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Full Length Research Paper

Zinc, nitrogen and salinity interaction on agronomic traits and some qualitative characteristic of canola

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A 2-year greenhouse experiment was carried out at the East Azerbaijan Agronomy and Natural Resources Research Center, Tabriz, Iran, during the two seasons of 2009 to 2010 and 2010 to 2011.The experimental design aimed to study the response of canola to different nitrogen and zinc fertilizer levels under two doses of salinity stress. The experimental design was randomized complete block design (RCBD) with three replications. The obtained results from two years showed that nitrogen increased seed yield, but there was no significant differences between two nitrogen levels. Zinc application had no significant effect on seed yield. Conversely, salinity decreased seed yield and yield components dramatically. Moreover, plant height increased due to nitrogen and zinc application. Nitrogen fertilizer application improved seed number per silique and 1000-seed weight over two years. Among yield components, only number of seed per silique was affected by zinc and increased in the first year. Oil percentage decreased due to nitrogen, zinc and salinity in the first year but zinc had no significant effect on oil in the second year. Salinity stress increased glucosinolate and protein content. Other treatments, however, had no significant effect on these traits. Salt stress induction decreased N, P, K, Ca and Mg content, but increased Na, Cl and Na/K ratio.

Key words: Canola, nitrogen, salinity, yield and yield components, zinc.

INTRODUCTION

Salt stress is a major abiotic stress reducing the productivity of crops in many areas of the world (Yamaguchi and Blumwald, 2005). This is particularly the case in semi-arid and arid zones, where already over a decade ago 50% of the cropland was salt affected (Flower and Yeo, 1995). Soil salinity occurs from natural processes or from crop irrigation with saline water. Salinity affects the water balance and results in osmotic damage; however, osmotic adjustment is a plant adaptation mechanism used to maintain their water balance in plant (Sairam and Tyagi, 2004). This can lead to a reduction of biodiversity and land degradation (Ghassemi et al., 1995). In many plant species, soil salinity is known to reduce growth and development through osmotic stress, ion toxicity, mineral deficiencies and induced physiological and biochemical disorders in metabolic processes (Hasegawa et al., 2000). Salinity stress is often associated with nutritional imbalance. The interaction between salt stress and other environmental factors influence the plant's response to the stress (Ashraf and McNeilly, 2004). Soil salinity may reduce micronutrients uptake due to stronger competition by salt cations at the root surface (Marschner and Romheld, 1994).

Ion uptake and compartmentalization are crucial not only for normal growth but also for growth under saline conditions (Adams et al., 1992). Nitrogen and zinc are essential minerals required for normal physiological processes of plants. Nitrogen fertilizer is the most important element for crop growth and high yield with good quality as it causes an increase in photosynthesis rate, metabolites synthesized, meristematic activity and assimilates transport to the seed. Especially, nitrogen fertilizer is inevitable to promote the growing in critical periods. However, this positive reaction continues increasing to a certain level and declines (Kusvuran, 2011). Seed yield and yield attributes increased by increasing nitrogen levels (Ali and Zaman, 1997). Nitrogen fertilizer increases yield by influencing a variety of growth parameters such as the number of branches

per plant, the number of pods per plant, the total plant weight, the leaf area index. Also, it increases the number and weight of pods, seeds and flowers per plant, and overall crop assimilation, contributing to increased seed yield (Al-Barrak, 2006).

Zinc is an essential microelement required by higher plants, and is mainly absorbed in the form of Zn^{++}. Increasing evidence suggests that zinc status of plants plays a critical role in increasing plant resistance to environmental stresses (Marschner, 1995). Zinc plays an important role in contributing to the survival of crop plants under environmental stress conditions. Zinc also plays an important role in the production of biomass (Cakmak, 2008). In addition, Zn plays other indirect and significant roles as stabilizer of proteins, membranes, and DNA-binding proteins such as Zn-fingers (Aravind and Prasad, 2003). Furthermore, zinc may be required for chlorophyll production, pollen function and fertilization (Pandey et al., 2006). Zinc is required for the biosynthesis of the plant growth regulator such as indole acetic acid (IAA) and for carbohydrate and N metabolism which leads to high yield and yield components. Zinc is an essential micronutrient for higher plants especially oil crops as it is required for activity of various types of enzymes (dehydrogenases, RNA and DNA polymerases), carbohydrate metabolism and protein synthesis. Zinc also plays an important role in the production of biomass (Kaya and Higgs 2002). Furthermore, zinc may be required for chlorophyll production, pollen function and fertilization (Pandey et al., 2006). Zinc deficiency also affects carbohydrate metabolism, damages pollen structure, and decreases the yield (Fang et al., 2008).

Bybordi and Malakouti (2007) found that application of zinc had a significant effect on seed yield, seed oil content and 1000-seed weight. Zinc deficiency is one of the most widespread micronutrient deficiencies in Iran as a result for the alkaline soil condition. So, it is very important to apply zinc fertilizer for increasing crop yields and improving crop quality. The family Brassicaceae includes a number of species that have considerable nutritional and economic values and that have been under cultivation since 1500 B.C. These crops are extensively grown as cash crops, fodder and industrial/medicinal crops (Ashraf and McNeilly, 2004). The most common Brassica oilseed crops grown in the world for industrial purpose are rape-seeds, *Brassica campestris* and *Brassica napus*. Therefore, this investigation aimed to study the response of canola (*B. napus* L.) to nitrogen and zinc fertilizer under conditions of salt stress.

MATERIALS AND METHODS

Two pot experiments were conducted at the east Azerbaijan Agronomy and Natural Resources Research Center, Tabriz, Iran, during the growing seasons of 2009 to 2010 and 2010 to 2011 to study the response of canola to different nitrogen and zinc fertilizer levels under two doses of salinity stress. The experiment was conducted in a glasshouse in plastic pots (30 cm diameter and 40 cm depth) containing 20 kg soil. The zinc deficient soil was collected from an agricultural field. The soil was sandy loam, moderately calcareous, low in nitrogen, low in organic matter and alkaline in reaction having a pH of 7.7.

Treatments consisted of different nitrogen levels (0, 50 and 100 $kgha^{-1}$ nitrogen from urea), different zinc levels (0, 5 and 10 $kgha^{-1}$ zinc from zinc sulfate) and two dose of saline water (8 and 16 dSm^{-1}).The amount of urea and zinc sulfate was calculated based on pot surface. All the pots were fertilized with above mentioned fertilizers so that fertilizers were incorporated into the soil before seeding. The pots were arranged according to three-factor randomized complete block design (RCBD) in three replications. The growth conditions were as follows: photoperiod of 14/10 h (day/night), temperature of 25/18°C (day/night), and the maximal photosynthetic photon flux density of 600 µmol $m^{-2}s^{-1}$. The pots were labeled and randomly arranged in the greenhouse and rearranged several times during the growth period. The seed surface was sterilized by immersion in 2% sodium hypochlorite solution for 10 min and 96% ethanol for 30 s then seeds were washed with distilled water for several times. Ten seeds were sown directly in plastic pots.

Application of salt stress

Immediately after sowing, soils were watered and watering was carried out regularly every two days during the experiment. The canola seedlings were thinned to four uniform stands two weeks after planting. One week after sowing, the salt treatments were applied. Salt treatments were applied by adding appropriate amount of NaCl in irrigation water. The EC of the irrigation water was 8 and 16 dS m^{-1}. To avoid early plant death by a sudden salt stress shock of the young seedlings, the salt stress was imposed gradually by applying half of the salt concentration over two weeks and increasing it to the final concentration for other weeks. The salinity treatments were maintained until final harvest.

Estimation of parameters

At harvest time, plants were sampled to estimate, plant height, and number of silique per plant, number of seeds per silique, 1000-seed weight, and seed yield per plant. Seed oil percentage was analyzed according to A.O.A.C. (1980) with Soxhlet apparatus.

Glucosinolate was extracted and analyzed as previously described with minor modifications (Jia et al., 2009). Samples were boiled in 4 ml water for 10 min. After recovery of the liquid, the residues were washed with water, and the combined aqueous extract was applied to a DEAE-Sephadex A-25 column (pyridine acetate form). The column was washed three times with 20 mM pyridine acetate and twice with water. The glucosinolates were converted into their desulpho-analogues by overnight treatment with 100 µL of 0.1% (1.4 units) aryl sulphatase, and the desulphoglucosinolates were eluted with 2 × 0.5 ml water. HPLC analysis was performed using an HPLC system consisting of a Waters 2695 separations module and a Waters 2996 photodiode array detector (Waters Corp., Milford, MA, USA). The HPLC system was connected to a computer with Empower Pro software. A Hypersil C18 column (5 m particle size, 4.6 mm 250 mm; Elite Analytical Instruments Co. Ltd., Dalian, China) was used with a mobile phase of acetonitrile and water at a flow rate of 1.0 ml/min. The procedure employed isocratic elution with 1.5% acetonitrile for the first 5 min; a linear gradient to 20% acetonitrile over the next 15 min followed by isocratic elution with 20% acetonitrile for the final 10 min. A 40-ll sample was injected onto the column by an auto sampler. Absorbance was detected at 226 nm. Sinigrin was used as an internal standard for HPLC analysis. Desulphoglucosinolates were identified by comparison of retention time and quantified by

Table 1. Analysis of variance on canola traits affected by nitrogen, zinc and salinity at first year.

S.O.V	d.f	Seed yield	Plant height	Silique per plant	Seed per silique	1000-seed weight	Oil	Glucosinolate	Protein	N	P	K	Ca	Mg	Na	Cl	Na/K
B	2	**	**	ns	ns	ns	ns	**	ns	**	ns	ns	ns	ns	ns	ns	ns
N	2	**	**	ns	**	**	**	ns	ns	**	ns	ns	ns	ns	ns	ns	ns
Z	2	ns	**	ns	**	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S	1	**	**	**	**	**	**	**	**	*	ns	**	**	ns	**	**	*
NZ	4	ns	ns	ns	ns	ns	*	ns	ns	ns	**	ns	ns	ns	ns	ns	ns
NS	2	*	**	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
ZS	2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
NSZ	4	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Error	34	527.09	14.30	0.04	5.69	0.02	0.92	28.56	0.04	0.02	0.00	0.01	0.01	0.00	4.63	3.96	0.98
C.V. %		20.87	4.83	1.68	7.16	5.73	2.34	19.26	1.68	7.42	6.75	4.59	10.11	4.62	23.02	18.90	24.74

B, Block; N, nitrogen; Z, zinc; S, salinity; *, ** and ns: Significant at 0.05, 0.01 probability level and not significant, respectively.

peak area. For calculation of molar concentrations of individual glucosinolates, the relative response factors reported by Tokuhisaet al. (2003) were used to correct for absorbance differences between the standard and the other glucosinolates. The glucosinolate concentration was expressed as Lmolg-^{1} fresh weight of radish sprouts.

Protein percentage was estimated by using Inframatic 8620 Percor. Total N and P were determined through titration method by Kjeltec Auto 1030 Analyzer, Tecator and calorimetric method by spectrophotometer, 6505 JenWay, respectively. For ion determination, fresh samples were extracted in 0.1 N nitric acid. Na^{+}, Mg^{2++}, and Ca^{2+} contents in the samples were detected by automatic absorption. K^{+} was detected by flame photometry in the samples from canola plants. For chloride determination, Cl^{-} was determined by the silver ion-titration method with an automatic chloride meter (Buckhler-Cotlovechlorido meter).

Statistical analysis

All data were analyzed from analysis of variance (ANOVA) using the SAS version 9.1. Duncan's multiple range test was used to measure statistical differences between treatments. Also the charts were drawn by Excel, Microsoft Office 2003.

RESULTS AND DISCUSSION

The analysis of variance showed that the application of nitrogen had significant effect on seed yield, plant height, seed number per silique, 1000 seed-weight, oil percentage and seed nitrogen content in the first year (Table 1), while in the second year, effect of nitrogen fertilizer was significant on seed yield, plant height, seed number per silique, 1000 seed-weight, oil percentage and seed nitrogen content (Table 2). Regarding zinc application, we observed that there were significant effects on plant height, seed number per silique and oil percentage in the first year, and plant height in the second year (Tables 1 and 2). In case of salt stress, results indicated that salinity had significant effect on all traits except for phosphorus and magnesium content in the first year (Table 1). In the second year, salinity showed no significant effect on phosphorus, calcium, magnesium and sodium to potassium ratio (Table 2). In some traits, interactions were significant as illustrated in Figures 1 to 9.

Estimated parameters

Seed yield

Comparison of means demonstrated that nitrogen application increased seed yield compared to without nitrogen in both years, although, there was no significant difference between two doses of nitrogen (Tables 3 and 4). In addition, increase of salinity up to 16 dS m^{-1} significantly decreased seed yield over two years. Increased seed yields due to nitrogen application were also reported by Nourai (1982). The increase in yield and yield attributes may be due to nitrogen fertilizer resulting in increase in photosynthesis rate, metabolites synthesized and assimilates transport from source to the sink consequently increase of yield. In addition, El Hilo et al. (1970) reported that flower stalks and caused burning of leaf tips.

Table 2. Analysis of variance on canola traits affected by nitrogen, zinc and salinity at second year.

S.O.V	d.f	Seed yield	Plant height	Silique per plant	Seed per silique	1000-seed weight	Oil	Glucosinolate	Protein	N	P	K	Ca	Mg	Na	Cl	Na/ K
B	2	**	**	**	**	ns	ns	**	**	ns	**	**	ns	ns	*	ns	*
N	2	**	**	ns	**	**	*	ns	ns	**	ns	ns	ns	ns	ns	ns	ns
Z	2	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S	1	**	**	**	**	**	**	**	**	**	ns	**	ns	ns	*	**	ns
NZ	4	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	**	ns	ns	ns	ns	ns
NS	2	*	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
ZS	2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
NSZ	4	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Error	34	527.09	14.3	0.15	9.29	0.07	8.45	6.01	0.15	0.01	0	0.04	0	0	29.23	47.03	4.03
C.V. %		21.45	5.09	3.09	8.29	15.93	7.27	7.07	3.09	5.71	5.05	6.37	4.54	4.82	19.82	35.5	23.78

B, Block; N, nitrogen; Z, zinc; S, salinity; *, ** and ns: Significant at 0.05, 0.01 probability level and not significant, respectively.

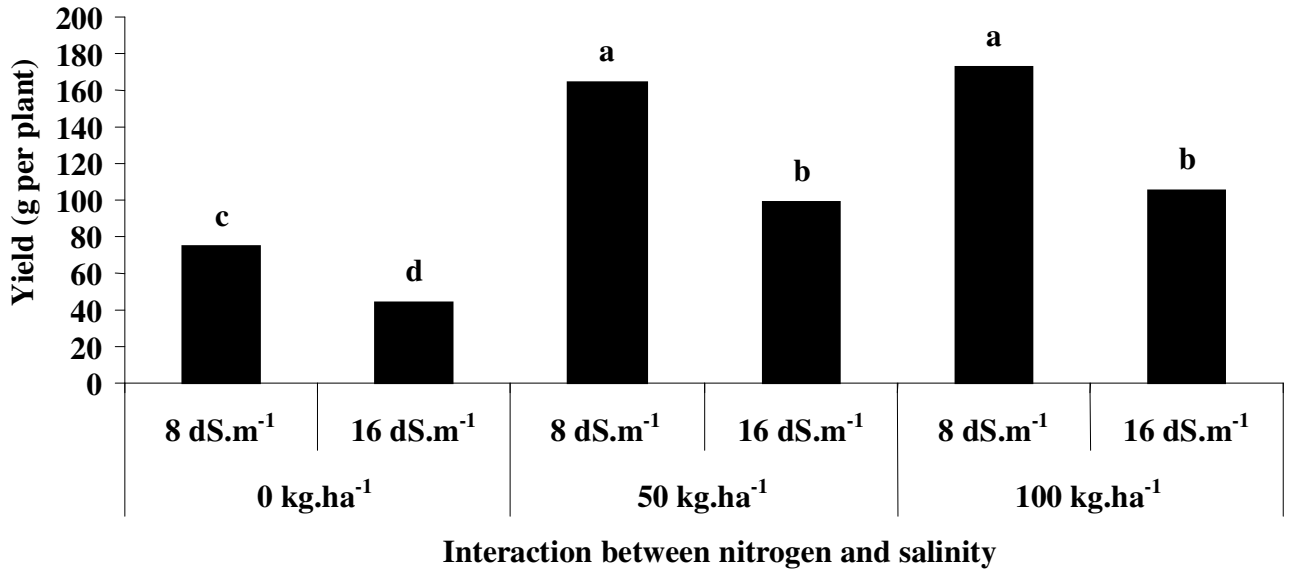

Figure 1. Interaction between different nitrogen levels and salinity stress in the first year showed that seed yield was strongly affected by these treatments so that increase of salinity level decreased seed yield under each level of nitrogen fertilizer. In addition, there was no significant different between 50 and 100 kgha^{-1} nitrogen.

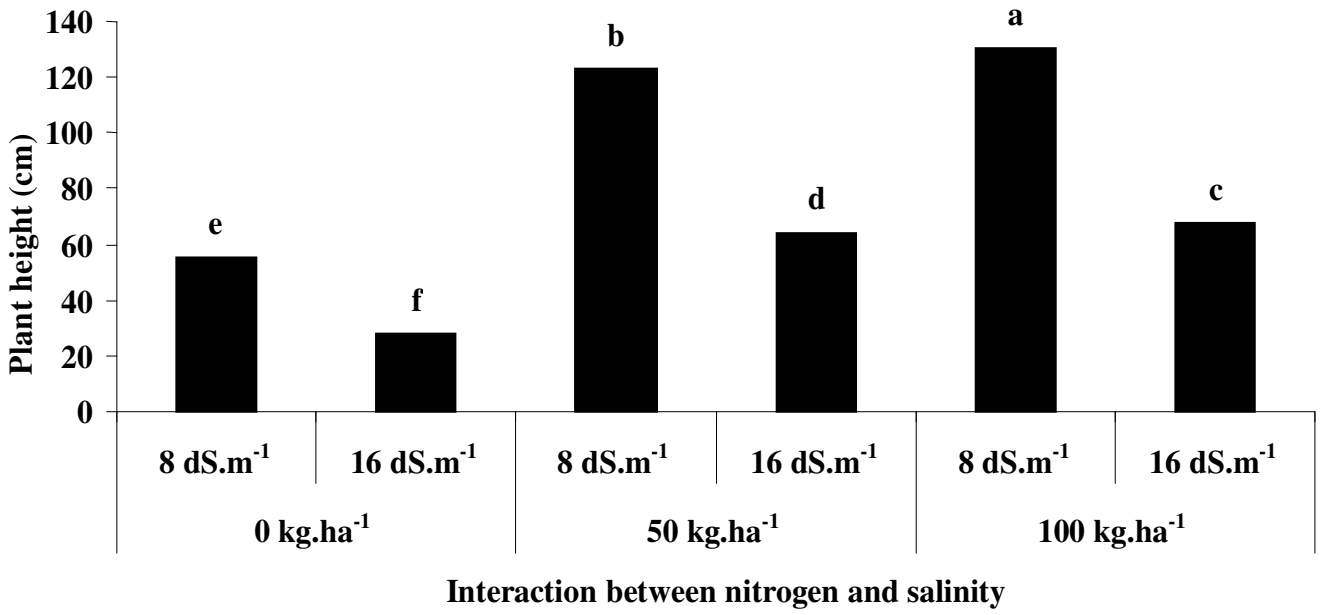

Figure 2. Interaction between different nitrogen levels and salinity stress in the first year. It was showed that plant height was affected by these treatments so that increase of salinity level decreased plant height under each level of nitrogen fertilizer. Application of 100 kgha^{-1} nitrogen significantly increased plant height whether under 8 or 16 dSm^{-1} salinity stress.

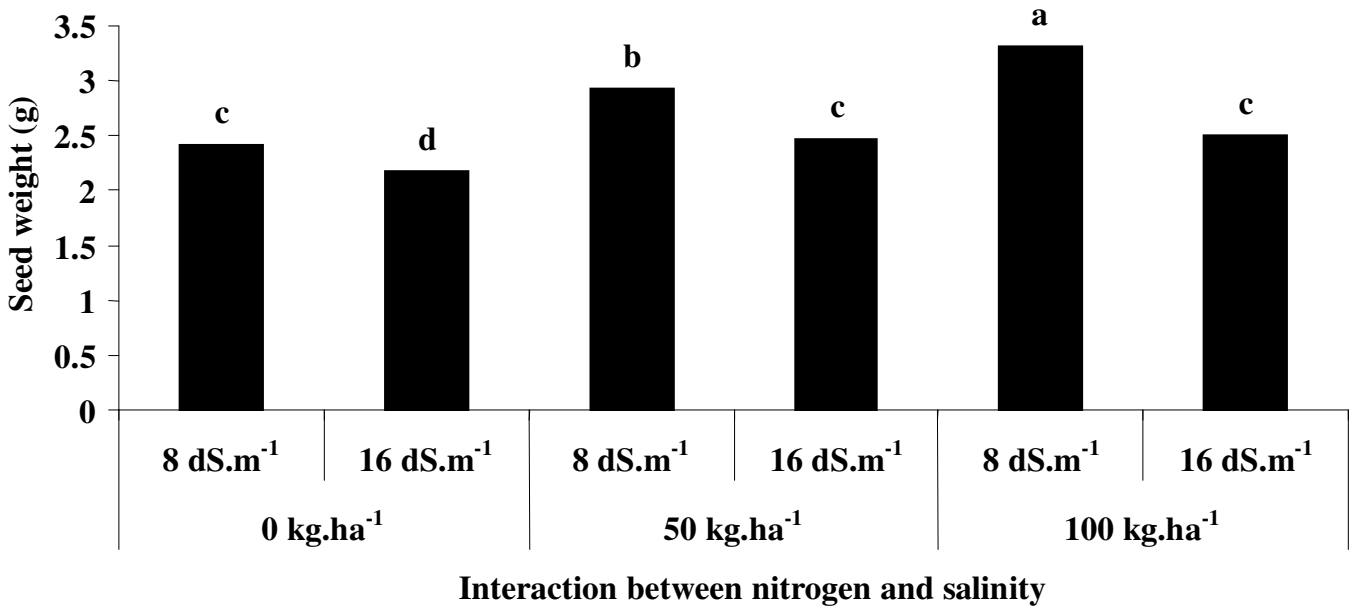

Figure 3. Interaction between different nitrogen levels and salinity stress in the first year. Results showed that seed weight was affected by these treatments so that increase of salinity level decreased canola seed weight under each level of nitrogen fertilizer. Application of 100 kgha^{-1} nitrogen significantly increased seed weight under conditions of 8 dSm^{-1}salinity stress, while application of this amount of nitrogen under conditions of 16 dSm^{-1} salinity stresses had no significant effect on seed weight.

The results (Tables 3 and 4) showed that NaCl adversely affected the seed yield. The salt stress caused significant reductions in all the growth variables including dry weights. Salinity has both osmotic and specific ion effects on plant growth (Dionisio-Sese and Tobita, 2000). In the study, salt stress caused a significant decrease in the plant height, dry weights of root, shoot and leaf of canola (data not shown) followed by decrease in seed yield.

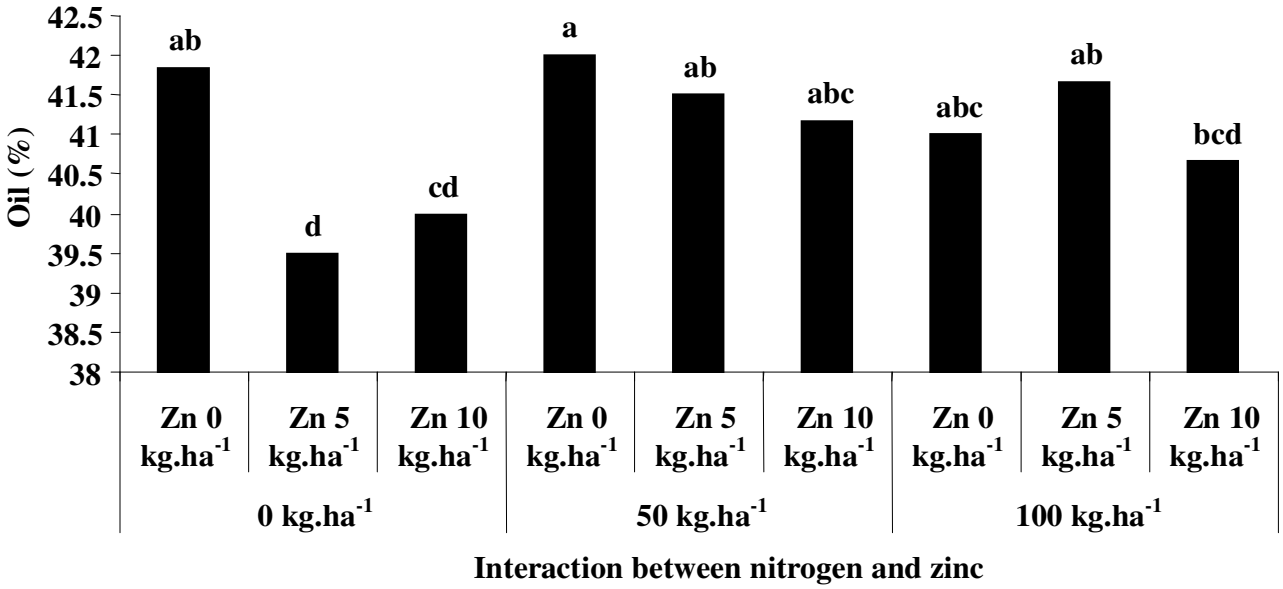

Figure 4. Interaction between different nitrogen and zinc levels in the first year. Results showed that zinc application deceased oil percentage under nitrogen free treatments, while nitrogen application increased oil percentage. The highest oil percentage was obtained when plants were treated by 50 kgha^{-1} nitrogen with no zinc application, although there was no significant difference between 50 and 100 kgha^{-1} nitrogen levels.

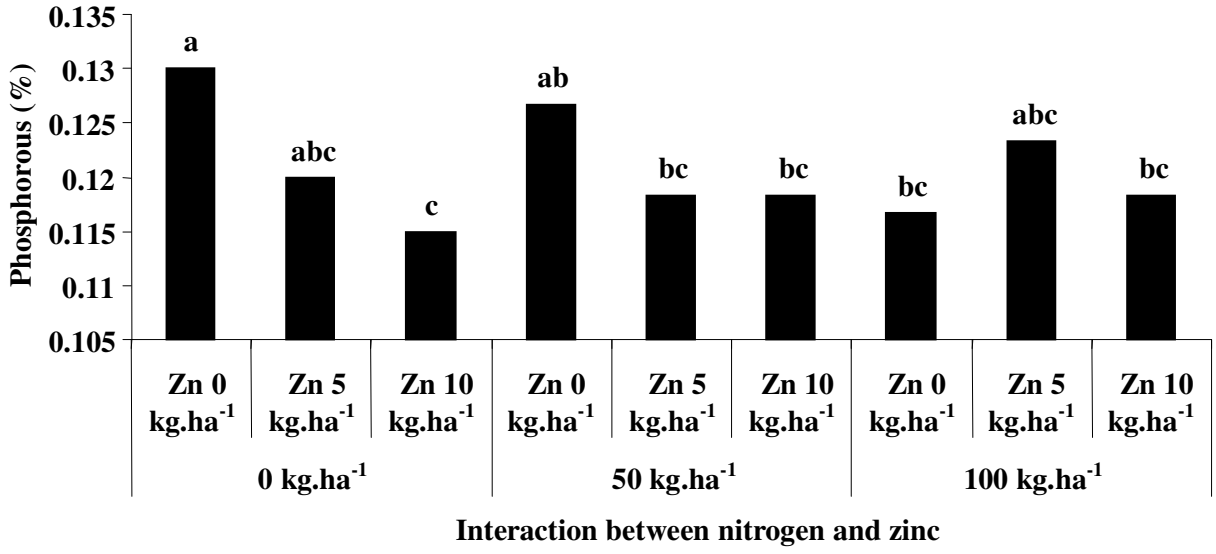

Figure 5. Interaction between different nitrogen and zinc levels in the first year. Results showed that zinc application deceased phosphorus content under nitrogen free treatments because of competitiveness effect between zinc and phosphorus, while nitrogen application increased phosphorus accumulation.

Reduction in plant growth as a result of salt stress has also been reported in other plant species (Ashraf and O'leary, 1997; Turkmen et al., 2008).

Plant height

Means of plant height under the nitrogen fertilizer rates in both years showed an increase in plant height as nitrogen fertilizer rate increased (Tables 3 and 4). A previous result might (Kusvuran, 2011) be due to the positive effect of nitrogen on the growth development of stem and leaf, which was reflected into taller plants. By contrast, salinity mitigated plant growth. Similar results were found in both years. The most common adverse effect of salinity on the crop of *Brassica* is the reduction in

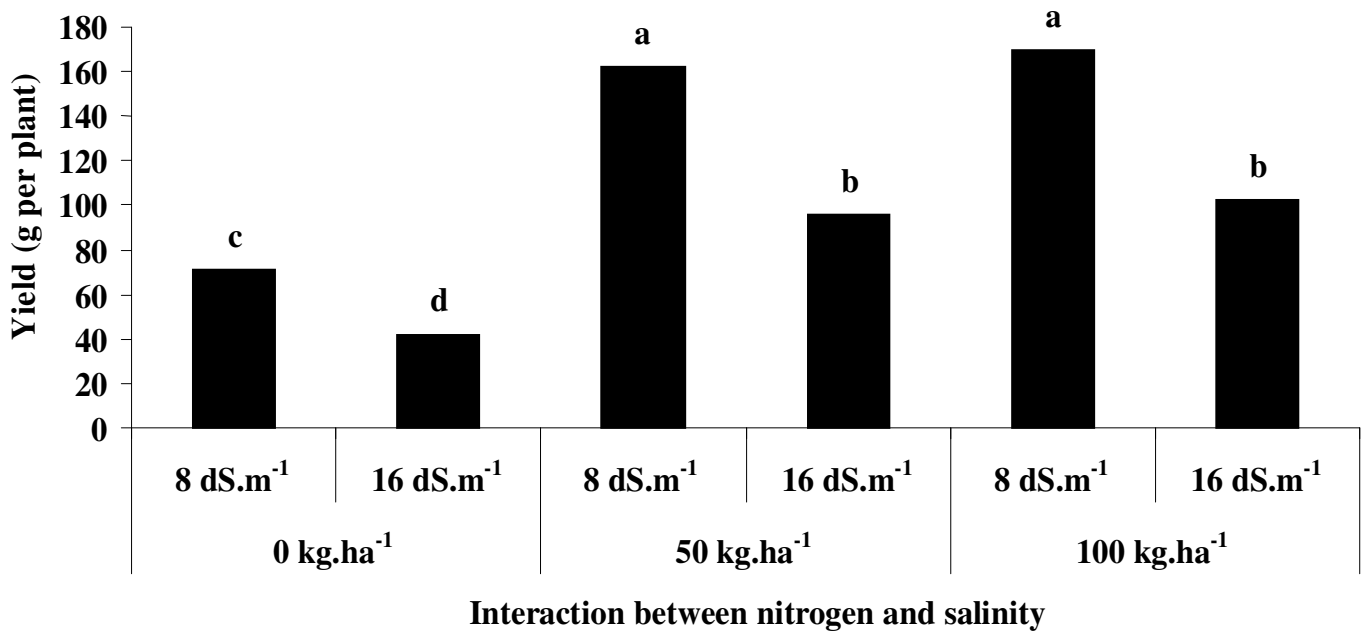

Figure 6. Interaction between different nitrogen levels and salinity stress in the second year showed that seed yield was strongly affected by these treatments so that increase of salinity level decreased seed yield under each level of nitrogen fertilizer. In addition, there was no significant different between 50 and 100 kgha^{-1} nitrogen.

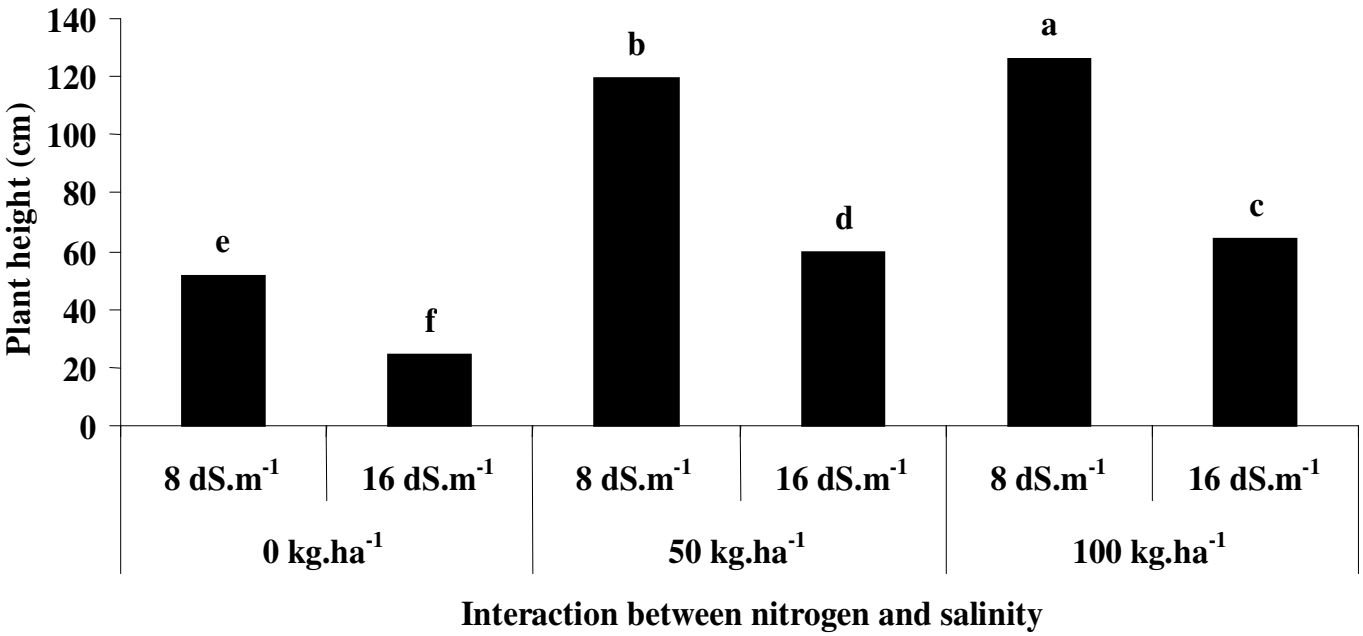

Figure 7. Interaction between different nitrogen levels and salinity stress in the second year. Result showed that plant height was affected by these treatments so that increase of salinity level decreased plant height under each level of nitrogen fertilizer. Application of 100 kgha^{-1} nitrogen significantly increased plant height whether under 8 or 16 dSm^{-1} salinity stress.

plant height, size and yield as well as deterioration of the quality of the product.

Our results are also in accordance with previous finding of Viégas et al. (2001) who reported that salinity inhibits shoot growth. On the other hand, zinc improved pant growth and increased plant height. Zinc plays important role in tryptophan biosynthesis, acting as precursor of auxin; it is expected that zinc will increase plant growth

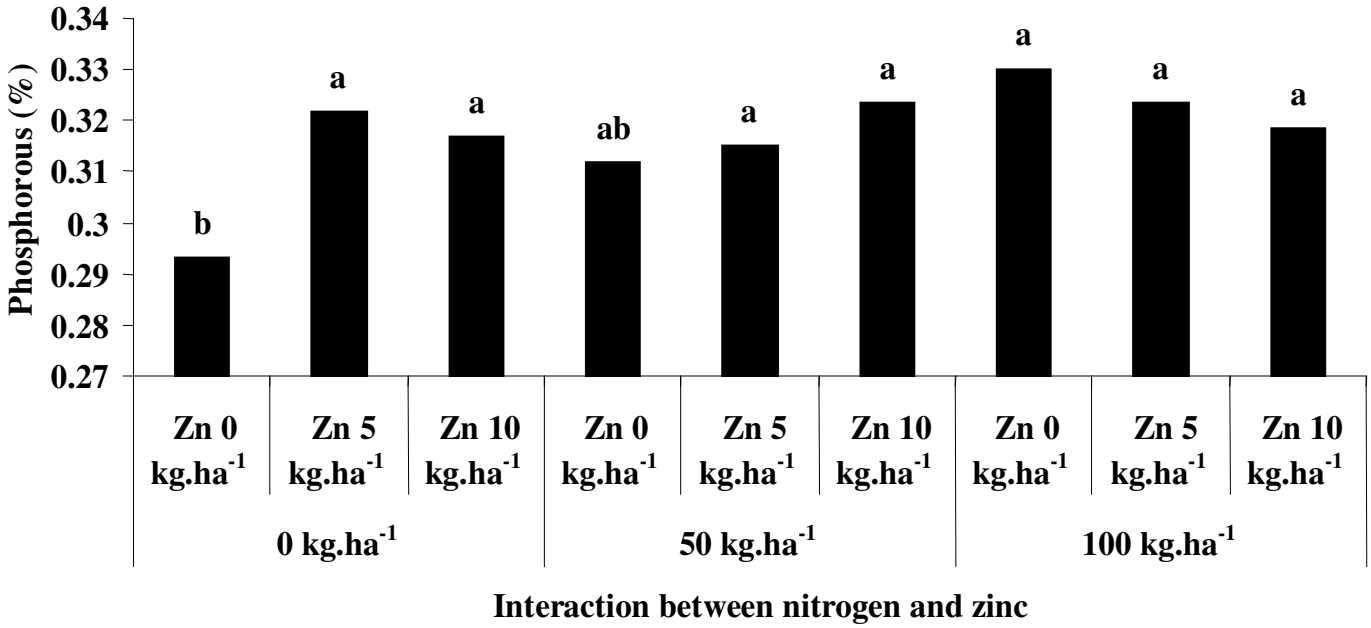

Figure 8. Interaction between different nitrogen and zinc levels in the second year showed that nitrogen and zinc increased phosphorus content compared with control treatment although there was no significant difference among treatments.

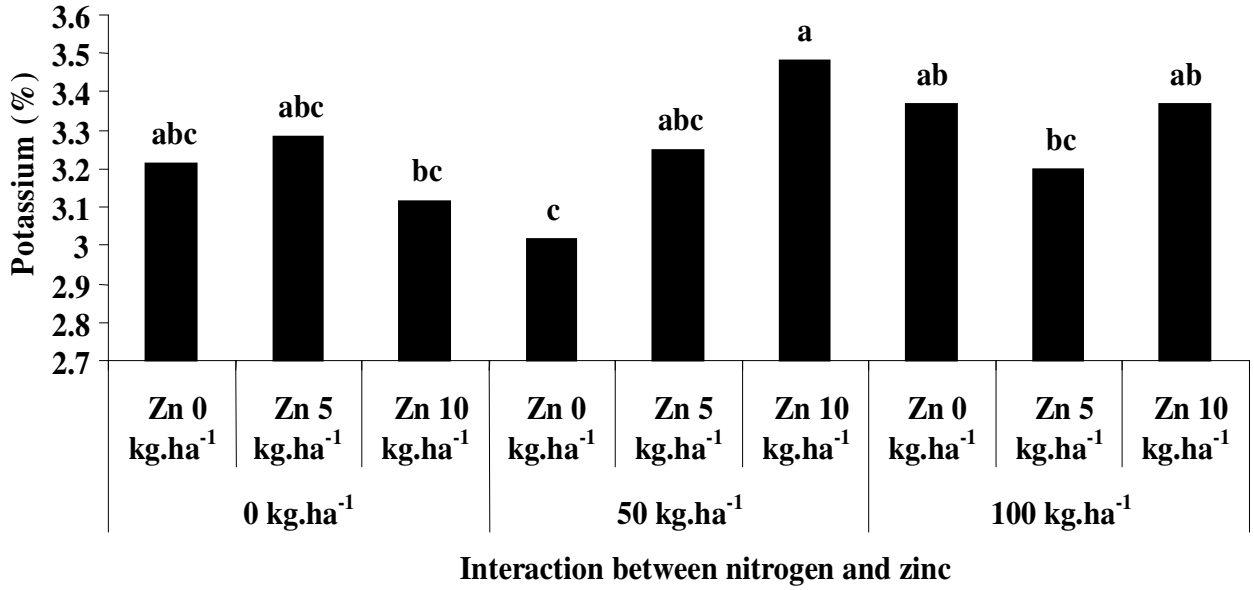

Figure 9. Interaction between different nitrogen and zinc levels in the second year. Results showed that potassium content increased as result of increase of zinc in those plants which were treated by 50 kg.ha^{-1}nitrogen. When 100 kg ha^{-1} nitrogen was applied zinc had no positive effect on potassium accumulation.

and elongation. It has been reported that zinc plays an important role in the production of biomass (Kaya and Higgs, 2002). Welch et al. (1982) stated that zinc is necessary for root cell membrane integrity. As suggested by Marschner and Cakmak (1986), root cell membrane permeability is increased under zinc deficiency which might be related to the functions of zinc in cell membranes. Hence, external zinc application could increase the integrity of cell membrane and play important role in plant enzyme systems by increasing the assimilation. Also zinc is founded in phosphoenolpyruvate carboxylase structure.

Table 3. Main effects of nitrogen, zinc and salinity on canola traits (first year).

Treatment	Level	Seed yield	Plant height	Silique per plant	Seed per silique	1000-seed weight	Oil	Glucosinolate	Protein	N	P	K	Ca	Mg	Na	Cl	Na/K
Nitrogen	0 kg ha^{-1}	59.38^{b}	41.88^{c}	11.86^{a}	23.38^{c}	2.29^{c}	41.55^{a}	28.83^{a}	11.86^{a}	1.36^{c}	0.12^{a}	2.42^{a}	1.31^{a}	0.33^{a}	9.90^{a}	10.83^{a}	4.26^{a}
	50 kg ha^{-1}	131.88^{a}	93.55^{b}	11.94^{a}	35.55^{b}	2.70^{b}	41.11^{a}	27.00^{a}	11.94^{a}	2.25^{b}	0.12^{a}	2.43^{a}	1.34^{a}	0.33^{a}	8.95^{a}	10.44^{a}	3.78^{a}
	100 kg ha^{-1}	138.72^{a}	99.05^{a}	11.88^{a}	41.00^{a}	2.90^{a}	40.44^{b}	27.38^{a}	11.88^{a}	2.66^{a}	0.11^{a}	2.38^{a}	1.28^{a}	0.34^{a}	9.17^{a}	10.30^{a}	3.97^{a}
Zinc	0 kg ha^{-1}	100.44^{a}	75.83^{b}	11.96^{a}	31.27^{c}	2.61^{a}	41.61^{a}	27.83^{a}	11.96^{a}	2.10^{a}	0.11^{a}	2.37^{a}	1.28^{a}	0.34^{a}	9.15^{a}	10.22^{a}	3.99^{a}
	5 kg ha^{-1}	113.61^{a}	78.16^{ab}	11.87^{a}	33.50^{b}	2.60^{a}	40.88^{b}	28.27^{a}	11.87^{a}	2.08^{a}	0.12^{a}	2.41^{a}	1.32^{a}	0.33^{a}	9.44^{a}	11.00^{a}	4.04^{a}
	10 kg ha^{-1}	115.94^{a}	80.50^{a}	11.85^{a}	35.16^{a}	2.68^{a}	40.61^{b}	27.11^{a}	11.85^{a}	2.08^{a}	0.12^{a}	2.45^{a}	1.32^{a}	0.33^{a}	9.44^{a}	10.36^{a}	3.98^{a}
Salinity	8 dSm^{-1}	137.14^{a}	102.96^{a}	12.45^{a}	32.70^{a}	2.88^{a}	40.88^{a}	27.00^{b}	12.45^{a}	2.14^{a}	12.45^{a}	2.54^{a}	1.35^{a}	0.33^{a}	4.11^{b}	6.79^{b}	1.62^{b}
	16 dSm^{-1}	82.85^{b}	53.37^{b}	11.34^{b}	28.92^{b}	2.37^{b}	37.18^{b}	29.48^{a}	11.34^{b}	2.03^{b}	11.34^{b}	2.28^{b}	1.27^{b}	0.34^{a}	14.57^{a}	14.25^{a}	6.39^{a}

Values within the same column and followed by the same letter are not different at P < 0.05 by an ANOVA protected Duncan's multiple range test.

Table 4. Main effects of nitrogen, zinc and salinity on canola traits (second year).

Treatment	Level	Seed yield	Plant height	Silique per plant	Seed per silique	1000-seed weight	Oil	Glucosinolate	Protein	N	P	K	Ca	Mg	Na	Cl	Na/K
Nitrogen	0 kg ha^{-1}	56.38^{b}	37.88^{c}	12.46^{a}	34.83^{b}	1.16^{c}	41.55^{a}	33.83^{a}	12.46^{a}	2.23^{b}	0.31^{b}	3.20^{a}	1.45^{a}	0.34^{b}	27.33^{a}	17.66^{a}	8.54^{a}
	50 kg ha^{-1}	128.88^{a}	89.55^{b}	12.64^{a}	36.44^{b}	1.66^{b}	39.22^{b}	35.27^{a}	12.64^{a}	2.28^{b}	0.31^{ab}	3.25^{a}	1.46^{a}	0.33^{b}	26.44^{a}	20.66^{a}	8.28^{a}
	100 kg ha^{-1}	135.72^{a}	95.05^{a}	12.63^{a}	39.00^{a}	2.64^{a}	39.16^{b}	34.83^{a}	12.63^{a}	2.43^{a}	0.32^{a}	3.31^{a}	1.42^{a}	0.33^{b}	28.05^{a}	19.61^{a}	8.51^{a}
Zinc	0 kg ha^{-1}	97.44^{a}	71.83^{b}	12.65^{a}	37.00^{a}	1.73^{a}	40.00^{a}	34.66^{a}	12.65^{a}	2.31^{a}	0.31^{a}	3.20^{a}	1.47^{a}	0.34^{a}	26.50^{a}	19.16^{a}	8.36^{a}
	5 kg ha^{-1}	110.61^{a}	74.16^{ab}	12.56^{a}	36.66^{a}	1.64^{a}	39.77^{a}	34.50^{a}	12.56^{a}	2.29^{a}	0.32^{a}	3.24^{a}	1.44^{a}	0.34^{a}	27.94^{a}	19.11^{a}	8.68^{a}
	10 kg ha^{-1}	112.94^{a}	76.50^{a}	12.53^{a}	36.61^{a}	1.68^{a}	40.16^{a}	34.77^{a}	12.53^{a}	2.34^{a}	0.31^{a}	3.32^{a}	1.42^{a}	0.34^{a}	27.38^{a}	19.66^{a}	8.28^{a}
Salinity	8 dSm^{-1}	134.14^{a}	98.96^{a}	12.85^{a}	39.48^{a}	1.81^{a}	41.37^{a}	33.51^{b}	12.85^{a}	2.45^{a}	0.32^{a}	3.41^{a}	1.45^{a}	0.34^{a}	25.74^{b}	15.40^{b}	8.40^{b}
	16 dSm^{-1}	79.85b	49.37^{b}	12.31^{b}	34.03^{b}	1.56^{b}	38.59^{b}	34.77^{a}	12.31^{b}	2.18^{b}	0.31^{b}	3.09^{b}	1.34^{b}	0.34^{a}	28.81^{a}	23.22^{a}	8.49^{a}

Values within the same column and followed by the same letter are not different at P < 0.05 by an ANOVA protected Duncan's multiple range test.

Table 5. Pearson Correlation Coefficients among different canola traits (first year).

Trait	Seed yield	Plant height	Silique per plant	Seed per silique	1000-seed weight	Oil	Glucosinolate	Protein	N	P	K	Ca	Mg	Na	Cl	Na/K
Yield	1															
Height	0.87**	1														
Silique per plant	0.47**	0.64**	1													
Seed per silique	0.61**	0.59**	0.76**	1												
Seed weight	0.81**	0.88**	0.81**	0.54**	1											
Oil	0.16^{ns}	0.14^{ns}	0.21^{ns}	0.26^{ns}	0.07^{ns}	1										
Glucosinolate	0.18^{ns}	-0.02^{ns}	0.18^{ns}	-0.11^{ns}	0.00^{ns}	0.23^{ns}	1									
Protein	0.47**	0.64**	0.45**	-0.06^{ns}	0.58**	-0.07^{ns}	0.15^{ns}	1								
N	0.69**	0.68**	0.82**	0.90**	0.67**	0.27*	-0.04^{ns}	0.10^{ns}	1							
P	0.47**	-0.10^{ns}	-0.09^{ns}	-0.06^{ns}	-0.08^{ns}	-0.12^{ns}	-0.12^{ns}	-0.11^{ns}	-0.10^{ns}	1						
K	0.39**	0.50**	0.33*	-0.10^{ns}	0.45**	-0.25^{ns}	0.13^{ns}	0.73**	0.00^{ns}	-0.05^{ns}	1					
Ca	0.18^{ns}	0.22^{ns}	0.09^{ns}	-0.09^{ns}	0.16^{ns}	-0.07^{ns}	0.18^{ns}	0.26^{ns}	-0.03^{ns}	-0.01^{ns}	0.29*	1				
Mg	-0.11^{ns}	-0.04^{ns}	0.00^{ns}	0.05^{ns}	-0.09^{ns}	0.24^{ns}	-0.13^{ns}	-0.16^{ns}	0.05^{ns}	-0.08^{ns}	-0.34*	-0.28*	1			
Na	-0.51**	-0.65**	-0.48**	0.03^{ns}	-0.62**	0.06^{ns}	-0.11^{ns}	-0.90**	-0.14^{ns}	0.07^{ns}	-0.74**	-0.29*	0.18^{ns}	1		
Cl	-0.49**	-0.63**	-0.44**	0.00^{ns}	-0.53**	0.08^{ns}	-0.16^{ns}	-0.87**	-0.13^{ns}	0.27*	-0.73**	-0.30*	0.26^{ns}	0.84**	1	
Na/K	-0.52**	-0.65**	-0.48**	0.03^{ns}	-0.62**	0.09^{ns}	-0.12^{ns}	-0.89**	-0.14^{ns}	0.07ns	-0.80**	-0.29*	0.22^{ns}	0.99**	0.84**	1

*, ** and ns: Significant at 0.05, 0.01 probability level and not significant, respectively.

Number of silique per plant

As for the number of silique per plant, the mean values in Tables 3 and 4 revealed that the lowest value was produced from the plants treated with 16 dSm^{-1} saline water. According to the findings of Lin et al. (2004), salinity stress lead to abscisic acid synthesis and transport to shoots and finally abscission of flowers and siliquae. No significant difference was shown between the nitrogen and zinc rates in the number of silique per plant. It can be concluded that the similarity in silique number of different nitrogen and zinc rates may be attributed to their genetic potential. The number of silique branches per plant is the result of combined effect of genetic make up of the crop and environmental conditions, which plays a remarkable role towards the final seed yield of the crop (Sana et al., 2003).

Seed number per silique

Seed number per silique was affected by nitrogen, zinc and salinity stress in the first year, while in the second year zinc had no significant effect on seed number per silique. Seed number was increased along with increasing of nitrogen or zinc rate. Nitrogen fertilizer increases yield by influencing a variety of growth parameters such as the number of branches per plant, the number of pods per plant, the total plant weight, the leaf area index. Also, it increases the number and weight of seeds (Al-Barrak, 2006). One of the most important reasons for decreasing seed number per silique is that salinity decreases length of siliques. Sakret al. (2007) reported that seed number per silique is sensitive to salinity stress. In this regard, Mendham and Salisbury (1995)

Table 6. Pearson's correlation coefficients among different canola traits (second year).

Trait	Seed yield	Plant height	Silique per plant	Seed per silique	1000-seed weight	Oil	Glucosinolate	Protein	N	P	K	Ca	Mg	Na	Cl	Na/K
Yield	1															
Height	0.87**	1														
Silique per plant	0.25^{ns}	0.14^{ns}	1													
Seed per silique	-0.02^{ns}	0.15^{ns}	0.11^{ns}	1												
Seed weight	0.19^{ns}	0.18^{ns}	0.43**	0.16^{ns}	1											
Oil	0.25^{ns}	0.39^{ns}	-0.06^{ns}	0.35**	0.00^{ns}	1										
Glucosinolate	0.22^{ns}	0.19^{ns}	0.10^{ns}	-0.33*	-0.17^{ns}	-0.09^{ns}	1									
Protein	0.25^{ns}	0.44^{ns}	-0.08^{ns}	0.43**	-0.13^{ns}	0.12^{ns}	-0.03^{ns}	1								
N	0.37**	0.43**	0.18^{ns}	0.43**	0.12^{ns}	0.24^{ns}	-0.00^{ns}	0.34*	1							
P	0.14^{ns}	0.21^{ns}	-0.05^{ns}	0.04^{ns}	0.17^{ns}	0.16^{ns}	-0.15^{ns}	-0.11^{ns}	0.21^{ns}	1						
K	0.37**	0.49**	0.22^{ns}	0.39**	0.02^{ns}	0.18^{ns}	0.14^{ns}	0.44**	0.18^{ns}	-0.01^{ns}	1					
Ca	0.00^{ns}	0.00^{ns}	-0.02^{ns}	-0.02^{ns}	0.39**	0.22^{ns}	-0.15^{ns}	-0.07^{ns}	-0.01^{ns}	0.03^{ns}	-0.31*	1				
Mg	0.12^{ns}	0.05^{ns}	0.10^{ns}	-0.11^{ns}	0.06^{ns}	0.25^{ns}	0.12^{ns}	-0.07^{ns}	-0.20^{ns}	-0.16^{ns}	0.07^{ns}	0.22^{ns}	1			
Na	0.26^{ns}	0.15^{ns}	0.42**	0.04^{ns}	0.32*	-0.26^{ns}	-0.02^{ns}	0.11^{ns}	0.28*	-0.05^{ns}	0.02^{ns}	0.08^{ns}	-0.13^{ns}	1		
Cl	-0.15^{ns}	-0.25^{ns}	-0.21^{ns}	-0.29*	-0.30*	-0.08^{ns}	-0.11^{ns}	-0.20^{ns}	-0.41**	-0.07ns	-0.34*	-0.13^{ns}	0.07^{ns}	-0.15^{ns}	1	
Na/K	0.10^{ns}	-0.04^{ns}	0.27*	-0.13^{ns}	0.27^{ns}	-0.29*	-0.07^{ns}	-0.06^{ns}	0.19^{ns}	-0.05^{ns}	-0.39**	0.22^{ns}	-0.14^{ns}	0.90**	-0.00^{ns}	1

*, ** and ns: Significant at 0.05, 0.01 probability level and not significant, respectively.

showed that ovum number per silique is about 30 at seed set stage, but will decrease due to osmotic and other environmental stresses.

1000 seed-weight

In both years, nitrogen rate increased 1000-seed weight, while salinity decreased it. Moreover, 1000 seed-weight did not change due to zinc application. The lowest value was obtained when salt stress occurred or no nitrogen was applied which can be attributed to deficiency of resources (photosynthesis from lower number of leaves and pods) to support pod filling (Angadiet et al., 2003). Olsson (1960) found that 1000-seed weight was not strongly or slightly influenced by environmental conditions, respectively. However, Krogman and Hobbs (1975) concluded that 1000-seed weight was increased with irrigation and nitrogen levels.

Oil percentage

As for oil percentage, the data of Tables 3 and 4 revealed that nitrogen free treatments had the

highest and then significantly decreased under the higher nitrogen rates. Similarly, salinity stress decreased oil percentage. Obtained results from zinc application differed from year to year; in other words zinc decreased oil content in the first year, but had no significant effect on oil content in the second year (Tables 3 and 4). Several reasons have been given for the decrease in oil content with increasing nitrogen rates. This may be due to a better supply of nitrogen, which increases the formation of nitrogen-containing protein precursors so that protein formation competes more strongly for photosynthates; as a result less of the latter are available for fat synthesis (Holmes, 1980). Kutcher et al. (2005) also stated that it might be due to the dilution effect of increased seed yield with increased nitrogen fertilization and the inverse relationship of protein and oil content. These results are in harmony with Ahmadi and Bahrani (2009).

Glucosinolate

Nitrogen and zinc had no significant effect on glucosinolate accumulation in seed over the two-year period, while salt stress significantly increased glucosinolate content (Tables 3 and 4). Little is known about glucosinolates accumulation in response to salt stress, although, previous studies indicated that environmental factors such as light (Engelen-Eigles et al., 2006), temperature (Velasco et al., 2007) and heavy metals (Tolra et al., 2006) altered the glucosinolate content and composition. Recently, several studies showed that salt stress dramatically increased the total glucosinolate content in broccoli florets (Lopez-Berenguer et al., 2008, 2009), suggesting that they could be involved in the osmotic adjustment under low water potential (Qasim et al., 2003).

Protein content

The increase of nitrogen fertilizer rate might increase the absorbed nitrogen by plant root. Accordingly, nitrogen increases in protein metabolism and is reflected in increased protein content in canola seeds. Conversely, our results showed that increased nitrogen levels had no significant effect on seed protein content (Tables 3 and 4). Our findings are in consistent with results of Singh and Singh (2002), which suggest that protein content is not affected by fertilizers treatment and is related to genetic potential. Similarly, zinc application had not significant effect on seed protein content, while salinity stress significantly decreased protein content. De-creasing protein percentage with increasing salinity could be attributed to the disturbance in nitrogen metabolism or to inhibition of nitrate absorption. It has been stated that the reduction in nitrogen under saline conditions might be due to the reduction of absorbed water and a decrease in root permeability (Strogonov et al., 1970). Medhat (2002) reported that salinity stress induce changes in the ion content of plant's cells, which in turn induce changes in the activity of certain metabolic systems that might have serious consequences for protein.

Mineral composition

Nitrogen content was not affected by zinc application, while nitrogen fertilizer increased nitrogen content especially in the first year. This could be due to the fact that nitrogen availability increases nitrogen uptake. By contrast salinity diminished protein synthesis and accumulation (Tables 3 and 4). Higher accumulations of Na^+ and Cl^- concentrations result in decreased protein content (Sultana et al., 1999) and inhibit the growth of plants. Phosphorus content increased on account of nitrogen fertilizer just in the second year, but it decreased due to salinity in both years (Tables 3 and 4). As for K and Ca, salinity significantly decreased concentration of these elements. Remarkable increase in Na^+, Cl^- concentrations and Na/K ratio was observed due to salinity stress. However, despite large accumulation of Na^+ and Cl^- in plant tissues, effects of nitrogen and zinc on tissue concentrations of K, Ca, Mg, Na, Cl and Na/K ratio were not significant. Lacking decline in root uptake efficiency of these nutrients with salt application may be attributed to internal osmotic adjustment of the seedlings in response to osmotic stress (Yang et al., 1990; Saneoka et al., 2001). These findings are in agreement with the other reports suggesting that salt stress increases the Na/K ratio of green bean (Yasar et al., 2006), wheat (Hu et al., 2006) and legume (Amador et al., 2007). We can therefore state that root cell membrane permeability decreased under salinity stress, which might be related to the functions of Ca in cell membranes. From this point of view, external Na concentrations could demolish cell membrane and inhibit nutrition uptake or translocation.

Pearson correlation coefficients among different canola traits in the first and second year are given in Tables 5 and 6. Briefly, the results demonstrated that there was positive and significant correlation between seed yield and yield components. By contrast, correlation between seed yield and Na, seed yield and Cl and seed yield and Na/K showed a negative and significant correlation. According to these results plant height as an important growth index had direct relationship with yield components; in other words increase in plant height produced more seed yield, while this trait revealed negative and significant relationship with Na, Cl and Na/K ratio. 1000 seed-weight as most important yield components decreased as a result of increased Na, Cl and Na/K ratio so that there was negative correlation between these traits as shown in Tables 5 and 6. In addition, we found that there was a negative and significant correlation between seed protein content and

Na, Cl or Na/K ratio.

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