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Effect of Salinity, Selenium and Boron on Chemical Composition of *Brassica napus* L. plants Grown under Sandy Soil Conditions

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ABSTRACT

Canola is one of the most important medicinal food plants. Salinity induces physiological and metabolic disturbances in plant and causes a great reduction in growth development, yield and chemical constituents. The purpose of this study was to evaluate the impact of salt stress (0, 2.5, 5 and 7.5 dS m^{-1}), selenium (0, 2 and 4 mg l^{-1} as sodium selenate) and boron (0, 2, and 4 mg l^{-1} as boric acid) on some chemical composition of canola plants at vegetative and flowering stages grown under sandy soil conditions. The results demonstrated that the lowest level of salinity (2.5 dS m^{-1}) resulted in the highest total soluble phenols, total free amino acids and protein concentrations in leaves of canola plants, while it was decreased with increasing salinity levels. Moreover, increasing salinity levels up to 5.0 and 7.5 dS m^{-1} increased total sugars, proline and nitrate concentrations. The plants irrigated with salinity up to 5 dS m^{-1} and sprayed with the highest levels of selenium and boron (4 mg l^{-1} + 4 mg l^{-1}) recorded the maximum total sugars concentration. The addition of 2 mg l^{-1} of selenium combined with 2 mg l^{-1} of boron increased total soluble phenols and protein concentration while increasing boron level to 4 mg l^{-1} increased total free amino acids concentration in the plants irrigated with the lowest level of salinity (2.5 dS m^{-1}). The plants irrigated with the highest level of salinity (7.5 dS m^{-1}) and sprayed with the lowest level of selenium (2 mg l^{-1}) increased nitrate concentration while proline concentration increased with the highest level of selenium (4 mg l^{-1}). It was clear that selenium and boron played an important role in enhancing the tolerance of the plants to salinity stress.

Keywords: Canola, salinity, selenium, boron, chemical constituents.

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INTRODUCTION

Brassica napus L., is commonly known as canola, oilseed rape and rapeseed. It contains many phytochemical constituents; phenolic and flavonoid contents [1], amino acids as lysine, methionine, and threonine [2]. Canola is a good source of natural antioxidants due to high levels of carotenoids, tocopherol and ascorbic acid [3] and some antioxidant enzymes [1]. The seeds residues, leaves and stems have high protein content and can be used as animal feed [4].

Salinity is a severe problem results in a noticeable reduction in the productivity of crops [5]. Over 6% of the total land area [6] and 19.5% of the agricultural land in the World is adversely affected by salinity [7]. Approximately 33% of the cultivated land and most extensions of agricultural land in Egypt are already salinized due to low precipitation, high temperature, high surface evaporation, poor drainage system, and irrigating with low quality water [8]. Plants differ in their ability to grow successfully under saline conditions and to accumulate high concentration of salts in their tissues. Increasing the level of the soluble salts in the soil solution tends to increase its osmotic pressure and/or cause individual ion toxicity [9].

Selenium is a beneficial element for higher plants and has a positive effect and performance on plants growth [10]. It plays an important role in enhancing the resistance of plants to certain abiotic stresses, e.g. salinity [11]. The protective role of low selenium concentrations in plants exposed to stress conditions has been attributed to various defense mechanisms which can stimulate plant growth [12].

Boron is essential for plant growth, development and yield as well as the quality of harvested crops. It is required by plants in small quantities that involve several physiological and biochemical processes in the plants [13]. Canola has a high demand for boron and is extremely sensitive to boron deficiency [14].

The aim of this investigation was to study the effect of salinity, selenium and boron on chemical composition of *Brassica napus* plants grown under sandy soil conditions.

MATERIALS AND METHODS

The experiments were carried out at the National Research Centre, Dokki, Cairo, Egypt during the two successive seasons of 2012/2013 and 2013/2014 to study the effect of salinity, selenium and boron on chemical composition of canola grown under sandy soil conditions.

Canola "*Brassica napus* L. cv. Serw 4" seeds were obtained from the Ministry of Agriculture "Oil Crop Research Center", Giza, Egypt. The seeds were sown on 5th November 2012 and 12th November 2013 in plastic pots (30 cm height x 25 cm diameter) filled with 12kg of sandy soil. The seedlings were thinned twice, leaving two plants pot^{-1}.

The soil was prepared two weeks before sowing date. During soil preparation, all treatments were fertilized with compost at the rate of 15 ton fed^{-1}, 300 kg fed^{-1} calcium superphosphate (15.5% P_2O_5), 90 kg fed^{-1} potassium sulphate (48.5% K_2O) and 300 kg fed^{-1} agricultural sulphur (99.9%). Moreover, ammonium sulphate (20.5% N) was added at the rate of 150 kg fed^{-1} in three doses; during sowing date, one month later and at the end of vegetative growth stage.

The layout of the experimental was factorial arrangement in a completely randomized design of all combination between four levels of saline water (0, 2.5, 5 and 7.5 dS m^{-1} as 2 NaCl : 2 $CaCl_2$: 1 $MgSO_4$), three selenium concentrations (0, 2 and 4 mg l^{-1} as sodium selenate Na_2SeO_4) and three boron concentrations (0, 2, and 4 mg l^{-1} as boric acid) as well as the interaction between selenium and boron under salinity treatments. The experiment consists of 36 treatments and each treatment was replicated three times. The plants were sprayed with selenium and boron twice, after 40 and 70 days from sowing.

All plants were irrigated with 1.5 l pot^{-1} of tap water for 5 weeks and then irrigated with saline water. Every three times of irrigation with saline water, full-strength Hoagland's solution [15] were added to all plants. The plants were irrigated three times weekly in the summer and two times weekly in the winter.

The physical and chemical characteristics of the used sandy soil were determined according to Jackson [16] and are presented in Table (1).

Table (1): The physical and chemical properties of the experimental soil

Physical properties (particle size distribution %)										
		Sand	**Silt**	**Clay**	**Texture**					
		81.8	**7.0**	**11.2**	**Sandy**					
Chemical properties										
pH (1:2.5)	**E.C. (dSm^{-1}) (1:5)**	**(meq/l)**								
		Cations				**Anions**				
		Ca^{++}	**Mg^{++}**	**Na^{+}**	**K^{+}**	**CO_3^{--}**	**HCO_3^{-}**	**Cl^{-}**	**SO_4^{--}**
7.8	0.3	1	0.5	1.5	0.4	-	0.4	2.5	0.5

Data recorded in leaves of plants at vegetative growth and flowering stages of both seasons:

In ethanol extract of herb total sugars, total soluble phenols and total free amino acids were determined. Total sugars concentration was carried out by using phenol sulphoric acid according to Dubois *et al*. [17], total soluble phenols concentration was estimated by using folin-ciocalteau colorimetric method according to Swain and Hillis [18], total free amino acids concentration was determined by using ninhydrin reagent according to Moore and Stein [19], proline concentration was carried out by using ninhydrin reagent according to Carillo and Gibon [20], protein concentration was estimated by using biorad according to Bradford *et al*. [21] and nitrate concentration was determined by using salicylic acid-H_2SO_4 and sodium hydroxide according to Cataldo *et al*., [22].

Proline, protein and nitrate concentrations in the 2nd season were determined in Department of Agriculture, Food and Environment, Plant Physiology Group, Pisa Univ., Italy.

RESULTS AND DISCUSSION

Total sugars

The results illustrated in Table (2) indicate that, salinity significantly increased total sugars concentration in canola leaves at vegetative growth stage and flowering stage of both seasons. The lowest level of salinity (2.5 dS m^{-1}) gave the highest values in the vegetative growth stage. Whereas, increasing salinity levels to 5 and 7.5 dS m^{-1} increased total sugars concentration in plants at flowering stage of both seasons. These results agreed with those obtained by Qasim *et al*. [23] on *Brassica napus*, Khattab and Afifi [24] on canola and Sahar *et al*. [25] on *Salivia officialis* who revealed that salinity stress increased sugars concentration of these plants. Salt stress often results in accumulation of compounds such as sugars, termed osmolytes [26]. These osmolytes prevents macromolecules from denaturation by supporting them to retain their natural configuration [27] as well as their role in carbon storage, radical scavenging, osmotic adjustment and osmoprotection [28] counteracting the toxic effect of Na^+ and Cl^- in the shoot of many plant species [29].

Spraying the plants with selenium and boron significantly increased total sugars concentration in both seasons and the best treatment was selenium at 4 mg l^{-1} with boron at 4 mg l^{-1}. This result is in accordance with that of Abbas [30] on sorghum and Ibrahim and Ibrahim [31] on potato who pointed out that selenium at lower concentrations enhanced sugar level in plants. The favorable effect of selenium may be due to it results in higher photosynthetic rate and C assimilation. This could be confirmed by slightly or significantly higher starch concentration of Se-treated plants [32]. Increasing the total sugars by selenium application had been observed in alfalfa plants, this response was related to increase the activity of fructose 1,6 – bisphosphatase (F 1,6 – BPase), a key enzyme in carbohydrate metabolism [33]. Although the involvement of boron in sugars was previously reported, the mechanisms that control this relationship are not yet understood, and further research is needed to investigate this relationship [34].

In respect to the interaction between salinity and selenium with boron, it significantly increased total sugars concentration at vegetative growth stage of both seasons and flowering stage of the 1st season. The

Table (2): Effect of salinity, selenium and boron on total sugars concentration (mg g⁻¹ fresh weight) of *Brassica napus* herb at vegetative growth and flowering stages during 2012/2013 and 2013/2014 seasons

Foliar Application (F) mg l⁻¹	2012/2013 Salinity (S) dS m⁻¹ 0	2.5	5	7.5	Mean (F)	2013/2014 Salinity (S) dS m⁻¹ 0	2.5	5	7.5	Mean (F)
				Vegetative growth stage						
Control	10.3	16.0	14.8	12.3	**13.4**	11.9	19.6	12.7	20.5	**16.2**
Se_1	10.4	17.3	15.9	12.7	**14.1**	20.2	22.8	17.0	14.3	**18.6**
Se_2	17.4	17.3	15.1	13.4	**15.8**	23.7	25.9	24.8	24.6	**24.8**
B_1	13.7	15.8	14.2	12.5	**14.1**	16.5	22.6	15.4	13.0	**16.9**
B_2	13.2	17.4	15.9	13.9	**15.1**	19.0	14.9	19.4	16.1	**17.4**
Se_1B_1	16.9	16.3	18.2	13.1	**16.1**	18.4	25.9	27.5	26.4	**24.6**
Se_1B_2	15.6	20.1	13.8	14.5	**16.0**	24.0	21.9	22.8	17.8	**21.6**
Se_2B_1	16.4	15.3	16.3	11.8	**15.0**	25.6	26.0	17.8	19.3	**22.2**
Se_2B_2	14.1	21.8	17.3	13.3	**16.6**	26.5	28.4	25.6	20.2	**25.2**
Mean (S)	**14.2**	**17.5**	**15.7**	**13.1**		**20.6**	**23.1**	**20.3**	**19.2**	
LSD at 5%	**S 0.84**	**F 1.27**	**SF 2.12**			**S 0.94**	**F 1.41**	**SF 2.36**		
				Flowering stage						
Control	17.2	16.6	16.3	24.5	**18.7**	22.6	21.9	22.4	23.6	**22.6**
Se_1	17.3	17.8	16.6	20.4	**18.0**	23.7	22.0	22.9	23.0	**22.9**
Se_2	19.4	18.7	20.6	20.2	**19.7**	24.0	22.9	23.1	26.2	**24.1**
B_1	17.1	18.5	18.2	21.3	**18.8**	22.5	23.2	24.6	22.0	**23.1**
B_2	18.4	19.6	21.4	19.6	**19.7**	23.4	22.3	22.0	21.3	**22.2**
Se_1B_1	19.6	19.9	18.5	21.4	**19.8**	23.1	24.9	25.9	22.0	**24.0**
Se_1B_2	21.1	19.1	22.6	23.8	**21.7**	23.5	23.9	23.0	22.2	**23.1**
Se_2B_1	22.4	20.6	22.2	22.3	**21.9**	25.2	23.8	26.3	26.4	**25.4**
Se_2B_2	19.8	21.0	24.7	22.9	**22.1**	25.7	24.1	26.5	25.8	**25.5**
Mean (S)	**19.1**	**19.1**	**20.1**	**21.8**		**23.7**	**23.2**	**24.1**	**23.6**	
LSD at 5%	**S 0.93**	**F 1.39**	**SF 2.33**			**S ns**	**F 1.38**	**SF ns**		

Se_1 (Selenium 2 mg l⁻¹), Se_2 (Selenium 4 mg l⁻¹), B_1 (Boron 2 mg l⁻¹), B_2 (Boron 4 mg l⁻¹)

plants irrigated with 2.5 dS m^{-1} and sprayed with selenium at 4 mg l^{-1} and boron at 4 mg l^{-1} gave the highest values of total sugars in vegetative growth stage in both seasons. While at flowering stage of both seasons, the highest level of sugars was recorded from applying 4 mg l^{-1} selenium combined with 4 mg l^{-1} boron to plants irrigated with 5 dS m^{-1}.

1. Total soluble phenols

The results in Table (3) clarify that, salinity significantly increased leaves concentration of total soluble phenols at vegetative growth stage in the 1st season but decreased it in the 2nd season as well as at flowering stage of both seasons. The lowest level of salinity (2.5 dS m^{-1}) resulted in the maximum total soluble phenols concentration in vegetative growth stage of the 1st season and flowering stage of both seasons. While at vegetative growth stage of the 2nd season, the control treatment (0 dS m^{-1}) recorded the highest value. It was noticed that, there was no significant difference between using 0 and 2.5 dS m^{-1} at vegetative growth stage of the 2nd season. Moreover, there were no significant differences between using 0, 2.5 and 5 dS m^{-1} at flowering stage of both seasons.

In accordance with these results Hanafy Ahmed *et al*. [35] on wheat, Hanafy Ahmed *et al*. [36] on cotton and Sadak and Mostafa [37] on sunflower found that increasing salinity increased total phenols concentration in the plants. These results could be attributed to that phenol accumulation is a cellular adaptive mechanism for scavenging oxygen free radicals that formed during stress and these free radical scavengers could be oxidized for preventing sub cellular damages [38]. In this respect, Hanafy Ahmed *et al*. [35] mentioned that salinized wheat plants showed remarkable higher levels of soluble phenols for osmoregulation. This might be explained on the assumption that such salt-stressed plants could have less

Table (3): Effect of salinity, selenium and boron on total soluble phenols concentration (mg g^{-1} fresh weight) of *Brassica napus* herb at vegetative growth and flowering stages during 2012/2013 and 2013/2014 seasons

Foliar Application (F) mg l^{-1}	2012/2013 Salinity (S) dS m^{-1} 0	2.5	5	7.5	Mean (F)	2013/2014 Salinity (S) dS m^{-1} 0	2.5	5	7.5	Mean (F)
					Vegetative growth stage					
Control	2.57	2.58	2.09	2.24	**2.37**	2.43	2.21	2.43	1.52	**2.15**
Se_1	2.33	2.74	2.49	3.77	**2.83**	2.80	3.09	2.32	2.08	**2.57**
Se_2	2.96	2.82	2.75	3.22	**2.94**	3.12	3.89	2.49	2.47	**2.99**
B_1	3.35	4.28	1.90	2.65	**3.05**	2.11	2.48	1.80	2.63	**2.25**
B_2	2.48	2.71	4.25	3.03	**3.12**	3.67	2.40	1.89	1.75	**2.43**
Se_1B_1	2.39	4.87	4.54	3.01	**3.70**	2.79	3.95	3.06	2.33	**3.03**
Se_1B_2	2.65	2.50	2.99	2.87	**2.75**	3.33	2.46	1.59	2.67	**2.51**
Se_2B_1	2.90	3.31	2.55	3.20	**2.99**	3.95	2.32	3.02	2.73	**3.00**
Se_2B_2	2.45	3.01	3.94	3.22	**3.16**	3.15	2.83	2.19	2.52	**2.67**
Mean (S)	**2.68**	**3.20**	**3.06**	**3.02**		**3.04**	**2.85**	**2.31**	**2.30**	
LSD at 5%	**S 0.017**	**F 0.025**	**SF 0.042**			**S 0.130**	**F ns**	**SF 0.327**		
					Flowering stage					
Control	0.83	0.84	0.71	0.58	**0.74**	0.73	0.64	0.51	0.70	**0.65**
Se_1	0.78	0.86	0.78	0.72	**0.79**	0.88	0.67	0.79	0.73	**0.77**
Se_2	0.98	0.89	0.76	0.80	**0.86**	0.84	0.68	0.82	0.73	**0.77**
B_1	0.97	1.09	0.87	0.63	**0.89**	0.78	0.72	0.58	0.80	**0.72**
B_2	0.91	0.98	0.84	0.62	**0.84**	0.49	0.79	0.71	0.64	**0.66**
Se_1B_1	1.17	1.27	1.01	0.94	**1.10**	0.82	1.15	1.13	0.73	**0.96**
Se_1B_2	0.98	1.04	1.08	0.70	**0.95**	0.78	0.84	0.83	0.77	**0.81**
Se_2B_1	0.97	1.09	0.98	0.89	**0.98**	0.84	1.06	1.03	0.91	**0.96**
Se_2B_2	0.94	1.11	1.11	0.83	**0.99**	1.14	0.88	0.79	0.85	**0.92**
Mean (S)	**0.95**	**1.02**	**0.90**	**0.75**		**0.81**	**0.83**	**0.80**	**0.76**	
LSD at 5%	**S 0.007**	**F 0.011**	**SF 0.019**			**S 0.023**	**F 0.034**	**SF 0.057**		

Se_1 (Selenium 2 mg l^{-1}), Se_2 (Selenium 4 mg l^{-1}), B_1 (Boron 2 mg l^{-1}), B_2 (Boron 4 mg l^{-1})

efficiency to condensate simple organic compounds into more complex ones. Margna [39] stated that the main determinant of phenol production is the supply of the prerequisites for its synthesis, namely the amino acid precursor`s phenylalanine and tyrosine.

The results show that the foliar spray of selenium and boron significantly increased total soluble phenols concentration in both seasons. The addition of selenium at 2 mg l^{-1} combined with boron at 2 mg l^{-1} produced the highest values of total soluble phenols in vegetative growth and flowering stages of both seasons. The accumulation of total soluble phenols may due to the role of boron in increasing the synthesis of phenolic compounds used in cell wall synthesis [40]. In this concern, Mengel and Kirkby [41] mentioned that boron may modify the activity of the enzyme 6-phosphogluconate dehydrogenase by complexing with its substrate 6-phosphogluconate. This enzyme regulates the first step in the pentose phosphate pathway. Therefore it is argued that when boron is present the activity of the pentose phosphate pathway is decreased in favor of glycolysis. On the other hand, when boron is deficient the pentose phosphate pathway is favored and consequently induces the accumulation of phenolic compounds.

From Table (3), it could be observed that the plants irrigated with the lowest level of salinity (2.5 dS m^{-1}) and sprayed with 2 mg l^{-1} of selenium combined with 2 mg l^{-1} of boron recorded the highest concentration of total soluble phenol at vegetative growth and flowering stages of both seasons.

2. Total free amino acids

Data recorded in Table (4) reveal that increasing salinity levels significantly decreased total free amino acids concentration in both seasons. The lowest level of salinity (2.5 dS m^{-1}) recorded the highest values of

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Table (4): Effect of salinity, selenium and boron on total free amino acids concentration (mg g^{-1} fresh weight) of *Brassica napus* herb at vegetative growth and flowering stages during 2012/2013 and 2013/2014 seasons

Foliar Application (F) mg l^{-1}	2012/2013 Salinity (S) dS m^{-1} 0	2.5	5	7.5	Mean (F)	2013/2014 Salinity (S) dS m^{-1} 0	2.5	5	7.5	Mean (F)
Vegetative growth stage										
Control	6.5	4.8	4.4	3.2	**4.7**	4.1	7.2	4.8	3.9	**5.0**
Se_1	7.0	5.3	4.5	4.0	**5.2**	4.9	7.6	6.2	3.5	**5.6**
Se_2	7.0	5.3	3.3	4.2	**4.9**	6.5	9.1	6.5	4.5	**6.7**
B_1	8.9	4.7	3.1	3.9	**5.1**	6.2	7.8	5.0	3.9	**5.7**
B_2	7.5	3.7	3.7	3.5	**4.6**	5.5	9.1	5.2	3.0	**5.7**
Se_1B_1	8.7	4.5	3.5	4.0	**5.2**	7.0	8.3	5.4	4.2	**6.3**
Se_1B_2	6.0	3.6	3.8	3.8	**4.3**	6.0	10.7	5.3	4.3	**6.6**
Se_2B_1	8.1	3.7	3.6	3.9	**4.8**	6.4	10.4	5.9	4.2	**6.7**
Se_2B_2	5.7	4.3	3.8	3.6	**4.3**	6.2	10.2	7.2	3.6	**6.8**
Mean (S)	**7.3**	**4.4**	**3.7**	**3.8**		**5.9**	**8.9**	**5.7**	**3.9**	
LSD at 5%	**S 0.41**	**F 0.61**	**SF 1.02**			**S 0.29**	**F 0.44**	**SF 0.73**		
Flowering stage										
Control	11.3	10.8	10.3	8.9	**10.3**	11.7	11.6	11.6	10.8	**11.4**
Se1	11.7	11.7	10.5	9.2	**10.8**	12.3	14.4	13.4	11.8	**13.0**
Se2	11.6	12.0	11.2	9.8	**11.1**	14.9	13.7	11.6	11.1	**12.8**
B1	11.2	12.2	10.9	9.1	**10.8**	13.9	12.5	12.9	10.8	**12.5**
B2	11.4	13.5	9.6	9.6	**11.0**	15.0	15.1	14.3	10.4	**13.7**
Se1B1	11.9	12.8	10.9	10.1	**11.4**	14.2	13.2	14.3	10.5	**13.0**
Se1B2	11.9	14.8	10.3	10.6	**11.9**	15.2	15.5	14.3	10.2	**13.8**
Se2B1	12.6	13.5	11.2	9.7	**11.8**	14.2	13.3	13.2	15.2	**14.0**
Se2B2	13.2	14.7	11.0	10.5	**12.3**	13.6	14.9	15.4	13.3	**14.3**
Mean (S)	**11.9**	**12.9**	**10.7**	**9.7**		**13.9**	**13.8**	**13.4**	**11.6**	
LSD at 5%	**S 0.31**	**F 0.46**	**SF 0.78**			**S 0.60**	**F 0.90**	**SF 1.51**		

Se_1 (Selenium 2 mg l^{-1}), Se_2 (Selenium 4 mg l^{-1}), B_1 (Boron 2 mg l^{-1}), B_2 (Boron 4 mg l^{-1})

total free amino acids at vegetative growth stage of the 2nd season as well as at flowering stage of both seasons. Moreover, there were no significant differences between 0, 2.5 and 5 dS m^{-1} at flowering stage of the 2nd season. Same findings were obtained by Hussein *et al*. [42] on *Ambrosia maritime* as well as Baghizadeh *et al*. [43] on canola who stated that increasing salinity reduced amounts of free amino acids.

The application of selenium with boron significantly increased total free amino acids concentration in both seasons. The favorable treatment was 4 mg l^{-1} of selenium combined with 4 mg l^{-1} of boron which resulted in the maximum total free amino acids concentration. These results were agreed with Sharma *et al*. [44] on rape and Hajiboland and Keivanfar [10] on canola who revealed that the application of selenium increased amino acids content. The role of selenium may be because it incorporates into Seleno-amino acids such as Se-methylselenocysteine, selenocystathione and Se-methyl-selenomethionine and replaced sulfur-amino acids as components of proteins [45].

In respect to the combination between salinity, selenium and boron, it significantly affected leaves total free amino acids concentration in both seasons. The plants irrigated with the lowest level of salinity (2.5 dS m^{-1}) and sprayed with 2 mg l^{-1} of selenium and 4 mg l^{-1} of boron produced the highest concentration of total free amino acids.

3. Proline

Table (5) revealed that, increasing salinity levels significantly increased proline concentration of leaves in both seasons. The greatest proline concentration in vegetative growth and flowering stages of both seasons

Table (5): Effect of salinity, selenium and boron on proline concentration (mg g^{-1} fresh weight) of *Brassica napus* herb at vegetative growth and flowering stages during 2012/2013 and 2013/2014 seasons

Foliar application (F) mg l^{-1}	2012/2013					2013/2014				
	Salinity (S) dS m^{-1}					Salinity (S) dS m^{-1}				
	0	2.5	5	7.5	Mean (F)	0	2.5	5	7.5	Mean (F)
Vegetative growth stage										
Control	0.019	0.018	0.018	0.029	**0.021**	0.040	0.043	0.141	0.137	**0.090**
Se_1	0.024	0.025	0.021	0.018	**0.022**	0.033	0.040	0.123	0.096	**0.073**
Se_2	0.023	0.024	0.027	0.031	**0.026**	0.034	0.071	0.144	0.122	**0.093**
B_1	0.020	0.025	0.024	0.026	**0.024**	0.033	0.037	0.106	0.101	**0.069**
B_2	0.017	0.023	0.027	0.019	**0.021**	0.052	0.141	0.115	0.125	**0.108**
Se_1B_1	0.020	0.027	0.017	0.020	**0.021**	0.034	0.051	0.104	0.113	**0.076**
Se_1B_2	0.019	0.018	0.017	0.023	**0.019**	0.039	0.129	0.131	0.129	**0.107**
Se_2B_1	0.024	0.022	0.030	0.016	**0.023**	0.056	0.083	0.110	0.130	**0.095**
Se_2B_2	0.021	0.011	0.024	0.019	**0.018**	0.041	0.128	0.101	0.117	**0.097**
Mean (S)	**0.021**	**0.021**	**0.023**	**0.022**		**0.040**	**0.080**	**0.119**	**0.119**	
LSD at 5%	**S 0.0012**	**F 0.0018**	**SF 0.0029**			**S 0.0017**	**F 0.0025**	**SF 0.0042**		
Flowering stage										
Control	0.011	0.010	0.010	0.024	**0.014**	0.054	0.102	0.137	0.123	**0.104**
Se_1	0.011	0.009	0.008	0.013	**0.010**	0.065	0.042	0.140	0.105	**0.088**
Se_2	0.017	0.017	0.011	0.025	**0.018**	0.047	0.077	0.121	0.141	**0.096**
B_1	0.018	0.013	0.012	0.016	**0.015**	0.059	0.039	0.092	0.124	**0.078**
B_2	0.016	0.010	0.018	0.016	**0.015**	0.079	0.118	0.132	0.136	**0.116**
Se_1B_1	0.009	0.013	0.015	0.012	**0.012**	0.095	0.086	0.091	0.133	**0.101**
Se_1B_2	0.008	0.013	0.017	0.011	**0.012**	0.043	0.131	0.135	0.137	**0.112**
Se_2B_1	0.012	0.015	0.015	0.009	**0.013**	0.081	0.037	0.128	0.128	**0.094**
Se_2B_2	0.007	0.011	0.024	0.012	**0.014**	0.032	0.100	0.128	0.096	**0.089**
Mean (S)	**0.012**	**0.012**	**0.015**	**0.015**		**0.062**	**0.081**	**0.123**	**0.125**	
LSD at 5%	**S 0.0007**	**F 0.0010**	**SF 0.0017**			**S 0.0024**	**F 0.0036**	**SF 0.0060**		

Se_1 (Selenium 2 mg l^{-1}), Se_2 (Selenium 4 mg l^{-1}), B_1 (Boron 2 mg l^{-1}), B_2 (Boron 4 mg l^{-1})

were recorded with plants irrigated with the highest levels of saline water (5 and 7.5 dS m^{-1}). The impact of salinity was reported by many investigators, Hanafy Ahmed *et al.* [46] on wheat, Hanafy Ahmed *et al.* [35] on wheat, Khattab and Afifi [24] on canola, El-Danasoury *et al.* [47] on spearmint, Bybordi [48] on rapeseed, Nazarbeygi *et al*. [49] on canola, Alavi and Ranjbar [50] on rape and Yldz *et al*. [51] on canola who pointed out that salinity stress increased the concentration of proline. This accumulation may be caused by inhibition of proline catabolizing enzymes (proline oxidase and proline dehydrogenase) [52], enhancing activities of proline biosynthesis enzymes (ornithine aminotransferase and pyrroline-5-carboxylate reductase) [53], breakdown of proline rich proteins during stress [54], increase in proteolysis or decrease in protein synthesis [55].

Spraying selenium with boron significantly affected leaves proline concentration in both seasons. In the 1st season, using selenium alone at 4 mg l^{-1} resulted in the highest values in vegetative growth and flowering stages. On the other hand in the 2nd season, adding boron alone at 4 mg l^{-1} gave the maximum values in vegetative growth and flowering stages.

The role of selenium in increasing proline concentration might be due to the modifications induced by selenium in nitrogen assimilation, hormonal status, and/or expressions of proline genes [56]. Ibrahim and Ibrahim [31] mentioned that increasing the concentration of proline in the Se-treated potato plants could be attributed to that selenium alters the activity of some enzymes which involved in the biosynthesis of proline. For instance, Khan *et al*. [57] showed that applied selenium has caused increasing in the activity of glutamyl kinase (GK) and decreasing the activity of proline oxidase (PROX).

Moreover, the plants irrigated with the highest level of salinity (7.5 dS m^{-1}) and sprayed with the highest level of selenium (4 mg l^{-1}) recorded the highest proline concentration at vegetative growth and flowering stages of both seasons; selenium enhances tolerance of plants to stress by increasing endogenous proline content. The effect of selenium may be due to its role in enhancing tolerance of plants to stress by increasing endogenous proline content [58].

4. **Protein**

Data presented in Table (6) point out that increasing salinity levels significantly decreased protein concentration at vegetative growth and flowering stages of both seasons as compared with control. These results may be due to that salinity decreases protein synthesis and increases its hydrolysis due to the incapability to incorporate amino acids into proteins, increase in proteolytic enzymes, contribution of polysomes to monosomes, disturbance in nitrogen metabolism, inhibition of nitrate absorption, synthesis of absiccic acid which increases the activity of RNase, breaking of electrostatic bonds and increasing hydrophobic interactions thus inhibiting the protein synthesis [59, 60, 61]. Moreover, release of proteins under stress by the stressed cells involved in nutrient transport and other protein released is an important component for membrane structure providing cells the ability to withstand high degree of salinity [26, 62].

The foliar application of selenium with boron significantly affected protein concentration at vegetative growth and flowering stages of both seasons. The maximum values of proline were recorded by the lowest level of selenium (2 mg l^{-1}) combined with the lowest level of boron (2 mg l^{-1}). The promotive effect of selenium was previously investigated by Hajiboland and Keivanfar [10] on canola who recorded that selenium application increased protein content.

Table (6): Effect of salinity, selenium and boron on protein concentration (mg g^{-1} fresh weight) of *Brassica napus* herb at vegetative growth and flowering stages during 2012/2013 and 2013/2014 seasons

Foliar Application (F) mg l^{-1}	2012/2013 Salinity (S) dS m^{-1}					2013/2014 Salinity (S) dS m^{-1}				
	0	2.5	5	7.5	Mean (F)	0	2.5	5	7.5	Mean (F)
Vegetative growth stage										
Control	25.6	28.3	19.9	30.4	**26.0**	20.9	18.0	16.6	21.0	**19.1**
Se_1	40.3	37.7	32.2	31.1	**35.3**	29.6	19.6	17.5	23.0	**22.4**
Se_2	36.2	32.5	30.8	38.2	**34.4**	19.2	27.5	20.8	27.4	**23.7**
B_1	31.4	36.6	34.4	33.1	**33.9**	30.2	20.7	15.9	18.5	**21.3**
B_2	36.4	31.3	37.1	24.5	**32.3**	32.2	28.8	17.3	16.3	**23.7**
Se_1B_1	40.4	39.0	38.0	26.9	**36.1**	33.1	30.0	22.2	20.8	**26.5**
Se_1B_2	31.8	28.1	26.2	28.7	**28.7**	21.7	18.9	25.6	16.1	**20.6**
Se_2B_1	31.8	30.2	40.2	20.3	**30.6**	31.3	29.0	18.7	20.9	**25.0**
Se_2B_2	30.4	14.6	28.0	20.0	**23.2**	26.8	18.6	23.0	17.0	**21.4**
Mean (S)	**33.8**	**30.9**	**31.9**	**28.1**		**27.2**	**23.5**	**19.7**	**20.1**	
LSD at 5%	**S 1.36**	**F 2.05**	**SF 3.42**			**S 1.48**	**F 2.22**	**SF 3.71**		
Flowering stage										
Control	35.9	34.4	24.4	33.7	**32.1**	19.6	15.1	25.8	27.0	**21.9**
Se_1	31.8	32.9	21.6	24.9	**27.8**	20.6	19.7	20.0	16.2	**19.1**
Se_2	32.8	40.6	19.8	18.3	**27.9**	18.8	19.2	20.9	25.9	**21.2**
B_1	40.6	37.4	27.1	23.2	**32.1**	18.7	25.2	18.1	25.4	**21.9**
B_2	40.6	34.9	39.3	24.1	**34.7**	32.3	33.0	28.6	15.2	**27.3**
Se_1B_1	46.4	29.3	38.4	25.9	**35.0**	18.7	18.9	25.0	24.6	**21.8**
Se_1B_2	17.3	40.1	26.8	22.2	**26.6**	24.3	35.3	29.8	21.0	**27.6**
Se_2B_1	45.5	23.0	34.6	18.5	**30.4**	23.5	21.1	23.7	19.9	**22.0**
Se_2B_2	24.5	32.8	27.2	21.4	**26.5**	16.3	27.5	21.4	21.5	**21.7**
Mean (S)	**35.0**	**33.9**	**28.8**	**23.6**		**21.4**	**23.9**	**23.7**	**21.9**	
LSD at 5%	**S 1.71**	**F 2.56**	**SF 4.28**			**S 1.58**	**F 2.38**	**SF 3.97**		

Se_1 (Selenium 2 mg l^{-1}), Se_2 (Selenium 4 mg l^{-1}), B_1 (Boron 2 mg l^{-1}), B_2 (Boron 4 mg l^{-1})

The favorable effect of selenium and boron on protein concentration may be due to that selenium increases protein synthesis [63] as well as the role of boron in the process of atmospheric nitrogen fixation [64] which in turn helps in the process of protein synthesis [65]. Moreover, Mengel and Kirkby [41] stated that boron may have possible effects on DNA and RNA synthesis, which could increase the rate of protein synthesis.

Generally, spraying 2 mg l^{-1} of selenium combined with 2 mg l^{-1} of boron on the plants irrigated with saline water resulted in the highest protein concentration.

5. Nitrate

The results in Table (7) state that, increasing salinity levels increased nitrate concentration at vegetative growth and flowering stages of both seasons. The highest level of salinity (7.5 dS m^{-1}) produced the maximum nitrate concentration in leaves of both seasons. While at the vegetative growth stage of the 2nd season, using saline water at 5.0 dS m^{-1} resulted in the highest concentration but there were no significant differences between 0, 2.5, 5 and 7.5 dS m^{-1}. The effect of salinity in increasing nitrate concentration may be due to its role in decreasing nitrate reductase activity [66].

Moreover, spraying the plants with selenium and boron decreased leaves nitrate concentration at vegetative growth and flowering stages of both seasons. The addition of selenium alone at 2 mg l^{-1} recorded the maximum values at vegetative growth stage of the 1st season as well as flowering stage of the 2nd season. Whereas the combination between selenium and boron lowered nitrate content in both seasons.

Table (7): Effect of salinity, selenium and boron on nitrate concentration (mg g^{-1} dry weight) of *Brassica napus* herb at vegetative growth and flowering stages during 2012/2013 and 2013/2014 seasons

Foliar Application (F) mg l^{-1}	2012/2013 Salinity (S) dS m^{-1} 0	2.5	5	7.5	Mean (F)	2013/2014 Salinity (S) dS m^{-1} 0	2.5	5	7.5	Mean (F)
Vegetative growth stage										
Control	2.11	2.26	3.04	3.34	**2.69**	0.27	0.13	0.29	0.35	**0.26**
Se_1	3.20	3.78	2.76	4.19	**3.48**	0.13	0.15	0.19	0.26	**0.18**
Se_2	2.27	2.15	3.47	2.81	**2.67**	0.27	0.15	0.22	0.16	**0.20**
B_1	3.88	2.51	3.45	3.14	**3.25**	0.21	0.23	0.22	0.17	**0.21**
B_2	3.19	3.88	3.17	3.56	**3.45**	0.15	0.22	0.28	0.20	**0.21**
Se_1B_1	2.22	3.06	2.89	3.00	**2.79**	0.16	0.32	0.19	0.29	**0.24**
Se_1B_2	2.71	2.60	2.65	3.13	**2.77**	0.13	0.24	0.21	0.13	**0.18**
Se_2B_1	2.60	2.78	3.37	2.33	**2.77**	0.23	0.18	0.20	0.17	**0.19**
Se_2B_2	3.28	3.09	3.06	2.66	**3.02**	0.16	0.27	0.18	0.11	**0.18**
Mean (S)	**2.83**	**2.90**	**3.10**	**3.13**		**0.19**	**0.21**	**0.22**	**0.20**	
LSD at 5%	S ns	F 0.620	SF ns			S 0.015	F 0.023	SF 0.038		
Flowering stage										
Control	0.72	1.00	1.47	1.59	**1.19**	0.22	0.20	0.29	0.23	**0.23**
Se_1	1.00	1.03	0.72	1.13	**0.97**	0.21	0.17	0.33	0.37	**0.27**
Se_2	0.95	0.73	1.23	1.02	**0.98**	0.19	0.14	0.16	0.16	**0.16**
B_1	1.06	1.02	1.01	1.09	**1.05**	0.23	0.35	0.23	0.15	**0.24**
B_2	0.84	1.01	0.91	1.06	**0.95**	0.12	0.27	0.11	0.16	**0.16**
Se_1B_1	1.01	0.81	1.03	1.00	**0.96**	0.12	0.11	0.12	0.14	**0.12**
Se_1B_2	0.93	1.00	1.53	0.99	**1.11**	0.18	0.17	0.24	0.22	**0.20**
Se_2B_1	1.07	1.31	0.95	1.01	**1.08**	0.30	0.15	0.18	0.22	**0.21**
Se_2B_2	0.67	0.90	0.77	1.05	**0.85**	0.13	0.15	0.15	0.33	**0.19**
Mean (S)	**0.92**	**0.98**	**1.07**	**1.10**		**0.19**	**0.19**	**0.20**	**0.22**	
LSD at 5%	S 0.041	F 0.062	SF 0.103			S 0.012	F 0.018	SF 0.030		

Se_1 (Selenium 2 mg l^{-1}), Se_2 (Selenium 4 mg l^{-1}), B_1 (Boron 2 mg l^{-1}), B_2 (Boron 4 mg l^{-1})

In regard to selenium, Hajiboland and Keivanfar [10] observed higher nitrate reductase activity in Se-treated canola plants. The influence of boron on nitrate content may be as a result of its indirect effects in increasing translocation of nitrate from vacuoles (inaccessible nitrate for reduction) to cytoplasm (accessible nitrate for reduction) and inducing nitrate availability to nitrate reductase [13, 67].

Concerning the interaction between salinity with selenium and boron, generally, plants irrigated with the highest level of salinity (7.5 dS m^{-1}) and sprayed with the lowest level of selenium (2 mg l^{-1}) increased nitrate concentration in leaves of the plants in both seasons. It was observed that selenium and boron played an important role in enhancing the tolerance of the plants to salt stress.

COCLUSION

Generally, from the above mentioned results of both successive seasons, it can be suggested that selenium and boron either alone or in combination under both non-saline and saline water conditions significantly increased total sugars, total soluble phenols, total free amino acids and proline concentrations at the vegetative growth and flowering stages of canola plants. The selenium-boron combinations have the superiority effect. In this respect, the favorable effects of selenium or boron alleviating the harmful effects of salinity on canola plants could be directly through increase the ability of plants under salinity stress to accumulate substances; selenium and or boron might be an adaptive response to salinity, through raising the concentration of these simple organic substances.

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