

Full Length Research Paper

Effect of ammonium : nitrate ratio on fatty acid composition and proline accumulation of canola cultivars grown under salinity stress

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The effects of NaCl salinity stress (100, 150 and 200 mM) and $\text{NH}_4:\text{NO}_3$ ratio (100:0, 25:75, 50:50 and 75:25) on fatty acid composition and proline accumulation of canola (*Brassica napus* L.) cultivars SLM₀₄₆, Licord and Okapi were studied when salinity stress and ammonium and nitrate ratio were applied together. The results show that there were significant differences among cultivars in response to salinity stress and nitrogen sources. In addition, fatty acids composition varied as a result of different salinity levels. Generally, among different ratios of ammonium and nitrate nitrogen sources, 50:05 ratios produced more oleic, linoleic and linolenic. Also, the results indicate that there was a negative correlation between oleic acid and linoleic acid and similarly between linoleic and linolenic acid, while there was positive correlation between oleic and linolenic acid. Proline accumulation increased dramatically due to salt stress. Finally, application of ammonium and nitrate at 50:50 ratios is recommended to earn high quality canola seed.

Key words: Ammonium nitrate ratio, canola, fatty acid composition, proline, salinity stress.

INTRODUCTION

Salinity is one of the most important environmental factors that influence both yield and quality of crop plants in arid and semi-arid regions of the world. Nearly half of the irrigated surface is seriously affected by salinity and/or secondary alkalinity (Flagella et al., 2002). Also in Mediterranean areas, salinity is an increasing problem (Hamdy et al., 1995). Salinity may occur when there is irregular irrigation, inadequate drainage, wrong fertilizer application, and it extremely increases particularly in protected cultivation (George et al., 1997). Generally, plants growing in saline media come across with major drawbacks. The first is the increase in the osmotic stress due to high salt concentration of soil solution that decreases water potential of soil. The second is the increases in concentration of Na and Cl, exhibiting tissue accumulation of Na and Cl and inhibition of mineral nutrients uptake, thus causing ionic imbalance (Mmrschener, 1995). Accumulation of metabolites that act as compatible solutes is one of the probable universal responses of plants to changes in the external osmotic potential. Metabolites with osmolyte function like sugar

alcohols, complex sugars and charged metabolites are frequently observed in plants under unfavourable conditions (Sotiropoulos, 2007). Proline and glycine betaine are known to serve as compatible osmolytes, protectants of macromolecules and also as scavengers of reactive oxygen species under stressful conditions (Ashraf and Foolad, 2007; Bybordi and Tabatabaei, 2009; Bybordi, 2011).

Canola a New World plant has been developed into a valuable source of edible oil and meal. Canola refers to cultivars of rapeseed that have been selected to contain low levels of erucic acid and glucosinolates. The seed contains about 40% oil and produces a meal containing about 38% protein that is used as a supplement in animal rations (Bell, 1984). Canola is classified as a tolerant crop on the basis of the estimation of the crop water stress index. The most common adverse effect of salinity on the crop of *Brassica* is the reduction in plant height, size and yield as well as deterioration of the quality of the product (Kumar, 1995). Fatty acid composition is the most important quality characteristic in oil quality of oilseed

Table 1. The concentrations of salts (mM) used to prepare nutrient solutions at $\text{NH}_4^+:\text{NO}_3^-$ ratio of 100:0, 75:25, 50:50 and 25:75.

Salt	$\text{NH}_4^+:\text{NO}_3^-$ ratio in the solution			
	100:0	75:25	50:50	25:75
KNO_3	0	5.4	0.0	0.0
$\text{Ca}(\text{NO}_3)_2$	0	2.7	3.6	1.8
MgSO_4	2.0	2.0	2.0	2.0
KH_2PO_4	1.0	0.0	0.0	0.0
NH_4HPO_4	5	1.0	1.0	1.0
NH_4Cl	4.5	2.6	6.2	9.8
KCl	1.0	2.3	7.7	7.7
CaCl_2	0.0	1.5	0.7	2.4

crops. In fact, canola is a rich source of oleic, linoleic and linolenic acid. One advantage of these fatty acids is their higher degree of oxidative stability (Fuller et al., 1967), which is desirable for frying purposes, refining and storage. Moreover, a number of investigators have shown significant effects of agronomic practices on oil content and fatty acid composition of canola cultivars. For many oil seed crops, variable environmental conditions may produce wide differences in oil quantity and composition (Shafii et al., 1992). Salinity is one of the key environmental factors that modifies fatty acid composition, and is hence important to salt tolerance of oilseed plants. Changes in the ratio of fatty acid saturation to unsaturation in response to salt stress, and a reduction in the concentrations of triacylglycerols containing primarily unsaturated fatty acids has been reported (Smaoui and Chérif, 2000).

Nitrogen is one of the most important mineral nutrients for plants and plants can utilise it in both anionic nitrate and cationic ammonium forms. Once absorbed, ammonium can be rapidly utilised in the synthesis of amino acids and other nitrogenous organic compounds, but nitrate must be reduced to ammonium before it can be assimilated (Barker and Mills, 1980; Bybordi, 2010). There is also evidence that the form of nitrogen supplied to plants not only affects their growth under non-saline conditions, but also shows an interaction with the salinity tolerance of plants (Hawkins and Lewis, 1993). Several researchers have studied the combined effect of salinity and the nitrogen source type added to the nutrient solution on productivity, photosynthesis and nitrogen metabolism (Hawkins and Lewis, 1993; Bybordi et al., 2010a). Recently, the effects of nitrate and ammonium ions and the combined effect of salinity with different nitrogen sources (Bybordi et al., 2010b) on certain biochemical adjustments to stress have been reported. Canola has a high demand for crop nutrients, including nitrogen and nitrogen deficiencies commonly limit canola yield (Grant and Bailey, 1993). Therefore, proper nitrogen fertilization is important in optimizing canola yield and quality especially under environmental stresses.

The aim of this study was to evaluate the changes in fatty acid composition and proline accumulation in three canola cultivars submitted to different levels of salinity and different ammonium and nitrate ratio.

MATERIALS AND METHODS

In order to study the effects of different combinations of NH_4^+ and NO_3^- on fatty acid composition and proline accumulation of three canola cultivars, a hydroponic pot experiment was conducted under three salinity levels. The experiment was conducted under controlled conditions in a glasshouse at Faculty of Ecology and Soil Science, Baku State University, Baku, Azerbaijan during 2010 growing season.

Experimental design was a completely randomized design (CRD), arranged in factorial with three replications. Treatments included salinity in three levels (100, 150 and 200 mM), different ratios of ammonium and nitrate (100:0, 75:25, 50:50 and 25:75) and three canola cultivars (SLM₀₄₆, Licord and Okapi).

Plastic pots were filled with autoclaved perlite and vermiculite (1:1 volume). Pots were put in glasshouse under conditions of $25/18 \pm 3$ C day/night temperature and supplementary photon flux density of $250 \mu\text{mol m}^{-2} \text{s}^{-1}$. Seeds were sterilized by sodium hypochlorite for 5 min and 96% ethanol for 30 s then seeds were washed by distilled water. Perlite was raked using fingers and six seeds were sown in each plastic pots at the depth of 3 cm. Pots were irrigated by nutrition solution free of nitrogen. All pots received half strength of Hoagland's solution. The greenhouse was under natural sunlight during spring and summer and the temperature was set to 25 ± 3 and 18 ± 3 °C, day and night, respectively. The concentration of nutrients in the solutions was as follows (in mg.L^{-1}): 330 K, 170 Ca, 50 Mg, 33 P, 1.5 B, 0.1 Cu, 2 Mn, 12 Fe (Fe- DTPA) and 0.1 Mo. Nitrogen at 200 mg.L^{-1} was provided as NO_3^- and NH_4^+ forms to give $\text{NH}_4^+:\text{NO}_3^-$ ratios of 0:100, 25:75, 50:50 and 75:25 (Table 1).

Then days after sowing, plants were thinned to three plants in each pot. Salt stress induced by different concentration of NaCl during experiment until harvesting time.

At flowering, leaf samples were picked in order to determine proline accumulation in leaf tissues. Proline content of leaves was determined according to a modification of the method of Bates et al. (1973). Samples of leaves (0.2 g) were homogenized in a mortar and pestle with 3 ml sulphosalicylic acid (3 % w/v), and then centrifuged at 18 000 g for 15 min. Two millilitres of the supernatant was then added to a test tube, to which 2 ml glacial acetic acid and 2 ml freshly prepared acid ninhydrin solution (1.25 g ninhydrin

Table 2. Effect of different nitrate and ammonium ratios on fatty acid composition and proline accumulation of canola cultivars grown under salinity stress.

Salinity	NH ₄ :NO ₃	cultivar	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Proline (mmol.g ⁻¹)	
100 mM NaCl	100:0	SLM ₀₄₆	58.0 ± 5.02	26.6 ± 2.12	22.0 ± 1.89	0.98 ± 0.087	
		Licord	57.5 ± 5.01	22.5 ± 1.89	20.0 ± 1.75	0.84 ± 0.080	
		Okapi	57.0 ± 5.01	22.1 ± 1.80	20.4 ± 1.71	0.85 ± 0.075	
	75:25	SLM ₀₄₆	59.1 ± 5.04	27.3 ± 2.30	19.9 ± 1.65	0.85 ± 0.076	
		Licord	59.3 ± 5.04	20.2 ± 1.75	18.0 ± 1.80	0.80 ± 0.074	
		Okapi	59.0 ± 5.04	20.8 ± 1.76	14.2 ± 1.40	0.82 ± 0.080	
	50:50	SLM ₀₄₆	60.2 ± 5.55	28.2 ± 2.14	18.8 ± 1.80	1.2 ± 0.095	
		Licord	56.8 ± 5.05	22.0 ± 1.85	16.0 ± 1.55	1.0 ± 0.085	
		Okapi	56.4 ± 5.03	21.9 ± 1.84	12.6 ± 1.20	1.0 ± 0.086	
	25:75	SLM ₀₄₆	48.3 ± 4.02	24.1 ± 1.95	18.6 ± 1.80	0.99 ± 0.087	
		Licord	42.4 ± 4.021	22.0 ± 1.42	16.3 ± 1.60	0.95 ± 0.086	
		Okapi	40.6 ± 3.89	22.0 ± 1.55	14.1 ± 1.39	0.95 ± 0.087	
	150 mM NaCl	100:0	SLM ₀₄₆	60.4 ± 5.87	27.8 ± 2.21	12.3 ± 1.22	2.8 ± 0.16
			Licord	63.0 ± 5.89	25.5 ± 2.04	11.5 ± 1.16	2.4 ± 0.12
			Okapi	58.1 ± 5.41	22.8 ± 1.89	10.1 ± 1.09	2.5 ± 0.13
75:25		SLM ₀₄₆	61.9 ± 5.95	27.0 ± 2.21	10.2 ± 1.08	2.9 ± 0.18	
		Licord	61.0 ± 5.90	27.9 ± 2.22	12.0 ± 1.02	2.8 ± 0.18	
		Okapi	56.8 ± 5.21	22.6 ± 1.98	10.0 ± 1.01	2.2 ± 0.10	
50:50		SLM ₀₄₆	64.6 ± 5.89	30.0 ± 2.87	14.2 ± 1.42	4.2 ± 0.56	
		Licord	56.5 ± 5.54	22.9 ± 1.89	9.6 ± 0.97	3.0 ± 0.41	
		Okapi	52.4 ± 5.02	35.8 ± 3.01	11.8 ± 1.15	2.9 ± 0.17	
25:75		SLM ₀₄₆	58.3 ± 5.45	22.7 ± 1.88	15.3 ± 1.15	3.2 ± 0.31	
		Licord	52.9 ± 5.21	32.5 ± 2.87	12.6 ± 1.25	2.8 ± 0.21	
		Okapi	51.2 ± 4.87	34.5 ± 2.55	10.3 ± 0.99	2.0 ± 0.19	
200 mM NaCl		100:0	SLM ₀₄₆	54.2 ± 5.21	33.5 ± 2.74	12.2 ± 1.12	3.8 ± 0.39
			Licord	50.1 ± 4.56	31.2 ± 2.45	10.6 ± 0.98	3.6 ± 0.35
			Okapi	48.7 ± 4.21	39.7 ± 3.26	0.3 ± 0.057	3.5 ± 0.29
	75:25	SLM ₀₄₆	55.3 ± 5.23	38.2 ± 3.18	6.5 ± 0.54	3.5 ± 0.30	
		Licord	51.3 ± 5.10	39.7 ± 3.28	9.0 ± 0.85	3.3 ± 0.28	
		Okapi	46.4 ± 4.23	44.2 ± 4.11	8.5 ± 0.80	3.2 ± 0.28	
	50:50	SLM ₀₄₆	58.6 ± 5.21	29.5 ± 2.21	11.9 ± 1.11	4.6 ± 0.36	
		Licord	47.8 ± 4.32	43.3 ± 3.98	8.9 ± 0.74	3.9 ± 0.30	
		Okapi	44.4 ± 5.11	44.4 ± 4.10	8.7 ± 0.85	3.8 ± 0.28	

Table 2 Cont

	SLM ₀₄₆	56.6 ± 5.32	33.8 ± 2.87	5.4 ± 0.50	3.6 ± 0.27
25:75	Licord	43.8 ± 4.21	33.7 ± 2.86	5.1 ± 0.49	3.6 ± 0.26
	Okapi	41.8 ± 4.89	36.2 ± 2.98	8.2 ± 0.75	3.4 ± 0.24

dissolved in 30 ml glacial acetic acid and 20 ml 6 m orthophosphoric acid) were added. The test tubes were incubated in a water bath for 1 h at 100°C and then allowed to cool to room temperature. Four millilitres of toluene was then added to the tubes and then mixed on a vortex mixer for 20 s. The test tubes were allowed to stand for at least 10 min, to allow separation of the toluene and aqueous phases. The toluene phase was carefully pipetted out into a glass test tube and its absorbance was measured at 520 nm in a spectrophotometer. The content of proline was calculated from a standard curve, and was expressed as mmol g⁻¹ fresh weight.

At the end of growing season, crop was harvested and seed samples were collected. The fatty acid compositions of the canola seed oils were determined by gas chromatography (GC) (Metcalf et al., 1966). The contents of palmitoleic, linolenic, oleic and myristic acids were determined using a computing integrator. The effects of the independent variables on oil content and palmitoleic, linolenic, oleic and myristic acid concentrations of the oil were analyzed on a percentage basis.

All data were analyzed by SAS software. Differences between mean values were determined with Duncan's multiple range test and *P* value was *P* < 0.05.

RESULTS AND DISCUSSION

Fats are classified into saturated and unsaturated fats. Saturated fats tend to increase blood cholesterol levels, while unsaturated ones show the reverse direction; they are mostly from plant sources. The most common saturated fatty acids found in plant lipids contain 16 or 18 carbon atoms. Low content of saturated fatty acids is desirable for edible uses. Usually only palmitic acid (C16) and stearic acid (C18) are present in significant amount, but the saturated fatty acids collectively account for only 20% of the total fatty acid content of most plants, while those with one or more double bonds (unsaturated fatty acids) account for the remaining 80%. In many fatty seeds, oleic [18:1(9C)], linoleic [18:2 (9C, 12C)] and linolenic [18:3 (9C, 12C, 15C)] acids frequently account for more than 70% of the fatty acid content (Anderson and Beardall, 1999).

Results show that oleic acid was the dominant unsaturated fatty acid and then linoleic acid was the most abundant acids. Maximum mean values for oleic acid (64.6%) were obtained from SLM₀₄₆ treated by 50:50 ammonium nitrate ratio and 150 mM NaCl, while the minimum values (40.6%) were obtained from Okapi treated by 25:75 ammonium nitrate ratio and 100 mM NaCl (Table 2). The minimum mean values for linoleic acid (20.2%) was obtained from licord treated by 75:25 ammonium nitrate ratio and 100 mM NaCl, while the highest values (44.4%) was obtained from Okapi treated

by 50:50 ammonium nitrate ratio and 200 mM NaCl. In addition, the highest mean values for linolenic acid (22.0%) was obtained from SLM₀₄₆ treated by 100:1 ammonium nitrate ratio and 100 mM NaCl, while the lowest means value (0.3%) was obtained from Okapi treated by 100:0 ammonium nitrate ratio and 200 mM NaCl (Table 2). In case of proline accumulation, the highest and the lowest accumulation were found in SLM₀₄₆ and licord cultivars when they were treated by 50:50 ammonium nitrate ratio and 200 mM NaCl and 75:25 ammonium nitrate ratio and 100 mM NaCl, respectively (Table 2). Accumulation of solutes especially proline and sugars is a common observation under stress condition (Qasim et al., 2003). Proline is an important osmolyte synthesized in many micro organisms and plants exposed to salinity and drought stress, thus it as an osmotic protector in plant. Proline accumulating in plants exposed salinity stress is due to low activity of oxidant enzymes (Sudhakar, 2001).

Furthermore, there was a negative relationship between linoleic and oleic acid concentrations (Figure 1); this result is in agreement with Seiler (2007) who reported that if one increases the other decreases. In addition, results showed negative relationship between linoleic and linolenic (Figure 2). Conversely, linolenic and oleic had positive correlation to each other (Figure 3). An increase in oleic acid content was observed in high oleic hybrids submitted to stress (Baldini et al., 2002; Flagella et al., 2002), while no effect on oleic to linoleic ratio was observed by Salera and Baldini (1998). In general, under each level of salinity, 50:50 ammonium nitrate ratios produced the highest fatty acid percentage. The fatty acid composition of seed oil crops is mainly under genetic control, but can be affected to some extent by nitrogen nutrition (Holmes and Bennett, 1979). Nitrogen plays the most important role in building the protein structure (Frink et al., 1999). Seo et al. (1986) found that when sesame was given 0 to 160 kg nitrogen, oleic acid content was highest at the highest nitrogen rates and linoleic acid content was highest at the intermediate rates. Khan et al. (1997) indicated that oleic acid increased by increasing levels of nitrogen added to rapeseed-mustard. Kheir et al. (1991), in flax, found that higher nitrate increased the percentage of unsaturated fatty acids and decreased saturated fatty acids in the seed oil. In response to salinity, the oil content of safflower (*Carthamus tinctorius* L.) decreased, whereas its composition was unaffected (Irving et al., 1988). However, despite the fact that NO₃⁻ assimilation consumes more energy than NH₄⁺ assimilation, only a few species perform well when NH₄⁺

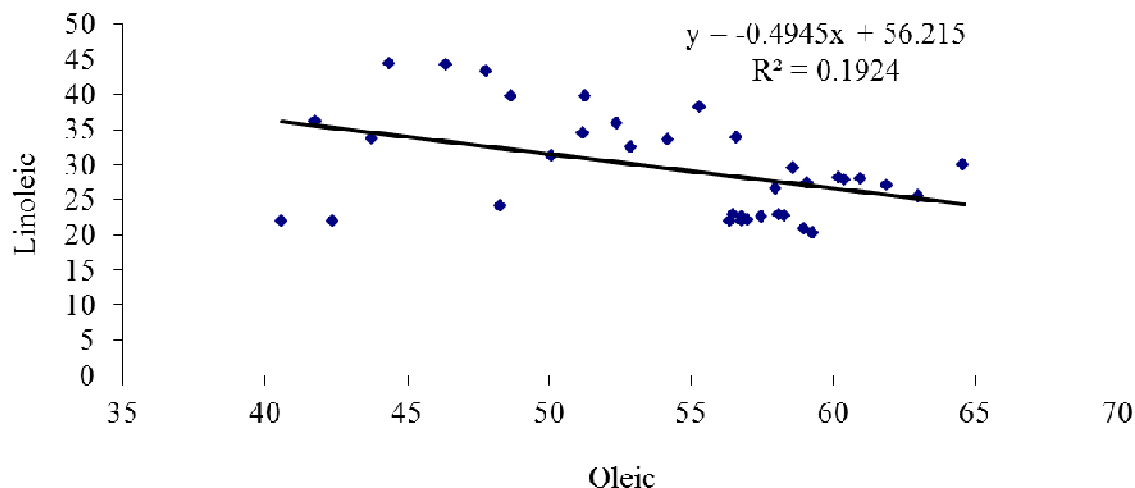


Figure 1. Correlation between linoleic acid and oleic acid content affected by different salinity stress and different ammonium and nitrate ratios. There is negative correlation between these fatty acids.

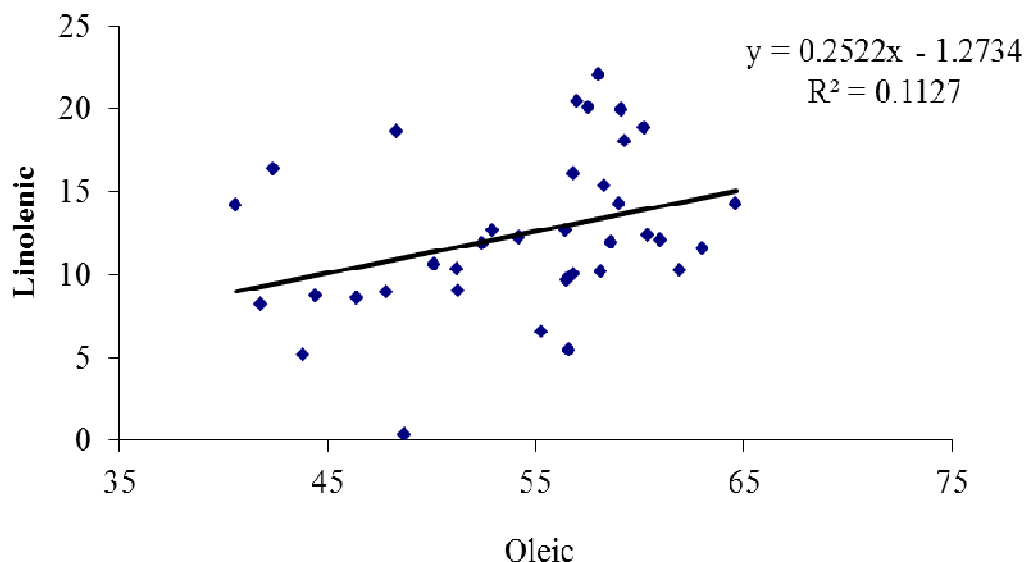


Figure 2. Correlation between linoleic acid and linolenic acid content affected by different salinity stress and different ammonium and nitrate ratios. There is negative correlation between these fatty acids.

is the sole nitrogen source (Marschner, 1995). Indeed, many plant species develop symptoms of toxicity when subjected to high concentrations of NH_4^+ , which are not detected when plants are grown with the same concentration of NO_3^- or in mixed N nutrition (Britto and Kronzucker, 2002). Although, NH_4^+ is an important intermediate in many metabolic reactions, it has been reported that high concentrations of NH_4^+ in the soil or in the nutrient solution may lead to an “ NH_4^+ syndrome”, which may include leaf chlorosis, net photosynthesis decrease, lower plant yield production and shoot to root ratio, lower cation content, acidification of the rhizosphere, and changes at several metabolite levels

such as amino acids or organic acids (Britto and Kronzucker, 2002).

Conclusion

Overall, this study concludes that fatty acid composition which is one of the most important oil qualitative characteristics varies due to salinity and different nitrogen sources. Although, genetic potential of cultivars determine fatty acid composition but some environmental factor like salinity stress and mineral nutrients can affect this traits. In general, we found that 50:50 ammonium

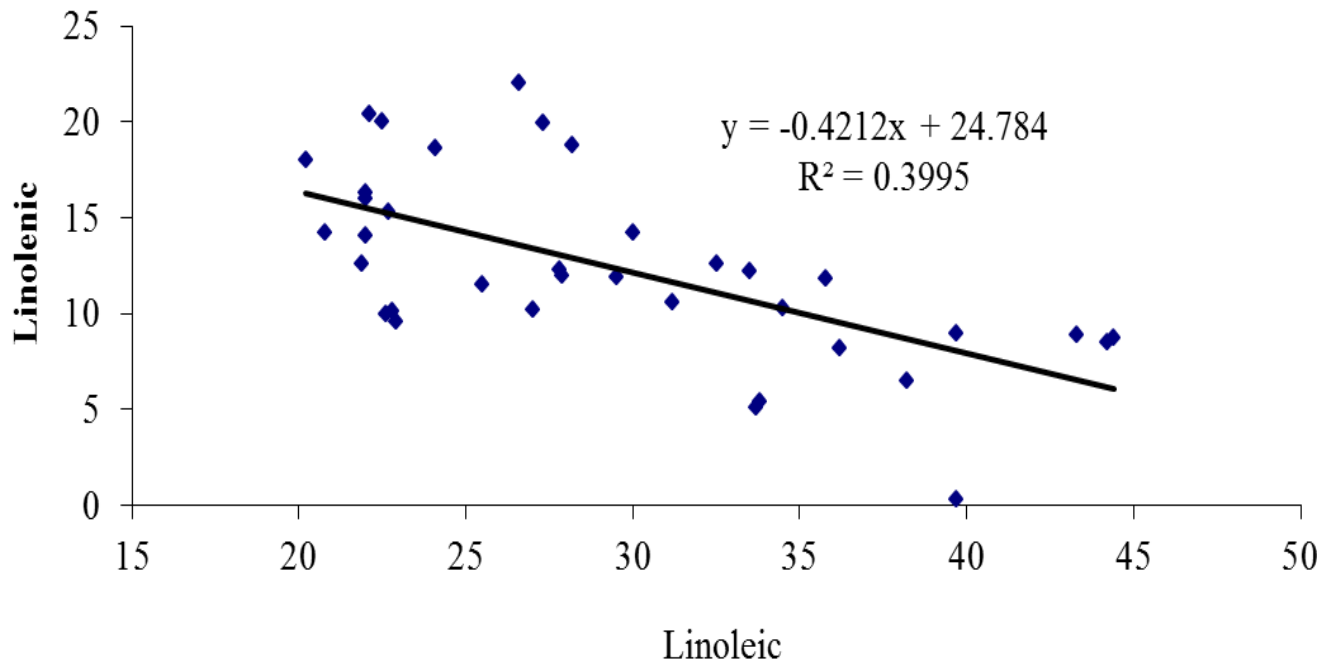


Figure 3. Correlation between linolenic acid and oleic acid content affected by different salinity stress and different ammonium and nitrate ratios. There is positive correlation between these fatty acids.

nitrate ratio is the best treatment to produce canola seeds with the best oil quality under salt stress conditions.

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