

Effect of salt stress on physiological and morphological parameters of rapeseed cultivars

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Abstract: This research was carried out in order to evolution effect of salt stress on physiological and morphological parameters of four rapeseed cultivars, slm04, Opera, Zarfam and SIm04 in 2010. Salt stress treatments were applied using salt solutions with EC values of 0.6(control), 4 and 8 ds/m⁻¹. The Results showed that the salt-tolerant cultivars, Modena and Zarfam showed an increase in peroxidase activity at high salinity level, whereas the salt-sensitive cultivars, SIm04 and Opera, did not show any increase in peroxidase activity at all. Salt stress decrease catalase activity in four rapeseeds cultivars but this decrease was slightly in Zarfam and Modena than other cultivars. With increasing level of salinity stress, the MDA content increased in the four cultivars, but this increase was higher in Opera and SIm04 than other cultivars. With increasing Salinization, in all cultivars Na⁺ was increase, but in Zarfam and Modena this increase were slowly than other two cultivars. With increasing Salinization, K⁺ was decrease in all cultivars, but this decrease was slowly in Zarfam cultivar. The lesser degree of membrane damage and the higher activity of peroxidase and catalase observed in NaCl treated plants of Zarfam and Modena indicated that these rapeseed cultivars had a higher capacity for the tolerant salinity in comparison with sensitive cultivars.

Key words: Rapeseed; Peroxidase; Catalase; Lipid prooxidation; Salt stress

1. Introduction

Abiotic stresses such as salt excess (NaCl) and drought are among factors most limiting to plant productivity (Bohnert et al., 1995). High salinity in soil or irrigation water is a common environmental problem affecting plant growth and productivity by provoking osmotic stress and ion toxicity together with induction of oxidative stress. There is an increasing body of evidence which suggests that together with osmotic adjustment and ion compartmentalization, an efficient antioxidant system is also important in combating salinity stress. Results have indicated that salinity affects growth and development of plants through oxidative, osmotic and ionic stresses. Because of accumulated salts in soil under salt stress condition plant wilts apparently while soil salts such as Na⁺ and Cl⁻ disrupt normal growth and development of plant (Noble et al., 2004; Yeo et al., 1983). Salt tolerance of wheat cultivars have a direct relationship with Na⁺/K⁺ ratio so that the ratio increased with the increase of salinity level but less increase is observed in tolerant cultivars, they concluded that Na⁺/K⁺ ratio can be a measure of salt stress tolerance (Poustini and Siosemardeh, 2004).

One of the biochemical changes occurring when plants are subjected to biotic or abiotic stresses is the production of reactive oxygen species (ROS)

(Neto et al., 2006). ROS are highly reactive and in the absence of any protective mechanism they can seriously disrupt normal metabolism through oxidative damage to lipids, protein and nucleic acids. In order to avoid the production of these reactive molecules plants have evolved an effective scavenging system involving antioxidant molecules like carotenoids, ascorbate, glutathione and tocopherols as well as antioxidant enzymes such as super oxide dismutase, catalase and glutathione reductase. Malondialdehyde (MDA) as the decomposition product of polyunsaturated fatty acids of biomembranes, showed greater accumulation under salt stress (Nillei et al., 2002). Cell membrane stability has been widely used to differentiate stress tolerant and susceptible cultivars of some crops and in some cases higher membrane stability and prolin content could be correlated with abiotic stress tolerance (Premachandra et al., 1992).

The aim of this study was to evaluate the effects of salt stress on the activity of antioxidative enzymes; the lipid membrane peroxidation; the prolin content and the Na⁺ and K⁺ content in four rape seed cultivars, in order to better understand their differences on salt stress tolerance.

2. Material and methods

2.1. Plant material

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A research was carried out to evolution effect of salt stress on physiological and morphological parameters of four rapeseed cultivars, SIm04, Opera, Zarfam and Modena in University of Tehran and Islamic Azad University (Shoushtar Branch) in 2010. Salt stress treatments were applied using salt solutions with EC values of 0.6 (control), 4 and 8 ds/m⁻¹. These solutions were called S0, S1 and S2 respectively. Required amount of each solid salt for preparing one liter salt solution was calculated through the following formula first (Al-Ansari, 2002):

$$\text{TDS (mg/lit)} = \text{EC} \times 640$$

Where: TDS= total soluble solid salt amount (mg/lit)

EC= given electro conductivity value (ds/m)

Then EC value of each solution was read by means of EC meter and reached the desirable EC with addition of solid salt or distilled water. 10 Seeds of each cultivar were sown in germination boxes filled with perlite. The germination boxes were placed greenhouse where temperature ranged between 22°C and 25 °C for a period of 3 weeks. The boxes irrigated daily with three different Hoagland solutions by the use of NaCl. Electrical conductivities (EC) at 25 °C of the three salinity levels were 0.6(control), 4 and 8dS m⁻¹, respectively. After 21 days, plants were harvested for morphological, physiological and biochemical determinations. The layout of the experiment was a Factorial complete block Design. There were four replicates in each treatment group.

2.2. Enzyme determinations

For enzyme assays and estimation of lipid peroxidation, frozen leaf samples were ground to a fine powder with liquid nitrogen and extracted with ice-cold 50 nM phosphate buffer (pH 7.0). The extracts were centrifuged at 4°C for 30 min at 20000g and the resulting supernatants; hereafter referred to as crude extracts, was collected and used for protein content assay and enzyme activities. Protein content was determined according to Bradford (1976) with bovine serum albumin as the standard. Peroxidase activity was determined using the guaiacol oxidation method (Bandeoglu et al., 2004) in a 3 ml reaction mixture containing 10 mM phosphate buffer (pH 6.4), 8 mM guaiacol, 100–200 ml enzyme extract and 2.75 mM H₂O₂. The increase in absorbance was recorded at 470 nm within 30 s (linear phase) after H₂O₂ was added. CAT extraction was performed in a 50 mM Tris- HCl buffer. The enzyme activity was assayed by measuring the reduction of H₂O₂ at 240 nm and 25°C as described by Dionisio-Sese et al. (1998).

2.3. Lipid peroxidation and Electrolyte leakage

Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) formation using the thiobarbituric acid method described by Stewart and Bewley (1993). The crude extract

preparation was mixed with the same volume of a 0.5% (w: v) thiobarbituric acid solution containing 20% (w: v) trichloroacetic acid. The mixture was heated at 95°C for 30 min and the reaction was stopped by quickly placing in an ice-bath. The cooled mixture was centrifuged at 10000g for 10 min, and the absorbance of the supernatant at 532 and 600 nm was read. After subtracting the non-specific absorbance at 600 nm, the MDA concentration was determined by its extinction coefficient of 155 mM⁻¹ cm⁻¹.

To determine electrolyte leakage, 100 mg fresh leaf samples were cut into 5 mm length and placed in test tubes containing 10 ml distilled deionized water. The tubes were covered with plastic caps and placed in a water bath maintained at the constant temperature of 32°C. After 2 h the initial electrical conductivity of the medium (EC1) was measured using an electrical conductivity meter. The samples were autoclaved afterwards at 120°C for 20 min to completely kill the tissues and release all electrolytes. Samples were then cooled to 25°C and the final electrical conductivity (EC2) was measured. The electrolyte leakage (EL) was expressed following the formula $EL = \frac{EC1}{EC2} \times 100$ (Sreenivasulu, 2000).

2.4. Proline conten

Proline determination was carried out according to the method of Bates et al. (1977).

2.5. Sodium and Potassium determination

For the determination of sodium and Potassium in the leaf, 10 mg dried material was cut into 1 cm length, placed in test tubes containing 20 ml distilled deionized water, and heated in a boiling water bath for 1 h. The tubes were then autoclaved at 120°C for 20 min and cooled. The sodium content in 15 time's diluted extract was determined by atomic absorption spectrophotometry (Al-Ansari, 2002).

2.6. Statistical analysis

All data were subjected to ANOVA test and means were compared by the Duncan's. Comparisons with P values B/0.05 were considered significantly different.

3. Results

3.1. Effect of salinity on growth

Seedling Fresh and dry weight, shoot length and root length of four rapeseed cultivars subjected to 3week salinity treatments are shown in Table1. Cultivars which are considered as salt-tolerant, that is, Zarfam and Modena, showed higher Shoot length, fresh and dry weight at 4 dS m⁻¹ salinity level compared to the non-salt-treated plants. The salt sensitive cultivars, SIm04 and Opera, on the other

hand, did not show this growth stimulation at moderate salinity level. At 8 dS m⁻¹ salinity level Zarfam and Modena cultivars showed higher root length, shoot length, fresh and dry weight than

Opera and Slm04. In all cultivars decrease of shoot length in compared to root length was higher.

Table1: Effect of salt stress on seedling growth of four rapeseed cultivars

Salt level	Cultivar	Shoot length(cm)	Root length(cm)	Seedling FW(mg)*	Seedling DW(mg)*
S0	Slm04	6.75 a	11.3 a	230.8 a	24.2 a
	Opera	6.50 a	11.1 a	228.0 a	22.4 a
	Modena	6.60 a	11.2 a	226.7 a	23.7 a
	Zarfam	6.90 a	11.3 a	228.7 a	23.8 a
S1	Slm04	5.10 c	8.10 c	165.2 c	18.2 c
	Opera	5.10 c	8.30 c	161.2 c	18.9 c
	Modena	6.20 b	10.1 b	180.3 b	20.2 b
	Zarfam	6.30 b	10.2 b	181.1 b	20.1 b
S2	Slm04	3.70 d	6.20 d	90.3 e	14.3 e
	Opera	3.70 d	6.30 d	92.4 e	14.7 d
	Modena	5.20 c	9.20 b	134.7 d	16.7 d
	Zarfam	5.30 c	9.10 b	137.3 d	17.0 d

*FW: Fresh Weight

**DW: Dry Weight

Means followed by the same letter(s) are not significantly different at P = 0.01 according to Duncan test

3.2. Effect of salinity on catalase and peroxidase activities

Fig. 1 and Fig.2 showed the effect of increasing level of NaCl salinity on catalase and peroxidase activities of the four rapeseed cultivars after 3 week exposure to salinity. The Results showed that salt stress decrease catalase activity in four rapeseeds cultivars but this decrease was slightly in Zarfam and Modena than other cultivars. In fact decrease of catalase in Zarfam and Modena was very slightly in compared to other cultivars (we can say catalase activity unchanged in salt tolerance rapeseeds cultivars). The salt-tolerant cultivars, Modena and Zarfam showed an increase in peroxidase activity at high salinity level, whereas the salt-sensitive cultivars, Slm04 and Opera, did not show any increase in peroxidase activity at all. In Slm04 Cultivar peroxidase activity unchang under salinity condition and in Opera Cultivar peroxidase activity decrease in compared to non-salinity condition.

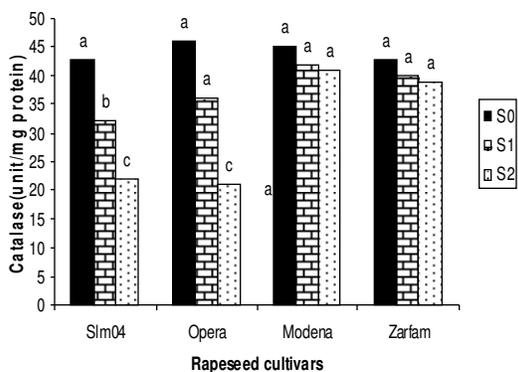


Fig. 1: Effect of salt stress on Catalase activity in leaf of four rapeseed cultivars

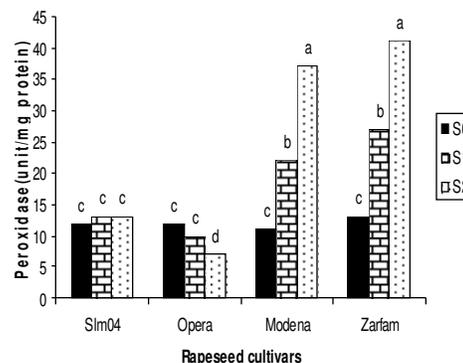


Fig. 2: Effect of salt stress on Peroxidase activity in leaf of four rapeseed cultivars

3.3. Effect of salinity on lipid peroxidation, Proline content and electrolyte leakage

The effect of increasing of salinity stress on MDA formation in the leaves of the four rapeseed cultivars after 3 week salinity treatment is shown in Table 2. With increasing level of salinity stress, the MDA content increased in the four cultivars, but this increase was higher in Opera and Slm04 than other cultivars. On the other hand, in Zarfam and Modena did not exhibit strongly increase in lipid peroxidation with a 3 week exposure to salinity stress? The amount of electrolyte leakage from the leaves of the four rapeseed cultivars subjected to increasing level of salinity stress is shown in Table2. Increasing of salinity stress increased the amount of electrolyte leakage from the leaves of Opera and Slm04 but this increase was slowly in Zarfam and Modena. The amount of proline from the leaves of the four rapeseed cultivars subjected to increasing level of salinity stress is shown in Table 2. Salinity significantly increase proline content in all rapeseed cultivars but this increase was not significantly in Slm04 and Opera.

Table 2: Effect of salt stress on some physiological trait of four rapeseed cultivars

Salt level	Cultivar	Proline (mg/grfw)	MAD (nmol/gr fw)	Electrolyte leakage (%)
S0	Slm04	22.7 a	4.7 a	8.30 a
	Opera	21.8 a	4.9 a	9.10 a
	Modena	20.9 a	5.1 a	8.20 a
	Zarfam	22.6 a	4.4 a	8.70 a
S1	Slm04	23.4 a	8.2 b	15.3 b
	Opera	21.6 a	9.0 c	16.1 b
	Modena	32.9 b	6.1 b	9.80 a
	Zarfam	31.8 b	5.8 a	10.10 a
S2	Slm04	27.2 a	12.1d	38.8 0c
	Opera	22.7 a	11.4d	37.0 c
	Modena	50.23 c	6.6 c	14.10 b
	Zarfam	51.0 c	6.3 c	15.00 b

Means followed by the same letter(s) are not significantly different at P = 0.01 according to Duncan test

3.4. Effect of salinity on Na⁺ and K⁺ uptake

There was a marked varieties difference in the accumulation of Na⁺ and K⁺ in rapeseed leaves in response to salinity. With increasing salinization, in all cultivars Na⁺ was increase, but in Zarfam and Slm04 this increase were slowly than other two cultivars. With increasing salinization, K⁺ was decrease in all cultivars, but this decrease was slowly in Zarfam Cultivar. Data showed that in Zarfam, K⁺ was higher than other cultivars and Higher K⁺: Na⁺ ratio showed in Zarfam than other cultivars (Table 3).

Table 3: Effect of salt stress on shoot Na⁺ and K⁺ percentage of rapeseed cultivars

Salt level	Cultivar	Na ⁺ %	K ⁺ %	K ⁺ /Na ⁺ ratio
S0	Slm04	3.6 c	10.6 a	2.9 a
	Opera	3.2 c	10.9 a	3.2 a
	Modena	3.4 c	10.5 a	3.1 a
	Zarfam	3.4 c	10.7 a	3.1 a
S1	Slm04	4.4 b	8.4 c	1.9 c
	Opera	4.4 b	8.2 c	1.8 c
	Modena	4.1 b	9.1 b	2.2 b
	Zarfam	3.8c	9.4 b	2.4 b
S2	Slm04	5.9 a	6.1d	1.3 e
	Opera	5.7 a	6.3d	1.1 e
	Modena	4.9 b	8.1 c	1.6 d
	Zarfam	4.6 b	8.2 c	1.9 c

Means followed by the same letter(s) are not significantly different at P = 0.01 according to Duncan test

4. Discussion

Morphologically, the most typical symptom of saline injury to a plant is retarded growth due to inhibition of cell elongation (Bandeoglu, et al., 2004). In this study Salinity decrease dry and fresh weight of rapeseed seedling but this decrease was lower in salt tolerance cultivars (Table 1). Researchers reported that accumulation of salts and ions in plant growth environment causes osmotic and drought stress leading to decrease of water absorption by plant tissues. Decrease of tissue water content results in reduction of cellular growth and

development. Therefore, restriction of water absorption and its consequences for cellular growth and development is one of the most important causes of decreased growth of stem and root. (Cavalacanti et al., 2007; Ashraf and Mcneilly, 1998; Munns, 1982). In supporting of our observation, Hernandez et al. (1999) reported a dose-dependent reduction in the growth of pea plants subjected to NaCl stress. Similarly, in rice leaves, under higher saline conditions, relative growth rate was decreased in salt sensitive cultivar whereas salt tolerant cultivars exhibited no significant change (Dionisio-Sese and Tobita 1998; Maribel et al., 1998). Data of this study showed salt stress decrease shoot and root length but this decrease was higher in shoot, especially in salt sensitive cultivars. Bandeoglu et al. (2004) showed salt stress decrease root and shoot growth of lentil seedling but decrease of shoot growth was higher than root growth.

Results of the analysis of Na⁺ and K⁺ percentage of leaves of rapeseed cultivars differing in salt tolerance agree with the view that there is an inverse relationship between shoot Na⁺ and K⁺ concentration and salt tolerance (Yeo, 1983; Umezawa, 2000). The salt-tolerant cultivars Modena and Zarfam did not exhibit a significant increase in Na⁺ accumulation in the leaves even at high salinity level whereas the salt-sensitive cultivars Slm04 and Opera showed pronounced accumulation. Our results showed that salt tolerant cultivars have higher K⁺ concentration and K⁺: Na⁺ than salt sensitive cultivars. On the other hand, this study showed composite correlation between increase of K⁺ and decrease of Na⁺ with growth of rapeseed seedling. Ashraf and McNilley (2004) showed that salt stress increase of Na⁺ and decrease of K⁺ in shoot of rapeseed and salt tolerance rapeseed have higher K⁺:Na⁺ ratio than salt sensitive cultivars. Chen et al (1996) studied soybean, wheat, maize and cotton and suggested that Na⁺ concentration increased with the increase in salinity level in all of these plants. Root Na⁺ content of cotton which is a salt stress tolerant plant was more than soybean root indicating preservation of Na⁺ in cotton root and lack of Na⁺ transportation to shoot. Data of our study showed negative correlation between Na⁺ concentrations in shoot of rapeseeds

cultivars with MAD concentration and electrolyte leakage and positive correlation between K^+ concentration and these parameters.

Researchers suggested that K^+ concentration observed in salt stress tolerant plants were more than that of susceptible cultivars led to decreased Na^+ toxicity. Increased Na^+ content led to decrease in seed germination level and seedling fresh weight in such plants Chen et al. (1996); Bayuelo-Jimenez and Debouk, 2003). Morant et al. (2003) working on Triticale cultivars suggested that $K^+ : Na^+$ ratio decreased with the increase in salt stress level in growth environment in all of investigated cultivars, however, more increase value was observed among salt stress susceptible plants and reported decreased K^+ absorption in presence of NaCl as the cause of this observation.

Proline is one of the most important osmoprotectant in plants. Under salt stress most plant species exhibit a remarkable increase in their proline content (Delauney and Verma 1993; Bandoğlu et al., 2004). In our experiments we also observed a similar behavior in the seedling of rapeseeds. Supporting findings come from other plants (alfalfa, soybean and pea) (Tramontano and Jouve 1997) where salt stress resulted in extensive proline accumulation. In support of our observations, recently in rice roots exposed to NaCl stress, a uniform accumulation of proline was shown to be related with increasing NaCl concentrations (Khan et al., 2002). Bandoğlu et al. (2004) showed that proline increase salinity tolerance in lentil seedling.

The extent of damage to the membrane was monitored by measuring the amount of MDA produced when polyunsaturated fatty acids in the membrane undergo peroxidation. Membrane structure and properties, this enhanced free radical formation and lipid peroxidation under salt stress in salt-sensitive cultivars may have also brought about an increase in membrane permeability or loss of membrane integrity, as evidenced by the increase in solute leakage (Table 2). Salt stress-induced electrolyte leakage has also been previously observed in foxtail millet (Sreenivasulu, 2000). Maribel et al. (1998) showed that salinity increase electrolyte leakage in rice cultivars but this increase was higher in salt sensitive cultivars.

An increase in MDA contents upon salt stress has been reported in different plant species (Dionisio-Sese and Tobita, 1998; Lee et al., 2001; Sudhakar et al., 2001; Sairam and Srivastava, 2002). This increase was shown to be related to the amount of stress and well correlated with lipid membrane damage. Our results also demonstrated a marked increase in MDA content in leaves of rapeseeds seedlings but this increase were higher in salt sensitive cultivars. Valentovic et al. (2006) showed salinity increase MAD content in corn salt sensitive cultivar but in tolerant cultivar, MAD unchanced. Mansour (1998) reported that application of proline prior to salt stress protected plasma membranes of onion cells from stress mediated oxidative damage.

Therefore, a higher percentage of increase in proline content and a lower extent of increase in MDA levels of seedling tissues as observed in our study was most probably the possible explanation of a reduced membrane damage of seedling tissues under salinity stress. Data of this study showed positive correlation between low of electrolyte leakage and MDA content with seedling dry weight (data don't show).

Various researchers dealing with plants (Santose, et al., 2003; Zhu and Seandalious, 1994) have also reported increase in antioxidant enzyme like as peroxidase (POD), super oxide dismutaz (SOD) and catalase activity in salt-tolerance cultivars under salt stress. In tolerant plant species, POD activity was found to be higher, enabling plants to protect themselves against the oxidative stress whereas such activity was not observed in sensitive plants (Scalet et al., 1995). In the present study, the POD activity significantly increased in Zarfam and Modena but remained unchanged in SIm04 and decrease in Opera. On the other hand, the salt-induced enhancement of POD activity in salt tolerant cultivars indicated that it had a higher capacity for the scavenge ROS. Data of this study showed positive correlation between peroxidase activity and low of MAD. Study of Bandoğlu et al. (2004) on Lentil showed under salt stress increase of peroxidase enzyme decrease effect of salt stress on growth of lentil seedling but activity of catalase decrease under salt stress in lentil. Meloni et al. (2003) showed under salinity, antioxidant enzyme like as peroxidase increase in salt tolerant cotton variety but in salt sensitive variety peroxidase activity was lower than salt tolerance variety. Increase in peroxidase in salt tolerant variety led to increase of photosynthesis of cotton in compared to salt sensitive variety. Conversely, as observed in our study, in potato (Benavides et al., 2000) and rice (Lin and Kao 2000) Catalase activity did not change under salt stress. Neto et al. (2006) showed salt stress reduced catalase activity of corn sensitive cultivar but did not effect on catalase activity of corn resistance cultivar. They suggested that catalase was sensitive antioxidant enzyme in compared to other enzymes such as peroxidase and super oxide desmutase under stress condition.

In conclusion, this study showed that the difference of antioxidant enzyme activities and ion content in the four cultivars could be described to the difference in mechanisms underlying salt stress injury and subsequent tolerance to salinity. Notably Zarfam and Modena cultivars, which exhibited higher salt tolerance, had also higher antioxidant enzyme activity and $K^+ : Na^+$ ratio than Modena and Opera. Data obtained of this study indicated that the relative NaCl stress tolerance of Zarfam and Modena may be due to a lower rate of peroxidation of its lipids and a higher constitutive activity of antioxidant enzymes. These results confirm that the scavenging system forms the primary defense line in protecting the plant tissue against ROS in rapeseed. MDA is produced when polyunsaturated fatty acids in the membrane undergo peroxidation. The results

reported here show that the degree of accumulation of MDA was higher in Opera and SIm04 than in Zarfam and Modena, indicating a high rate of lipid peroxidation in Opera and SIm04 due to salt stress. The lesser degree of membrane damage (as indicated by low MDA content and electrolyte leakage) and the higher activity of peroxidase and catalase observed in NaCl treated plants of Zarfam and Modena indicated that these rapeseed cultivars had a higher capacity for the tolerant salinity in comparison with sensitive cultivars.

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