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Response of spring canola cultivars to sodium chloride stress

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ABSTRACT

To study the effect of salt stress on quantitative and qualitative parameters of canola (Brassica napuse. L) cultivars, and determine the possible mechanisms of salt tolerance and the best salt tolerance indices, an experiment was conducted a hydroponic culture in Greenhouse of university of Tabriz and also To investigate the effect of salinity resulted from sodium chloride on rapeseed and selection of the most tolerant and susceptible genotypes, 12 canola (Brassica napus L.) cultivars were evaluated under three salinity treatment (0, 150 and 300 mM NaCl) arranged in split plot design under hydroponic culture system. Salinity stress was significantly affected all the traits under study. Proline is the major amino acid associated with environmental stresses (salinity, extreme temperatures, UV radiation and heavy metals). When exposed to drought or a high salt content in the soil (both leading to water stress), many plants accumulate high amounts of proline, in some cases several times the sum of all the other amino acids. Free proline content in leaves increased significantly by increasing of NaCl concentration. Accumulation of K+ in shoot instead of proline might be a way in which the genotypes perform osmotic adjustment under salinity. Tolerance index was identified as a good criterion to select the tolerant genotypes under high salinity stress. According to this index, Heros and Comet were identified as salt-sensitive and Craker and Amica as salt-tolerant genotypes. Results indicated that, pod per plant is the more influencing trait on seed yield under both normal and salinity conditions. Cluster analysis were classified the genotypes into the three groups in which Heros and Comet were blong to the cluster with low mean with respect to all the traits. These results suggest an ample genetic variability between rapeseed genotypes which could be used in breeding programs. Furthermore, by identification of contrasting genotypes, molecular dissection of tolerance to salinity stress and identification of corresponding genes would be amenable.

Keywords : *Brassica napus*, Correlation coefficient, Proline, Salt Stress, Sodium chloride.

INTRODUCTION

Salinity is a major abiotic stress reducing the yield of a wide variety of crops all over the world [1]. Although the level of salts in most irrigation waters is below the threshold for the more sensitive

crops, salt accumulation in irrigated soils from both irrigation and groundwater sources can increase salinity to levels which can reduce growth and yield of even the more tolerant crops. Overcoming salt stress is a main issue in these regions to ensure agricultural sustainability and continued food production [2]. Rapeseed (Brassica napus L.), from the Cruiceferae family, grows in about 42.2 million ha in 53 countries all over the 6 continents, yielding an average of 1451 kg ha-1. Asia alone owns 59.1% of the cultivated areas, but produces only 48.6% of the whole production. Canola (Brassica napus L.) has a high adaptability under the different environmental conditions especially under the drought, salinity and temperature stresses [3].

The research has shown that in response to soil salinity, seedlings growth, leaves area, root biomass and shoot biomass have all been reduced [4]. In oilseed Brassicas, for example, higher yield is closely associated with greater post-anthesis growth which, in turn, is correlated with a capacity for osmotic adjustment to drought. Differences in salt tolerance among plant species have also been long recognized. However, the role that salt tolerance plays in causing differences in nutrient uptake and metabolism between various plants, among plant species, at different stages of growth is still a major concern among investigators, and has not been fully understood. So it requires joint effort of agronomist, biochemist, geneticist, plant physiologist, soil scientists among others. Plant performance usually expressed as a crop yield, plant biomass or crop quality, may be adversely affected by salinity-induced nutritional disorders. These disorders may result from the effect of salinity on nutrient availability, competitive uptake, transport or partitioning within the plant. For example salinity reduces phosphate uptake and accumulation in crops grown in soil a primarily by reducing phosphate availability. High salinity is harmful to plant growth as it causes; (a) Nutritional disorders by decreasing the uptake of cations, such as potassium and calcium, but also of anions such as phosphorus and nitrate [5]. (b) Ion cytotoxicity mainly due to elevated concentrations of Na+, Cl-, plus SO- 4 (c) Osmotic stress [6]. The detrimental effects of salt on plants are a consequence of both a water deficit that results from the relatively high solute concentrations in the soil and a Na+-specific stress resulting from altered K+/ Na+ ratios and Na+ ion concentrations that are inimical to plants [7]. Rosielle and Hamblin [8] defined stress tolerance (TOL) as the differences in yield between the stress (Ys) and non-stress (Yp) environments and mean productivity (MP) as the average yield of Ys and Yp. Fischer and Maurer [9] proposed a stress susceptibility index (SSI) of the cultivar. Fernandez [10] defined a new advanced index (STI= stress tolerance index), which can be used to identify genotypes that produce high yield under both stress and non-stress conditions. Other yield based estimates of drought resistance are geometric mean (GM), mean productivity (MP) and TOL. The geometric mean is often used by breeders interested in relative performance since abiotic stress can vary in severity in field environment over years [11]. Several environmental factors adversely affect plant growth and development and final yield performance of a oilseed crop. Antioxidants enzyme and organic osmolytes such as proline are known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses. Under environmental stress conditions, many plants accumulate several kinds of compatible solutes such as proline, glycinebetaine, sugars and polyols [1]. Oilseed crop studies published to date have concentrated on the various species in response to different biotic and abiotic factors and many functional proteins have been identified, for example, many kinds of proteins up-regulated in response to stress in Suaeda aegyptiaca leaves [12], rice leaves [13] and rice roots [14] and proteins involved in responses to osmotic stress in Arabidopsis [15]. The responses of plants to stress conditions have evolved a variety of physiological and biochemical processes, for example, solute accumulation and the development of enzymatic antioxidant systems [1]. The amino acid

proline is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses [16]. Plants employ antioxidant defense mechanisms against oxidative damage of reaction oxygen species. Proline and betaine enhance antioxidant defense systems in plant responses to various oxidative stresses [17]. Increased accumulation of proline leads to the increase of enzyme activity of glutamate kinase and therefore increases biosynthesis proline [18].

This experiment was conducted to determine the seed yield and shoot development of canola cultivars at vegetative growth stage, association of drought tolerance indices with seed yield and contribution of proline to osmotic adjustment in response to salinity stress. Several indices have been utilized to evaluate genotypes for drought resistance based on grain yield.

MATERIALS AND METHODS

Plant Material and Growh Condition

The experiment was conducted in hydroponics culture system under greenhouse conditions at the Faculty of Agriculture, University of Tabriz. The experimental design consisted of 36 treatments replicated three times in split plot design, with salinity as main factor and cultivar as sub factor with 5 plants in each subplot. Twelve canola cultivars (B. napus) Olga , Wild cat, Sarigol, Heros, Cracker, Comet, Option 500, SW hotshot, Amica, SW5001, Eagle and RGS003 were subjected to three NaCl concentrations (0, 150 and 300 mM). Seeds were sterilized and germinated in petri dishes and seven day-oil seedling of uniform size were transferred into large sandbanks housed within an environmentally–controlled greenhouse (14h daily light, 600-800µmol m-2s-1photosynthetic photon flux density (PPFD) thermo-period 25\15ºC day\night, relative humidity 50\60% day\night). The P.V.C. tanks were sub irrigated and flushed four times daily with a modified Hogland nutrient solution. NaCl stress was imposed seven days after the seedlings were transferred. Three randomly selected plants per replicate were collected at the fruit set stage, divided into leaves, stems and roots, and dried in an oven at 70 °C for 2 days to determine dry weights and elemental concentrations. Analyses were carried out on a dry weight basis.

Tolerance Indices

Grain yield was determined under non-stress, mild stress and high stress conditions and indicated as Yp, Ys_1 and Ys_2 respectively.
Stress tolerance indices TOL1, MP2, SSI3, GMP4 and STI5 were calculated using the following relationships, respectively:

$$\mathbf{TOL = Y_P - Y_S} \quad (1)$$

1 - Tolerance

2 - Mean productivity

3 - Stress Susceptibility Index

4 - Genometric Mean Productivity

5 - Stress Tolerance Index

$$MP = \frac{(Y_P + Y_S)}{2} \quad (2)$$

$$SSI = 1 - \frac{(Y_S / Y_P)}{(\bar{Y}_S / \bar{Y}_P)} \quad (3)$$

$$GMP = \sqrt{Y_P \times Y_S} \quad (4)$$

$$STI = \frac{(Y_P)(Y_S)}{(\bar{Y}_P)^2} \quad (5)$$

$\bar{Y}_p$ = mean yield in non-stressed environment. $\bar{Y}_S$ = mean yield in salty stressed environment. Correlation between yield and salinity tolerance indices was evaluated by MSTATC and SPSS computer programs.

Proline content

Proline content was determined according to the method described by Bates et al. [19] Approximately, 0.5 g of fresh leaf material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and filtered through What man's No. 2 filter paper. Two milliliter of the filtrate was mixed with 2 ml of acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100 °C. The reaction mixture was extracted with 4 ml toluene and the chromophore containing toluene was aspirated, cooled to room temperature, and the absorbance was measured at 520 nm with a Shimadzu UV 1601 Spectrophotometer. Appropriate proline standards were included for the calculation of proline in the samples.

Statistical Analysis

Data for ion content and water relations were analyzed using ANOVA (Randomized completely Blok-design) to determine if significant differences were present among genotypes means and mean comparison was done using FLSD[6] test.

RESULTS

Effect of salinity on plant growth

This study indicates that, the traits of plant height, shoot fresh and dry weight, root dry weight, number of pods on the plant and seed yield were affected by salinity, so that reduction in these traits was significant. This reduction can be a result of increase in Osmotic pressure of the soil solution and imbalances in the elements. Reduction of Osmotic potential, as a result of salinity, is the most important deterrent factor in plant growth and the maximum reduction was observed in 300 ml mol.

6 - Fisher Least Significant Different

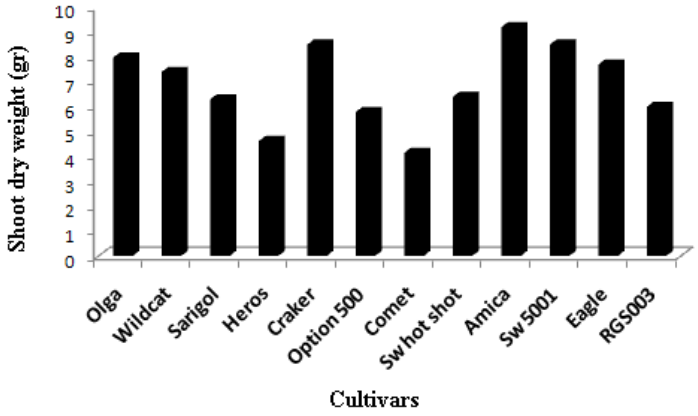

Figure 1: Average shoot dry weight of canola cultivars under salinity condition (Lsd 1% =3.54).

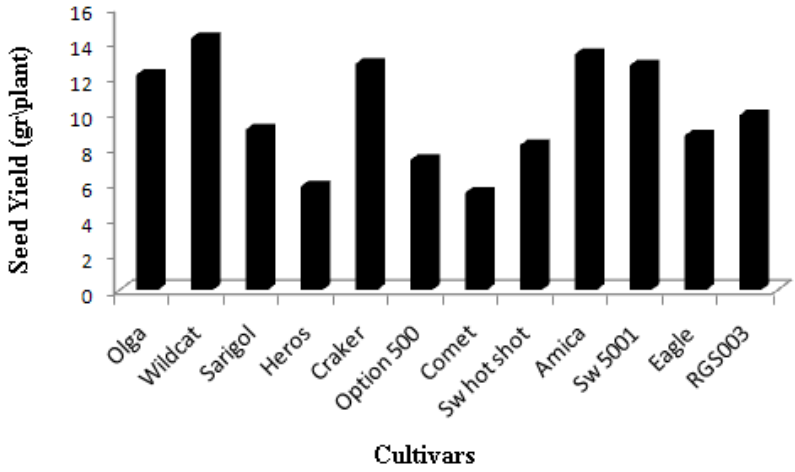

Figure 2: Seed yield of canola cultivars under salinity condition (Lsd 1% = 5.24).

Assessment of salinity Tolerance in canola cultivars

Determine the most desirable salinity tolerance criteria, the correlation coefficient between Yp, Ys_1 and other quantitative indices of salinity tolerance were calculated (Table 1). There were positive significant correlations among Yp and (MP, GMP, TOL and STI) and Ys_1 and (MP, GMP and STI). The correlation coefficients for TOL and SSI vs. seed yield under mild salinity stress (Ys_1) were r = -0.581, -0.49, respectively. No significant correlations were observed between TOL and GMP (r = 0.034, $p<0.05$) and TOL and STI (r = 0.143, $p<0.05$). A positive correlation (r = 0.331, $p<0.01$) was found between seed yield under mild stressed and non-stressed situations (Table 1).

Also determine the most desirable salinity tolerance criteria, the correlation coefficient between Yp, Ys_2 and other quantitative indices of salinity tolerance were calculated (Table 2). There were positive significant correlations among Yp and (MP, GMP, TOL and STI) and Ys_2 and (MP, GMP and STI). The correlation coefficients for TOL and SSI vs. seed yield under high salinity stress (Ys_2) were r = -0.43, -0.80, respectively. No significant correlations were observed between TOL and STI (r = 0.028, $p<0.05$). A positive correlation (r = 0.13, $p<0.05$) was found between seed yield under high stressed and non-stressed situations (Table 2).

Average STI index for canola cultivars were calculated (Table 3). Amica and Craker had the highest amount of this index in high salinity stress and demonstrate tolerant cultivars. Also Heros and Comet had the lowest values for this index and demonstrate sensitivity cultivars. This index is in relation with seed yield, in both environments. Any amount greater STI is more tolerant genotypes to represent the salinity.

Table 1: Correlation coefficients between Yp, Ys_1 and salinity tolerance indices

	Yp	Ys_1	MP	GMP	TOL	SSI	STI
Yp	1	0.331*	0.815**	0.67**	0.575**	0.36*	0.626**
Ys_1		1	0.817**	0.706**	-0.581**	-0.49**	0.788**
MP			1	0.844**	0.005	0.085	0.867**
GMP				1	0.034	-0.116	0.886**
TOL					1	-0.74**	0.143
SSI						1	-0.085
STI							1

*and **Means significant at 5 and 1% levels of probability, respectively. Yp: Yield under non-stresscondition, Ys_1: Yield under mild salinity conditions, TOL: Tolerance index, GMP: Geometric mean productivity, SSI: Stress susceptibility index, STI: Stress tolerance index.

Table 2: Correlation coefficients between Yp, Ys_2 and salinity tolerance indices

	Yp	Ys_2	MP	GMP	TOL	SSI	STI
Yp	1	0.13	0.873**	0.598**	0.33**	0.225	0.436**
Ys_2		1	0.597**	0.656**	-0.43**	-0.80**	0.768**
MP			1	0.807**	-0.46**	-0.241	0.731**
GMP				1	-0.181	-0.384**	0.904**
TOL					1	-0.65**	0.028
SSI						1	-0.41**
STI							1

*and **Means significant at 5 and 1% levels of probability, respectively. Yp: Yield under non-stresscondition, Ys_2: Yield under high salinity conditions, TOL: Tolerance index, GMP: Geometric mean productivity, SSI: Stress susceptibility index, STI: Stress tolerance index.

Table 3: STI index for canola cultivars

STI		cultivars
High salinity	Mild salinity	
0.87	2.77	Olga
0.39	1.91	Wildcat
0.85	2.72	Sarigol
0.28	1.4	Heros
1.37	3.18	Craker
0.43	2.38	Option 500
0.3	1.18	Comet
0.52	1.67	Sw hot shot
0.91	1.76	Amica
0.64	2.24	Sw 5001
0.55	1.77	Eagle
0.87	2.72	RGS003

PROLINE

Results showed that the prolin levels also was affected by salinity and The increase in salt stress, increased proline concentration in canola cultivars (Figure 3). The accumulation of free proline is a common response to a wide range of biotic and abiotic stresses [2]. Figure(4) shows the effect of salinity on canola species' leaves' proline mean in various salinity levels. The proline concentration percentage changes in high salinity level shows that increase in proline amount in Olga specis is more than increase in mild salinity level. By taking these different reports about proline's role in salinity stress resistivity into account, it's use as an indicator and standard has always been in doubt by the researchers, and more investigation in various plants is required [20].

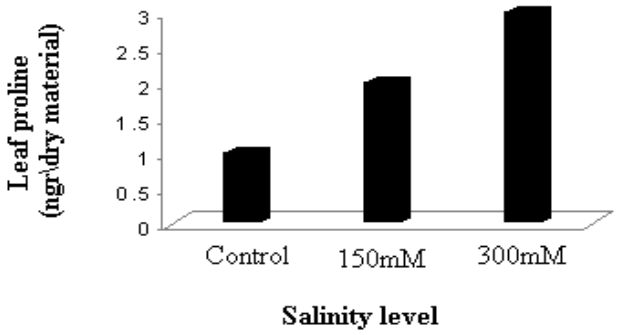

Figure 3: leaf proline canola cultivars in salinity condition (LSD 1% = 0.63).

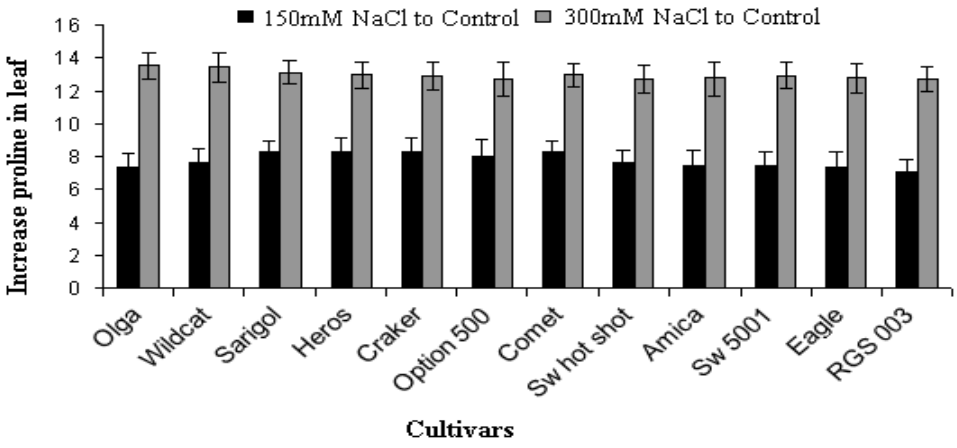

Figure 4: Effect of salinity on leaf proline in the canola cultivars.

DISCUSSION AND CONCLUSION

Effect of salinity on plant growth

Figure(1) and Figure(2), show shoot dry weight and Seed yield of canola cultivars under salinity condition. In a comparison made based on cultivars average traits, the reduction in Comet and Heros cultivars was more than other cultivars. In stress conditions Amica showed root length

growth. The reason is that, it devotes more photosynthesis energy for root growth. Negative effect of salinity on growth and root weight reductionwas also reported by Saqib et al. [21]. The root growth reduces and even stops, as a result of salinity, because it leads to a disturbance in distribution of mineral supplies and the root dry weight is reduced as a result of reduction in root growth [22]. here is significant difference between stress levels from the point of traits plant height, root length, fresh and dry shoot weight, fresh root weight, length of pod, 1000-seed weight, number of pods on the plant and plant yield. It can be said that, there is a high genetic diversity between these cultivars and it can be used in reform programs, especially in making hybrid done to produce appropriate breed. The cultivar interaction for salinity was non-significant for all of the traits of the study, that indicates that the similar reaction of cultivars in different levels of salinity. Keshta et al. [23] examined the salinity stress on different canola cultivars on a farm experiment. By increasing soil salinity from 2.5 to 6.5 mmohs flowering, number of racemes per plant, number of silliqua per plant, 1000-seed weight, seed yield per hectare, oil content, total dry matter and harvest index showed significant decrease. This reduction is due to increase in soil solution Osmotic pressure and the imbalances in needed elements.

Evaluated based on stress resistance index

The results indicated that there were positive and significant correlations among Yp and (MP, GMP and STI) and Ys_2 and (MP, GMP and STI) and they hence were better predictors of Yp and Ys_2 than TOL and SSI. The observed relationship between Yp and (MP and STI) and Ys_2 and (MP and STI) are in consistent with those reported by [10] in mung bean and [24] in maize. Ud-Din et al. [25] showed significant and positive correlation between Ys and TOL and Ys and Mp as well as between Yp and MP, while TOL was negatively correlated with Yp and MP. In the present study, the correlation coefficient for stress tolerance (TOL) vs. seed yield under high salinity stress (Ys_2) was ($r = -0.43$). Thus, selection for tolerance should decrease yield in the high salinity stress environment and increase seed yield under non-stress ($r = 0.33$). Thus, selection for tolerance will be worthwhile only when the target environment is non- stressed. The correlation coefficient for mean productivity vs. yields in high salinity stress and non-stress environments were 0.597 and 0.873. Thus, selection for MP should give positive responses in both environments. No significant correlations were observed between TOL and GMP ($r = -0.181$) and TOL and STI ($r = 0.028$). The lack of a correlation between TOL and GMP and between TOL and STI would indicate that the combination of high GMP and STI with a low to moderate TOL is biologically accessible in canola, thereby, combining different traits that associate with each index.

[10] proposed STI index which discriminates genotypes with high yield and stress tolerance potentials. Limitations of using the SSI and TOL indices have already been described in common bean [26]. The SSI does not differentiate between potentially drought-tolerant genotypes and those that possessed low overall yield potential. Although, low TOL has been used as a basis for selecting cultivars with resistance to water stress, the likelihood of selecting low yielding cultivars with a small yield differential can be anticipated [26].

Correlation analysis revealed that Yield potential (Yp) and high stress yield (Ys_2) had highly significant positive correlation coefficients with Stress Tolerance Index (STI), Mean Productivity (MP) and Geometric Mean Productivity (GMP). Moreover, the correlations among STI, MP and GMP exhibited same trend, thus they can be introduced as the most desirable indices for screening salinity tolerance genotypes. Stress Tolerance Index (STI) is calculated based on GMP and thus rank

correlation between STI and GMP is equal to 1. The higher value of STI means higher tolerance and yield potential for genotype. The stress intensity value is also incorporated in the calculation of STI. Thus, STI is expected to be the most desirable index for salinity tolerance. Same result was obtained by [27] for STI, MP and GMP.

Under most yield trial condition, the correlation between Ys and Yp is between 0 and 0.5 and genetic variance ratio is <1 [27]. Present results revealed that the correlation coefficient between Ys_2 and Yp was 0.13 (Table 2). Thus, genotypic selection for yield under a non-stress environment would increase the mean stress yield. MP is based on the arithmetic means and therefore, it has an upward bias due to a relatively larger difference between Yp and Ys_2, whereas, the geometric mean is less sensitive to large extreme values.

In the present study, Craker and Amica cultivars had the highest amount of STI and therefore, they may be known as desirable genotypes for both stressed and non-stressed environments. Also Heros and Comet had the lowest amount of STI and therefore, they may be known as undesirable genotypes for stressed environment.

PROLINE

The plants use increased proline content for biosynthesis of physiological specific proteins and/or stress proteins. Effect of salinity to proline content in canola, rice and wheat was reported previously [24]. The accumulation of proline oxidation or diminished incorporation of proline into protein is due to impaired protein synthesis and reduced growth. Accumulated proline may supply energy to increase salinity tolerance [28]. Accumulation of an osmoprotectant, proline, is enhanced in response to salinity in plants. One of these mechanisms depends on the capacity for osmotic adjustment, which allows growth to continue under saline conditions [2]. Plants may have evolved a mechanism to coordinate synthesis, catabolism, and transport activities for the accumulation of proline [29]. A positive correlation between the proline content and salt-stress tolerance has been previously observed in a wide range of plant species [30]. Many studies have suggested that proline is involved in intracellular osmotic adjustments between the cytoplasm and vacuole. It has also been proposed that proline can stabilize the cellular structure and scavenge free radicals or act as a storage compound for carbon and nitrogen to allow recovery of lipids from stress. Reviews indicate that although assembly Transgenic plants leads to increase in stress tolerance, but whether proline accumulation in transgenic plants resulted in increased stress tolerance through osmotic adjustment or other mechanisms is unknown [1]. Madan et al. [31] showed Salt stress caused differential enhancement in proline level in both seedlings and leaf tissue of plants at different developmental stages. Against Moghaieb et al. [32] view in study in Suaeda maritima that such sensitive species to salinity, amount of proline cumulative were in the leaves more than species Salicorina europaea tolerant to salinity. also have been reported negative relationship between proline accumulation and stress tolerance of tomato [33], and rice [34]. So it can be concluded that proline increase not only in sensitive cultivars but also can be seen tolerant cultivars, but show the amount of increase in tolerant cultivars more than the sensitive cultivars.

Proline is gathering canola cultivars under salinity in tolerant cultivars, especially in two Osmotic adjustment and physiological activities such as planning to build stable structures following cellular membranes and proteins such as, clearing free radicals and stabilize cell oxidation potential destructive effects of the adjustment to stress [1]. So we can conclude that the first role of proline is

physiological activities [35] and the next role, Osmotic adjustment. the first role in canola cultivares is more important than the second role. Role playing Osmotic adjustment is required for high proline concentration. If mentioned in 300 mmol saline in addition Na+, the proline active role in the amount Osmotic adjustment.

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