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Superoxide Dismutase (SOD) Activity in Nacl Stress in Salt-Sensitive and Salt-Tolerance Genotypes of Colza (*Brassica napus* L.)

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Abstract: The production of significant amount of reactive oxygen species (ROS) in salt stress condition causes damage to proteins, lipids, nucleic acids and other sites of cells this process is a lethal factor for salt sensitive plants. Tolerant plants involved an antioxidant defense system which protects them against oxidative damage, so antioxidant activity, especially Superoxide Dismutase, as scavengers of ROS can be used as biochemical marker to screen for salt tolerant resources. In these study two genotypes of colza, Quantum as a salt tolerant and Fornix as a sensitive, were chosen and germinated in Four NACL concentrations. SOD activity was assayed by monitoring the inhibition of photochemical reduction of nitro Blue tetazolium in roots and shoots of both genotypes in all concentrations. Analysis showed that amount of Superoxide Dismutase activity increased in both genotypes in relation to increase in Nacl concentration. Increase in amount of SOD activity in root of tolerant genotype was greater than sensitive genotype. In shoot SOD activity inducted in both genotypes up to 100 mmol concentration, increase in salt concentration from 100 to 150 mmol cause decrease in SOD activity in shot of both genotypes but decrease in salt sensitive genotype was greater than salt tolerant genotype.

Key words: Colza · Superoxide Dismutase · NaCl stress · Antioxidant defense system

INTRODUCTION

Salt tolerance associated with the adjustment mechanisms ionic, osmotic and antioxidants have been studied [1] salinity stress, like other non-living can lead to oxidative stress by increasing the reactive oxygen radicals (ROS) including Superoxide (O^-_2), hydrogen peroxide (H2O2) and hydroxyl radicals (OH), which are very active and can is through lipid oxidation [2, 3] proteins [4] and nucleic acid is [5-7] O_2 activation and formation of cells in many parts of the electron transport chain exists including mitochondria, chloroplast, micro some, glycosome, sitosoll and Praksysome done [8]. Although all parts of the cell to produce O^{-2} are suitable but, chloroplasts, Praksysome and mitochondria, as the most important producers of oxygen free radicals are known [2, 9-11].

Since the internal rate of O_2 during the process of photosynthesis is high, chloroplasts the main producer of oxygen free radicals know [12] Oxide to reduce the effects of salt stress plant cells equipped with antioxidant defense system are the low volume of antioxidant molecules (glutathione, ascorbate and carotenoids) and enzyme converts active oxygen radicals such as Superoxide Dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) has been established [9]. In a cell enzyme Superoxide Dismutase (SOD) in the first line of defense against reactive oxygen species is. O^{-2} enzyme to convert H_2O_2 catalyzed the beginning that their chain of reactions that convert host H_2O_2 produced by the enzyme catalase and other enzymes by the H_2O and preventing the damaging effects of oxygen is O_2 [9]. Increased rate of Superoxide Dismutase enzyme activity in salt stress in wheat roots [13], peas and flax [12, 14] has been studied. And is marked with increasing salt concentration in the rate of secretion of the enzyme Superoxide Dismutase also observed increases are [12], for example Agraval and Pound [15] increased the enzyme Superoxide Dismutase

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with increasing concentration of ions Na^+ and Cl^- in *Cassia angustifolia* seedlings were reported. The results of research conducted on tomato [16], wheat [13, 17], beet and cotton [12, 14] a significant increase in the Superoxide Dismutase enzyme activity in tolerant cultivars than susceptible cultivars under salt stress shows said. Also, complementary studies significant differences between the secretion of this enzyme in salinity in the root to the stem and leaves showed.

This Experiment aimed to study the activity of antioxidant system Superoxide Dismutase enzyme and secretion in sensitive and resistant canola varieties to salinity was performed.

MATERIALS AND METHODS

Among the 20 genotypes of canola, by seed germination test on two different levels of salinity, two genotypes of colza, Quantum as a salt tolerant and Fornix as a sensitive, were chosen. The seeds were planted in Petri dishes in 27°C temperatures to 14 days Period at zero salinity conditions, 50, 100 and 150 mm Nacl. The germinated seeds with roots and shoot in Petri dish choice and shoot them separately and according to the following method of enzyme extracted. Selected organs were powdered in liquid nitrogen and immediately were kept in eppendorf tube at -20°C. Then 0/5 grams of frozen powdered root and shoot, in 10 ml of potassium phosphate buffer 50 mM with PH = 7 containing 1 mM EDTA, 1% poly and poly vinyl poly pyrrolidone and was Homogenized. The Homogenized samples by iceman Centrifuge for 20 min in 4 Temperature with ×15000gr was centrifuged. And Obtained super netEnt as enzyme source was separated by sampler for next steps. The Rate of Superoxide Dismutase activity, were determined by photochemical nitro blue tetrazolium test method Beyer and fridovich [18,19]. In this method, Protect NBT reduction by extracted enzyme was determined by spectra photo metric method. thus, Identify solution Containing 27 ml 50 mM potassium phosphate solution with PH = 7/8, 1/5 ml L-methionine, 1 ml nitro blue tetrazolium and 1000ml Triton - X 100 was prepared. One ml of this solution was poured into small tubes and then the 20 microliters of Extracted enzyme was with 10 micro liter of riboflavin (4 / 4 mg in 10 ml) was added. The solution 7 minutes within the chamber that completely covered by aluminum foil and the two 20-watt fluorescent lamp was placed. Also, the two solutions as the control were considered. One of these solutions, contain all the substances in solution were identified and only the same amount of enzyme rather than the potassium phosphate buffer was added. The first solution to control any light away from the radiation and the second was distilled water as control without light absorption were used and the absorption of light at 560 nm by spectrophotometer shomadzo samples were tested. Uptake in control solution as 100% absorption was zero value in reducing absorption in the sample was recorded. 50% of NBT reduction as a unit of Superoxide Dismutase activity intended Superoxide Dismutase activity was obtained by the following formula:

$$\text{Percent inhibition of resuscitation} = \frac{\text{Uptake in control-Sample uptake}}{\text{Uptake in control-Distilled water uptake}}$$

RESULTS AND DISCUSSION

Amount of measured Superoxide Dismutase using spectra photo metric in 560 nm wavelength meter showed that between salinity levels of the enzyme Superoxide Dismutase level was a significant difference in the 0.06 percent level is observed. Thus, different levels of salinity in the amount of Superoxide Dismutase in roots and shoots vary between genotype and environment (salinity levels) a significant difference in the 0.01 percent level was observed. Thus, in sensitive and resistant cultivars to salinity differences significant amount of Superoxide Dismutase has been. Comparison of enzyme in root and shoot showed different levels of salinity (Table 1) with increasing salinity in the amount of Superoxide Dismutase in roots and shoots is increased. As the highest level of Superoxide Dismutase in the root was in 150mM Salinity levels and root was in 100mmol. Purpose, can be stated that the amount of salt in order to create stress amount of enzyme secreted Superoxide Dismutase in stem and roots were different. The amount of Superoxide Dismutase in roots secretion was higher than shoot. Comparing resistant and susceptible cultivars in terms of the amount of Superoxide Dismutase indicated that Superoxide Dismutase in roots and shoots of resistant cultivars than

Table 1: Average amount of enzyme activity in root and shoot inhibition of regeneration at different levels of salinity (in terms of percentage inhibition of CPR)

Salinity (Mmol)	Plumule	Root
Control	11^{d}	22.91^{d}
50	13.67^{c}	24^{c}
100	35^{a}	33.91^{a}
150	32.25^{b}	31^{b}
$^{-}SE_X$	0.0026	0.0039

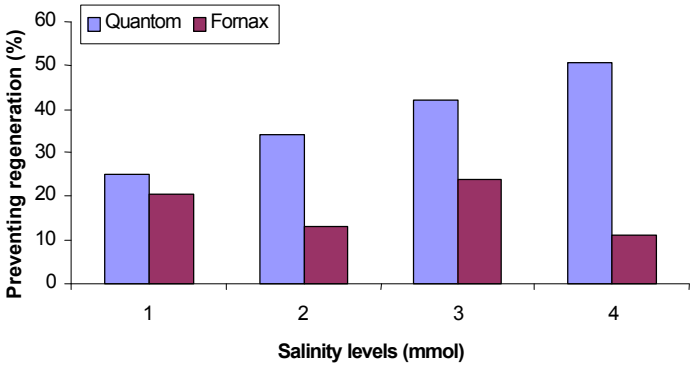

Fig. 1: Percent inhibition of nitro blue tetazolium reduction, in the salinity level in the roots of two cultivars

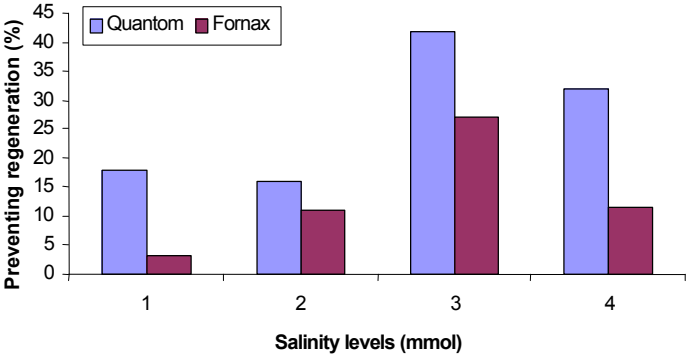

Fig. 2: Percent inhibition of nitro Blue tetazolium reduction, in the salinity level the shoots of two cultivars

the susceptible cultivar was. Considering the existence of significant interaction genotypes in salt levels, analysis of variance and mean for salt levels in both resistant and susceptible varieties were carried. The results of this comparison chart are the number one point. In The root, enzyme Superoxide Dismutase in the amount of resistant cultivars under salt stress significantly increases showed. So that the amount of uptake inhibition in the concentration 0 mmol, 35% and 100 mmol 43% and 150mmol showed 50%. In both figure in normal conditions the amount of control there was little enzyme that resistant varieties were slightly more susceptible.

In Sensitive genotype, amount of enzyme in the root in stress condition any of the levels was not significant, meaning that the rate of enzyme secretion in all conditions showed no significant change is.

In tolerant and resistant cultivars Shoots, Amount of enzyme under stress conditions was significantly increased (Fig. 1). But the reaction of susceptible and resistant cultivars showed salinity levels shoot both cultivars with increasing salt concentrations up to 100 mmol increase the absorption of preventing the existence of an indicator enzyme is observed, but the concentration of 150 mmol salt amount of enzyme than the enzyme concentration of 100 mmol amounts was reduced in both cultivars. Reduce the amount of enzyme in susceptible than resistant cultivars significantly in root and shoot were higher.

It has been frequently reported that salt stress induces oxidative damage to plant tissues [13]. The oxidative stress is considered to be due to increased production of O_2^-, $^{\bullet}OH$, H_2O_2 and O_2. Hence, constitutive and/or induced SOD is needed to prevent plant tissue from the oxidative damage. The tolerant genotype (*Quantum*) had higher constitutive levels of SOD activities than the sensitive genotype (Table 2). This indicates that *Quantum* has potentially a better protection system against the oxidative damage caused by salinity stress. Several previous studies have also reported that salt-tolerant cultivars of tomato and beet had higher constitutive levels of antioxidant enzymes [20, 21].

By NaCl treatment, SOD activity was enhanced in root of *Quantum*, However the activities of SOD in root of *Fornax* has not been changed significantly by increase in salt levels (Figure 1). These results are the same as reported by Nur Cicerali [7] and Rios-Gonzalez *et al.* [22]. Also, analysis of the SOD activity in shoots subjected to NaCl stress up to 100 mM NaCl revealed an increase in SOD activity of both genotypes, but increase in salt tolerant was higher than salt sensitive genotype (Fig. 2). High SOD activity was observed in shoot of tolerant genotypes of onions [23].

SOD activity was enhanced in roots and shoots of *Quantum* in relation to increase in salt concentration. The activity of SOD was also induced in shoots of *Fornax* although its constitutive activity was lower than those of *Quantum* (Table 1). So it is suggested that this enzyme fulfills the important role for the tolerance to NaCl especially in *Quantum* as the same as reported in several studies [13, 24].

In enzymatic scavenging systems, SOD is well known as O_2^- scavenger. The combined action of SOD converts the potentially dangerous O_2^- and H_2O_2 to water (H_2O) and molecular oxygen (O_2) [1].

Results of this study suggested that the high constitutive and induced levels of SOD in the roots of *Quantum*. This may indicate that their combined action is an effective scavenging mechanism to abate the toxic of O_2^- in the root cells.

CONCLUSION

Results of this study clarified the differences of Super Oxide Dismutase activity between *Quantum* (as a salt tolerant) and *Fornax* (as a salt sensitive). A high level of Super Oxide Dismutase, either constitutive or induced, has been reported to correlate with plant resistance to salt stress.

In addition the SOD activity increasing as a result of salt stress was stronger in the salt tolerant genotype compared to the salt sensitive one. Results obtained support the hypothesis the higher efficiency of the antioxidant enzymatic system of *Quantum* (tolerant) genotype could be considered as one of the factors responsible for its tolerance to salt stress. Therefore, it is suggested that superoxide dismutase activity could be used as a working hypothesis for a biochemical marker for salt tolerance in rapeseed.

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