tolerant plants by the genetic engineering of genes that encode the stress-induced proteins.

Conclusions

Wheat seedling showed negative response when treated with different concentration of NaCl. In order to overcome this negative effect wheat grains were presoaked in different concentrations of antioxidants (β-carotene, glutathione and uric acid). Pretreatment of grains stimulated activities of some antioxidant enzymes, increase some antioxidants contents and formation of new bands in the protein pattern. Overall, it can be concluded that antioxidants (β-carotene, glutathione and uric acid) could improve physiological properties of wheat seedlings under saline conditions

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