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Research Article

EVALUATION OF CHANGES IN ENZYMES OF TWO CANOLA GENOTYPES IN THE PRESENCE OF SALINE IN GREENHOUSE CONDITIONS

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ABSTRACT
Salinity is of the most important stress on the Earth and the medium culture. One of the most important research fields of modern plant science researchers is to investigate and detect the changes in plant life under stress and Psychological and morphological changes. It is also much easier and less costly to evaluate and select the traits that can be used to select more resistant cultivars or stress sustainable rather than yield traits. Since the planting of canola had a sharp growth rather other oilseeds and is considered as an important industrial and oil plants, so an experiment was done to compare 2 canola cultivars on salinity stress With various concentrations of a factorial complete randomized block design having three replications in a greenhouse environment. 15 traits were studied. Cobra canola and okati cultivar were tested. Analysis of the comparison showed no significant differences among cultivars and salinity levels but catalase enzyme was decreased. But Ascorbate and peroxidase enzyme was increased by increasing the salinity level But Super oxidize dismutase was decreased after slightly increased of salinity levels and had a half steadily process.

Keywords: *Canola, Salinity Stress, Physiological, Greenhouse*

INTRODUCTION
Canola plays an important role in ensuring global sweet oil. Canola oil in some cultivars are equal to 48% of the dry weight of the seed, are Currently providing 12% of people need in the world need to provide oils and thus has the third place among oil plants (Ahmadi, 1991). Not only the plants has different stress tolerance, but also on a particular species, the level of tolerance is varied to environmental abiotic stress for different cultivars of one species. Salinity would induce water shortages even in the presence of sufficient water by reducing the osmotic potential of the soil solution around the roots (Sairam *et al*., 2002). Salinity, a general term indicating the presence of various mixtures of saline in the soil. Nearly 50% of the cultivated area has different degrees of salinity, alkalinity, and flooding problem (Mir *et al*., 2001). Plant growth may be decreased due to salinity stress through Osmotic effect of in terms of water potential in the root or ion-specific effects in metabolic processes (Greenway and Munns, 1980). Ions would inevitably absorbed during the seed is exposed to saline, causing toxicity in, biochemical and physiological processes. Enzyme activity are impaired and result in synthesis of micro and macro molecules decrease and decrease the growing tissues. Salinity also impair enzyme activity and changes. Accumulation of soluble sugars, free protein and soluble proteins are other plant responses to salinity which may occur during germination. This effect is first act as by reducing the osmotic potential and secondly by providing a substrate for the growth of fetal tissues (Ragreys, 1995). Generally, The effect of salinity on the increase or decrease of enzymes activity, depending on the nature of the enzyme, and plant species and plant stress. The activity of some enzymes that are important in the synthesis of osmosis, would also increase. For example, amylase activity, Aldose redoctaze, effective enzymes that synthesize betaine and proline would increase too. Effective transfer of membrane enzymes such as ATPase in cotton seeds and Ca ATPase in tomato under salinity show more activity (Lechno *et al*., 1997). Salinity changes different enzyme activities such as protolithic amino lithic, nucleolthic, phosphor lithic, oxidative and antioxidant and photosynthetic in the seeds of growing plants germination (Evers *et al*., 2000). This defense includes enzymes that are capable to remove, neutralize and scave the oxygen mediating which the most important of which can be catalase and superoxide dismutase (Eslafer and Araoos, 1988). Free

radicals would damage cells through a mechanism of lipid peroxidation and occlusion of normal antioxidant systems (Mittler, 2002). Catalase is an enzyme, which uses the metal ions as a cofactor. Catalase also protects cells against hydrogen peroxide. Catalase is necessary for some types of cells under normal conditions and has crucial role in providing resistance to oxidative stress in adaptive response of the cells. Biochemical findings suggest that catalase in plant cells are settled just in peroxisome and galaxy zoom (Bartels and Sunkar, 2005).

Tom and Astrom believe that (Tan and Strum, 1999) tension and stress increase producing reactive oxygen species and increase the anti – oxidant defense. Studies show that antioxidant responses depends on the sensitivity and resistance of the cultivars (Bates *et al*., 2007).

The salinity stress decrease the activity of peroxidase, dismutase and peroxidase in the receiver plant, which in turn free oxygen radicals in the cell increases. Accumulation of free radicals cause lipid peroxidation and especially the membrane and would destroy its structure. Conduction electron-ion cells is increased due to release ion and decrease of photosynthesis and damages mitochondrial structure and chloroplasts the reduction of photosynthesis and respiration increase, causing lack of energy and eventually fall in growth.

MATERIALS AND METHODS

An experiment conducted on two cultivars of Canola in greenhouse to study the effects of salinity stress and potential mechanisms of possible tolerance. It was a factorial experiment based on randomized complete block with three replications. The seeds were obtained from the Department of Agriculture. Treatments were combination of three cultivars: vanguard, Okapi and 4 salinity concentration of sodium chloride zero (control), 50, 75, 150 and 100 mM) . Measurement was performed Forty days after planting to measure the traits.

Superoxide Dismutase

Superoxide dismutase activity was measured by Gianopolities and Raiz (1977), were measured.

$$UnitSOD = \frac{V}{v} - 1$$

V= absorption rate in free enzyme reaction (maximum response)

v = absorption rate of enzyme reaction

Catalase: Catalase activity was measured using chance and Maehly (1955) method. One unit of catalase, as the amount of enzyme required to oxidize 1 mM H2O2 per minute, were considered.

3-4-7-APX: Ascorbate peroxidase enzyme was measured according to Nakano and Asada method (1981). 1 unit of ascorbate peroxidase activity was considered as the amount of enzyme required to oxidize 1 mM ascorbic acid per minute. Enzyme activity was explained as a specific activities (mg fresh weight of leaves / enzyme unit). Data was performed for the analysis of variance using SPSS software and the average compare was done by MSTATC software and on Duncan's multiple range test basis.

Super Oxidase

Super oxidase activity is increased by increasing the amount of salinity concentration, saline concentration of 150 mM, with the average is 3681.02 has the highest amount of increase. The rate of Superoxide activity had an increasing mode in Okati cultivar so that it showed a 49.96% increase at the level of 150 mM to the control group. Superoxide activity in the vanguard cultivar showed a decrease at the salinity level of 75. So that it decreased to 0.86% to control group.

And it increased at the level of 75 mM to 100 mM so that salinity level of 100 showed an increase of 79.88 to 75 mM (Table 1).

Table 1: Compare Super oxidase in salinity stress × cultivar the interaction of using Duncan's multiple range tests at the 5% level

Superoxide	Cultivar	The salinity levels
1505 ± 06 / 182ab	talaye	control
1018 ± 06 / 182b	okati	
1464 ± 06 / 182ab	talaye	50 mM
1350 ± 06 / 182ab	okati	
1492 ± 06 / 182ab	talaye	75 mM
1254 ± 06 / 182ab	okati	
2684 ± 06 / 182ab	talaye	100 mM
1730 ± 06 / 182ab	okati	
1469 ± 06 / 182ab	talaye	150 mM
2034 ± 06 / 182ab	okati	

Average that at least one class in common, according to Duncan's multiple range test, no significant difference $\alpha \leq 0.05$

CAT

Comparing the average of Salinity levels of catalase by Duncan at the possible level of 0.5% showed the highest enzyme activity in the salinity levels of 150 mM with the average of 0.2910 and the control group (water without saline) with the average of 0.1550 had the lowest enzyme activity among salinity level. The rate of catalase activity is increased by an increase in salinity concentration. So that the salinity concentration of 150Mm of 0.35 had the highest increase. With the average of 0.35. Due to changes in catalase, okati had the lowest rate of changes at the highest salinity rate to the control group. And also due to the high levels of catalase, cobra cultivar had the highest salinity to the control group so it had the greatest impact in salinity. Comparing the average data suggest that in terms of controlling condition, cobra cultivar compared to Okati had the lower catalase and the rate of catalase activity in okati cultivar would be increasing to salinity level of150. So that it showed an increase of 26.08% to the control group. The CAT activity in cobra cultivar has been increasing to the salinity level of 150mM so it showed an increase of 43.33% compared to the control group (Table 2).

Table 2: Compare catalase in salinity stress × cultivar the interaction of using Duncan's multiple range test at the 5% level

Catalase	Cultivar	The salinity levels
171/0 ± 01 / 0cd	talaye	control
171/0 ± 01 / 0cd	okati	
179/0 ± 01 / 0cd	talaye	50 mM
203/0 ± 01 / 0bcd	okati	
195/0 ± 01 / 0bcd	talaye	75 mM
219/0 ± 01 / 0bcd	okati	
211/0 ± 01 / 0bcd	talaye	100 mM
219/0 ± 01 / 0bcd	okati	
299/0 ± 01 / 0ab	talaye	150 mM
227/0 ± 01 / 0bcd	okati	

Average that at least one class in common, according to Duncan's multiple range test, no significant difference $\alpha \leq 0.05$

APX

The rate of APX activity is increased by an increase of the salinity concentration which the salinity concentration of 150mM with the average of 12.0 had the highest increase. Due to the changes of Ascorbate peroxidase, cobra cultivar had the highest changing in salinity level to control condition.

Comparison of data indicates an equal Ascorbate peroxidase rate in all three cultivars in control condition. The rate of activity in Ascorbate peroxidase in cobra cultivar showed an increasing mode and the salinity level of 150 indicates an increase of 11.20% to the control group (Table 3).

Table 3: Compare Ascorbate peroxidase in salinity stress × cultivar the interaction of using Duncan's multiple range tests at the 5% level

Ascorbate peroxidase	Cultivar	The salinity levels
1029/0 ± 0008 / 0bc	talaye	control
1028/0 ± 0008 / 0bc	okati	
1041/0 ± 0008 / 0bc	talaye	50 mM
11/0 ± 0008 / 0abc	okati	
1062/0 ± 0008 / 0bc	talaye	75 mM
1020/0 ± 0008 / 0bc	okati	
1083/0 ± 0008 / 0abc	talaye	100 mM
1045/0 ± 0008 / 0bc	okati	
1158/0 ± 0008 / 0a	talaye	150 mM
1041/0 ± 0008 / 0bc	okati	

Average that at least one class in common, according to Duncan's multiple range test, no significant difference $\alpha \leq 0.05$

RESULTS AND DISCUSSION

Discussion

Many plants are remarkably sensitive to saline conditions which is due to sodium accumulation in the cell and its impact on disorder of ionic balance, osmosis regulation, and the activity of enzymes, cells metabolic and preventive toxicity. Canola is among the saline tolerant crops and has the ability to be grown in saline (Ashraf and McNeilly, 2004). Jooz *et al.*, (2004) concluded in the investigation that there was a significant and negative relationship between the catalase and proline enzyme. There was also a significant and positive correlation between proline and super oxidase. in the present study, an increase of super oxide was observed by an increase of salinity concentration which the salinity of 150mm with an average of 3681.02 had the highest increase.

Considering the super oxidase, cobra cultivar had the highest changing to the control group so this cultivar has more resistance against stress condition to the other cultivar. the rate of catalase has increased by an increase of salinity concentration which the salinity of 150 by the average of 0.35 has the highest increase. Due to changes in catalase was observed Okoth had the lowest rate of change relative to the control condition at the highest salinity. Since it had the highest rate of change, so is more resistant.

By an increase of saline concentration, the rate of Ascorbate peroxidase has increased too which the salinity of 150mM with the average of 0.12 experienced the highest increase. Here, Cobra cultivar had the highest rate of change to the control group, so it was the most resistant cultivar. Mihalvic et al, in a study of the Effect of salinity on canola plants, through The analysis of variance and the means concluded, catalase and Ascorbate peroxidase enzymes have decreased by an increase in salinity rate but super oxidase has increased.

A negative and meaningful relation was observed among catalase and proline enzyme. This result was true about catalase and super oxidase. Canola cultivars was influenced by salinity, type of cultivar and the interaction. Peroxidase enzyme activity has been low in free saline condition. The catalase activity decreased with an increasing of salinity levels (Ghalibaf, 1994).

Conclusion

Studies showed an increase of catalase and ascorbate peroxidase enzyme by an increase of salinity. But super oxidase dismutase has decreased after a slight increase of salinity level and had a semi- steady mode.

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