Effect of NaCl Induced Oxidative Stress on Growth and Antioxidant Enzymes of Strawberry (Fragaria *x ananassa*. Duch.) cv. Selva

Prasada Rao. K[1], A.M. Lall[1], G.Abraham[2] and P.W.Ramteke[3]

[1]Department of Biochemistry& Biochemical Engineering
[3]Department of Biological Sciences, Sam Higginbottom Institute of Agriculture Technology & Sciences, Deemed to-be University, Allahabad-211007, UP, India
[2]CCUBGA , Indian Agricultural Research Institute
New Delhi.-110012 India

Email: johnscritch@gmail.com

ABSTRACT

The modern cultivated strawberry is one of the most delicious, refreshing and soft fruits of the world. About 20% of irrigated arable land in arid and semi-arid regions world wide is salt affected .The possible involvement of antioxidant enzymes in relation to the tolerance to salt stress was investigated in the cultivated Strawberry *Fragaria x ananassa*.D. Selva.Five months old berry runners were grown and subjected to 0.00, 0.25.0.50, 0.75, 1.0, and 1.50% NaCl treatments. The Growth and antioxidant enzymes investigation was carried out in three important stages of crop such as pre-flowering, flowering and fruiting against salinity induction. Growth of the plant was gradually decreased with the increase in salinity whereas total soluble and proline content and the activities of superoxide dismutase (SOD), ascorbate Peroxidase (APOX), and Catalase (CAT) increased with increase in NaCl concentrations in external medium till 0.75% and other treatments showed decrease in activity. The activities of SOD, APOX and CAT were up regulated at fruiting stage. These results suggest that the cultivated strawberry *Fragaria x ananassa*.D.Selva exhibit a better protection mechanism against oxidative damage by up regulation of antioxidant enzymes.

Keywords: Strawberry (Fragaria x ananassa.Duch.) cv. Selva, Antioxidant enzymes, Salt stress

INTRODUCTION

The modern cultivated strawberry (*Fragaria x ananassa* Duch.) is an octoploid hybrid (2n=2x=56) of largely delicious (octoploid) species *i.e*. *Fragaria chiloensis* Duch and *Fragaria virginiana* Duch, which belongs to family Rosaceae. Strawberries are adapted to different climatic conditions *viz*., moderate, mediterranean and sub-tropical.

It can even be grown at higher altitudes under tropical climate. Purposeful breeding has played a leading role in the development of modern cultivars and resulted in a tremendous increase in strawberry production. Several cultivars have been developed in different countries and were evaluated under local conditions. *Fragaria x ananassa.* Duch *cv.* Selva is a day neutral variety developed in Belgium from Cal.70.3-117 x Cal.71.98-605 in 1987. Its average fruit weight is higher than Repella and considered to be a ray of hope in sub-tropical zones of India, because it has performed very well under north Indian plains in the recent years [1]. Most of the horticultural crops are glycophytes [2] and strawberry is also considered as salinity sensitive species [3].

Agricultural productivity is severely affected by soil salinity and the damaging effects of salt accumulation in agricultural soils have influenced ancient and modern civilizations. Up to 20% of irrigated arable land in arid and semi-arid regions world wide is salt affected [4]. Salinity adversely affects germination of seeds, plant growth and alters the overall metabolism of the plant. Plants growing in saline environment suffer injury due to water deficit, osmotic and ionic stress which induces nutrient imbalance and the secondary induced oxidative stress by the production of Reactive oxygen species (ROS) at different locations of the cell. ROS scavenging mechanisms are based on antioxidant enzymes that can efficiently destroy the ROS like superoxide radicals (O2 -) and hydrogen peroxide (H2O2). Enzymatic mechanisms include super oxide dismutase (SOD-EC 1.15.1.1) which converts O2 - to H2O2 and Catalase (CAT-EC 1.11.1.16) which converts H2O2 to water and molecular oxygen (O2). ROS scavenging systems also includes ascorbate peroxidase (APOX-EC 1.11.1.11) and glutathione reductase (GR-EC 1.6.4.2**)** ***etc*****.** [5]. India also faces the problem of salinity, which is developed due to primary and secondary salinisation processes. The climatic conditions of India enable continuous growing several crops through out the year, but increasing demand for irrigation water compels the growers to utilize water of poor quality. Gradual build up of sodium (Na) and Chloride (Cl) in the root zone may be detrimental to plant growth and yield [6]. The present study was conducted to investigate the effect of different levels of salinity upon growth and antioxidant enzymes of strawberry *cv.* Selva ,because this variety is considered a ray of hope in sub-tropical zone of India; as it has performed very well north Indian plains in the recent years.

MATERIALS AND METHODS

The experiment was conducted in the Department of Biochemistry, Allahabad Agricultural Institute– Deemed University, Allahabad during November 2007 – April 2008 to investigate the effect of NaCl stress on growth and antioxidant enzymes in strawberry *cv.* Selva. Five months old runners were transplanted in pots and maintained in green house condition. The runners were supplied with ½ Hoagland medium. Salt treatments (0.00, 0.25%. 0.50, 0.75, 1.00, and 1.50%) were given by dissolving sodium chloride in ½ Hoagland medium at four-leaf stage. Triplicate samples were used for the study. Pots irrigated only with the ½ Hoagland medium served as control.

Plant Sampling and Analysis

Leaves were sampled according to life-cycle of strawberry at pre-flowering, flowering

and fruiting stages. Leaf samples were standardized by using only fully expanded leaves from the middle part of plants in each replicate, as they reflect most clearly from the nutritional and metabolic standpoint and the effects of salinity. The material was rinsed three times in distilled water and then blotted on filter paper. At each sampling, leaf matter was used fresh for analysis of growth and antioxidant enzymes.

Measurement of Growth

Dry weight: Dry weight was estimated as difference between the DWf obtained (Constant) at the end of each sampling period after drying at 70 ^{o}C and the DW0 measuring just before drying at each stage.[7].

DW Production = (DWf - DW0), given in g.

Soluble Protein Content

Soluble protein content was quantified using folin ciocalteau reagent according to Lowery *et al.*,1951[8] with bovine serum albumin as a protein standard.

Antioxidant enzymes

Detached one gram of strawberry leaves from different treatments was homogenized on ice bath in 100 mM potassium phosphate buffer (pH 7.8), containing 5 mM EDTA and 1 % polyvinyl polypyrrolidone (PVPP). The homogenate was centrifuged for 30 minutes at 13,000 X g at 4^{0}C and the supernatant was used for the determination of antioxidant enzymes. The analysis of growth and antioxidant enzymes was carried out in three important stages of crop such as pre-flowering, flowering and fruiting against salinity induction.

Superoxide dismutase (SOD-EC 1.15.1.1) activity was determined by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium chloride (NBT). The reaction mixture contains 50 mM Na Phosphate buffer (PH 7.8), 33 mM NBT, 10 mM l- ethionine, 0.66 mM EDTA and 0.0033 mM riboflavin. Reactions were carried out at 25 ^{o}C under light intensity of about 300 m mol-1 m-1 s-1 through 15 min. One unit of SOD activity was defined as amount of enzyme requires causing 50% inhibition of photochemical reduction rate of NBT according to Giannopolitis *et al.*,1977 [9].

Ascorbate peroxidase (APOX-EC1.11.1.11) activity was measured according to Nakano and Asada [7] by reading the absorbance at 290 nm at the time of H_2O_2 addition and 30 sec later monitored the ascorbate oxidation. The reaction mixture contains 50 mM Na Phosphate buffer (PH 7.0), 0.5 mM ascorbate, 0.1 mM EDTA and 1.2 mM H_2O_2. The difference in absorbance (A290) was divided by ascorbate molar extinction coefficient (2.8 mM-1cm-1) and enzyme activity is expressed as m mol ml-1, taking into consideration that one mole of ascorbate is required for the reduction of 1.0 mol of H_2O_2.

Catalase (CAT-EC 1.11.1.16) activity was done according to the Aebis**,** 1984 [10] method. The reaction was initiated by adding 0.5 ml of H_2O_2 (12.5 mM). The reaction mixture contained 0.05 mM Na Phosphate buffer (PH 7.0) with 1mM EDTA and 3%

H_2O_2. Reading the absorbance at 240 nm at the time of H_2O_2 addition and 30 sec later monitored the decrease in H_2O_2. The difference in absorbance (A290) was divided by the H_2O_2 molar extinction coefficient (0.36/min/sec) and the enzyme activity was expressed as m mol of H_2O_2 min-1mg-1 protein. One unite of enzyme was the amount necessary to decompose 1 ìl of H_2O_2 per unit at 25 °C.

Proline was measured by taking 0.5 g of leaf from sample and homogenized in 1 ml, 3 % sulphosalicylic acid and residue was removed by centrifugation at 40,000 rpm for 15 min and by using filter paper ,according to Bates *et al.*, [11].. Two ml of extract was reacted with 2 ml glacial acetic acid and 2 ml acid ninhydrin (0.92 g ninhydrin and 30 ml methyl and 10 ml acetate buffer) for 1 hour at 100 °C and then the reaction was terminated in on ice bath. The reaction mixture was extracted with 1 ml toluene. The chromatophore containing toluene was warmed to room temperature till a bilayer (upper pink and lower yellow layer) was formed. Optical density was read at 520 nm. The amount of proline was determined from a standard curve in range of 20- 100 µg.

Statistical Analysis

Each data point in the mean of three replicates were obtained from the experiment (n=3). All data were subjected to a two-way analysis of variance and the mean differences were compared by lowest standard deviations test.

RESULTS AND DISCUSSION

Growth Response of *Fragaria x ananassa* Duch *cv.* Selva to NaCl

The dry weight production in strawberry *cv*. Selva plants was gradually decreased with increasing external salinity when it was compared with Control (Tab.1). At 0.75% NaCl, the dry weight production was reduced by about 50%. At pre-flowering stage the plant dry weight was not severely affected by salinity. As the crop grew

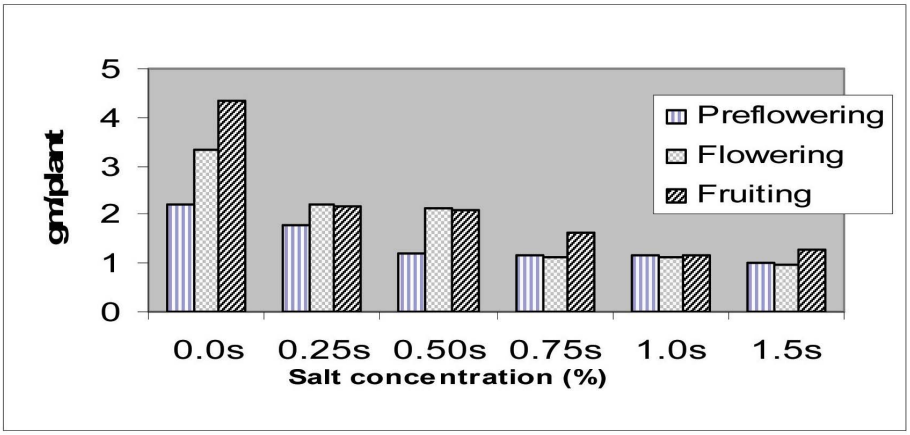

Fig.1: Effect of NaCl treatments (0, 0.25, 0.50, 0.75, 1.0 and 1.5%) on Dry weight at Pre-flowering,
Flowering and fruiting stages. Data are means of three replicates +_SE at 0.05 levels.

from pre-flowering to flowering and fruiting, the dry weight production gradually increased and showed some degree of adaptation to salinity. The highest dry weight production was observed at fruiting stage. It was noted that the increasing salinity in external medium led to glycophytic response that is the dry weight production gradually decreased with increase in the salinity (Fig.1)

Effect of NaCl on Soluble protein content:

After the NaCl treatment, the Soluble protein content at Pre-flowering stage in leaves of strawberry *cv*. Selva was 132±1.6 mg.g-1 for control, 155±3.6, 188±5.9, 185±0.8, 213±2.08 and 159±2.88 mg.g-1for 0.25, 0.50, 0.75, 1.0 and 1.5% NaCl treatments, respectively. At flowering stage after the NaCl treatment, the protein content in leaves of strawberry *cv*. Selva was 143±3.6mg.g-1 for control, 158±1.2, 192±1.6, 198±6.4, 254±2.08 and 142±2.88 mg.g-1for 0.25, 0.50, 0.75, 1.0 and 1.5% NaCl treatments, respectively. At fruiting stage after the NaCl treatment, the protein content in leaves of strawberry *cv*. Selva was 159±2.9 6 mg.g-1 for control and 195±3.8, 195±3.8, 220±1.2, 202±2.6, 272±1.6 and 186±4.3 88 mg.g-1 for 0.25, 0.50, 0.75, 1.0 and 1.5% NaCl treatments (Table2). This shows soluble protein content gradually increased till 1.0% NaCl but showed decrease in immediate treatment *i.e.* 1.5% NaCl with the increase in salinity (Fig.2). At 1.0% NaCl, the soluble protein content was noticed maximum. The insensitivity of this parameter to salt treatments suggests that protein biosynthesis was not affected significantly.

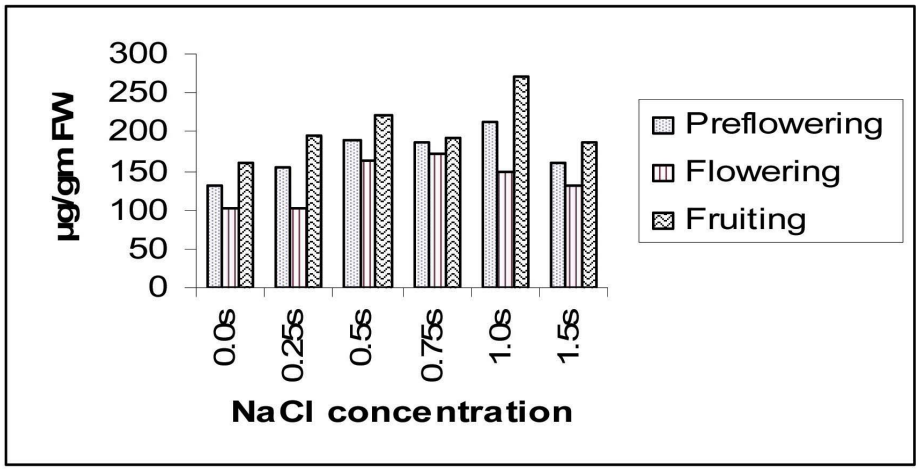

Fig.2: Effect of NaCl treatments (0, 0.25, 0.50, 0.75, 1.0 and 1.5%) on soluble protein content at Pre-flowering, Flowering and fruiting stages. Data are means of three replicates ±SE at 0.05 levels.

Effect of NaCl on Antioxidant metabolism:

Super oxide dismutase: SOD activities increased due to the increase in salt concentrations till 0.75% NaCl but 1.0% and 1.5% NaCl showed decrease in the leaves of Strawberry cv. Selva at pre-flowering stage. In flowering stage SOD activities decreased in Control and 0.25% NaCl concentrations and showed up-regulated activity

at 0.75% and 1.0% NaCl but 1.5% NaCl showed down regulated at pre-flowering stage whereas, at fruiting stage SOD activities increased than pre-flowering and flowering stage till 0.75 % NaCl, but 1.0% and 1.5% NaCl concentrations showed decreased SOD activity (Table 3). The rate of increase in SOD activity was higher after pre-flowering stage and flowering stage in all NaCl treatments except 0.75% NaCl.

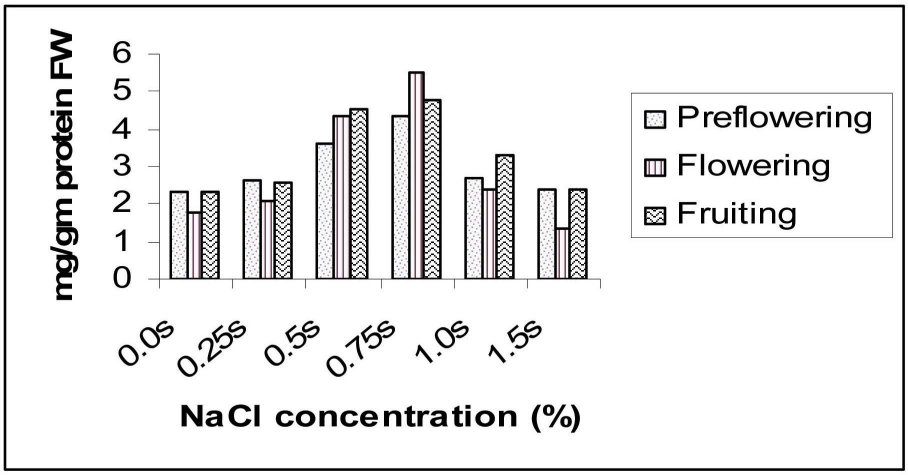

Fig.3: Effect of NaCl treatments (0, 0.25, 0.50, 0.75, 1.0 and 1.5%) on SOD activity at Preflowering,
Flowering and fruiting stages. Data are means of three replicates +_SE at 0.05 levels.

Ascorbate peroxidase: The activity of APOX, which decomposes the H2O2 produced by SOD also increased due to the increase in salt concentrations till 0.75%NaCl but 1.0%and 1.5% NaCl showed decrease in the leaves of Strawberry cv. Selva at pre-flowering stage Fig.4. However, salt induced APOX activity was significantly higher at pre-flowering stage at 0.75% NaCl when it was compared with control.

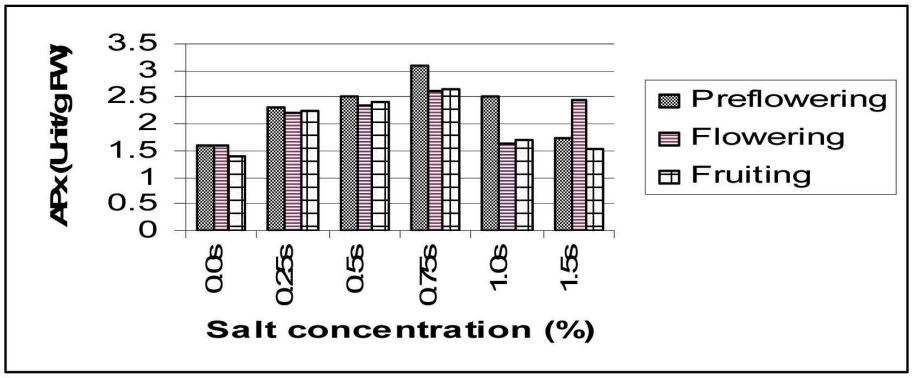

Fig.4: Effect of NaCl treatments (0, 0.25, 0.50, 0.75, 1.0 and 1.5%) on APOX activity at Pre-flowering,
Flowering and fruiting stages. Data are means of three replicates +_SE at 0.05 levels.

Catalase (CAT): Another scavenger of H2O2, CAT activity (Fig.5) increased in strawberry cv. Selva at flowering and fruiting. It was significantly increased in all the treatments when it compared with Control at pre-flowering stage. The activity was significantly higher after pre-flowering stage and flowering stage in all NaCl treatments. As a whole the CAT activity is significantly increased during the treatment period.

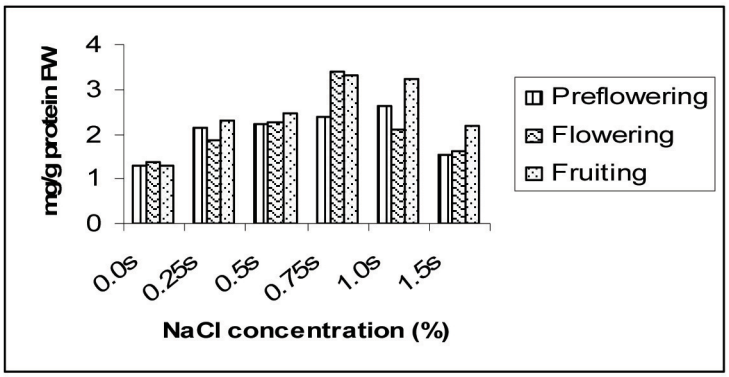

Fig. 5: Effect of NaCl treatments (0, 0.25, 0.50, 0.75, 1.0 and 1.5%) on CAT activity at Pre-flowering, Flowering and fruiting stages. Data are means of three replicates +_SE at 0.05 levels.

Proline: The content was significantly higher as compared to control .In *Fragaria x ananassa* Duch cv. Selva leaves there was significant increase in proline content observed. The proline content highest at pre-flowering stage and it was 3.77±0.21and at flowering stage it was 3.21±0.06, the fruiting stage also showed the increase in proline content which was highest at 0.75% salt concentration as given in Table 6**.**

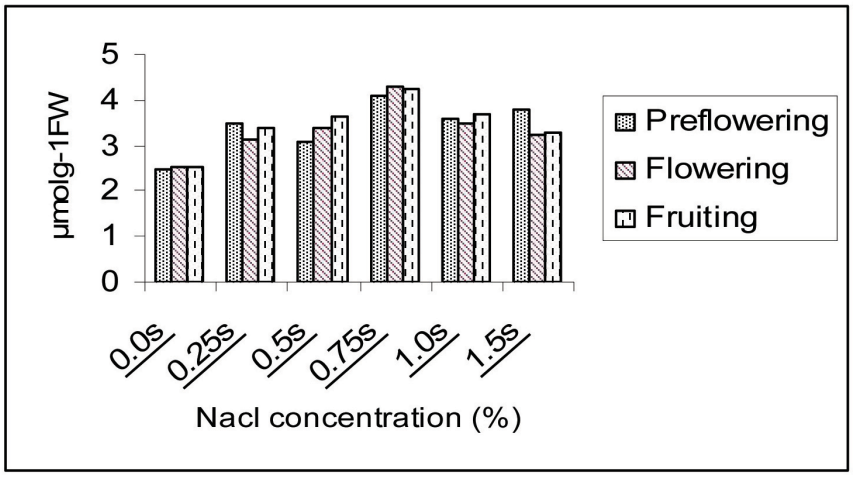

Fig. 6: Effect of NaCl treatments (0, 0.25, 0.50, 0.75, 1.0 and 1.5%) on Proline content at Pre-flowering,
Flowering and fruiting stages. Data are means of three replicates +_SE at 0.05 levels.

It has been demonstrated that salt treatment increases the induction of oxidative stress in strawberry cv. Selva plant tissues [12] [13]. This NaCl induced oxidative stress has initiated the generation of super oxide radicals (O_2 -) singlet oxygen ($^1O_2$), Hydroxyl free radical (OH^-) and hydrogen peroxide (H_2O_2). Hence, the constitutive of /or induced activity of SOD, CAT and APOX are essential [14][15]. The up regulated activity of these antioxidant enzymes in *Fragaria x ananassa* Duch *cv.* Selva under salinity has provided a better protection from oxidative damage caused by salt treatment. According to Scandalios, Particularly SOD and CAT are the most effective antioxidant enzymes in preventing cellular damage. Unlike the salt tolerant beet, it was also observed the increase in generation of ROS due to salinity in *Fragaria x ananassa* Duch *cv.* Selva (especially , at 1.5% in Fruiting) may result in an increase in membrane permeability or loss of membrane integrity leading to an increase in solute leakage , hence decreasing the resistance to salinity [16]. Growth of *Fragaria x ananassa* Duch *cv.* Selva to NaCl was exhibited that the increasing salinity in external medium led to the response of glycophytic response: the dry weight production was gradually decreased with the increase in salinity [17].

Soluble protein content was gradually increased till 1.0% NaCl but showed decrease in immediate treatment i.e. 1.5% NaCl with the increase in salinity (Fig.2). At 1.0% NaCl, the soluble protein content was maximum level. The insensitivity of this parameter to salt treatments suggests that protein biosynthesis was not affected.

SOD catalyzes the conversion of the super oxide anion to H_2O_2 and is the key enzyme in protecting the cells from oxidative stress. The salt induced SOD activity at 0.75% than Control was observed. This may led the *Fragaria x ananassa* Duch *cv.* Selva to resist the oxidative damage. The rate of increase in SOD activity was higher after pre-flowering stage and flowering stage in all NaCl treatment except 0.75% NaCl. The reduction in SOD activity at higher NaCl Concentrations may be attributed to an inactivation of enzyme by H_2O_2 which is produced in different cellular compartments and also from a number of enzymatic and Non- enzymatic processes in cell [18]. These results are in good agreement with the results of Gossett *et al.*, [19].

APOX was ascorbate as the electron donor for the reduction of H_2O_2 and is well known to be important in the detoxification of H_2O_2. It has been shown that over expression of APOX gene in plants increases protection against oxidative stress [20]. Activity of APOX was higher at 0.75% NaCl treatment than control throughout stress period. Since the up regulated activity of SOD under salt stress was accompanied by increase in APOX activity of *Fragaria x ananassa* Duch *cv.* Selva leaves. It may also be suggested that SOD and APOX are working more efficiently in concert to decompose oxidants such as super oxide radicals (O_2 -) and hydrogen peroxide (H_2O_2) which might possibly be produced during stress conditions. Hence it may also be suggested that H_2O_2 in the leaves of *Fragaria x ananassa* Duch *cv.* Selva is more efficiently eliminated by Ascorbate Cycle in which Ascorbate acts as a strong catalyst together with other Antioxidant enzymes. These results are in good agreement with the results of Shalata and Tal,[21] who reported inherently and induced levels of APOX in wild salt tolerant tomato and radish plants, respectively.

CAT activity together with SOD considered to be the most effective antioxidant enzymes in preventing cellular damage. The salt induced CAT activity levels were significantly higher at0.75% NaCl in leaves of *Fragaria x ananassa* Duch *cv.* Selva. Considering that similar results obtained in APOX activities, it may be suggested that CAT and APOX which of both responsible for detoxification of H_2O_2. Confirming our results, Sandalio *et al.*, [22] also observed salt induced activities of APOX and CAT in wild salt tolerant tomato. The increased proline content could be attributed to the stress factor of salinity. Its possible role in scavenging free radical is also not ruled out at least in plants exposed to lower NaCl concentration. The present finding was supported by Alia *et al.,* [23] who has reported that there is increase in proline content due to salt stress.

CONCLUSION

In our study, salt induced SOD activity was remarkably higher at 0.75% NaCl treatment at flowering stage than any other antioxidant enzymes. This may lead the strawberry to resist the potential oxidative damage without the requirement to increase the SOD activity more. A comparison of enzymatic values at 0.50% and 0.75% NaCl clearly indicates that Strawberry cv. Selva has a higher dismutating capacity under moderate and acute doses of salinity. These results corroborate the results of Gossett *et al*, [19] who found higher constitutive and induced levels of SOD in more tolerant cotton cultivars under salt stress conditions. In conclusion, higher SOD, APOX and CAT activities in strawberry cv. Selva under stress, which probably come from an increased capacity of oxygen radical scavenging, indicates the salt tolerance and antioxidant defense system. Salt tolerance differs according to the duration of stress and in this present investigation, 0.75% NaCl seemed to have an intermediate effect on antioxidant enzyme activities. The proportional and rational contribution of antioxidative enzymes activities differs in salt tolerant plants. Further investigations are necessary to put forward the effect of salt stress by means of ion distribution and osmotic adjustment and also sub-cellular compartmentation of antioxidative enzyme activities would be a useful tool for our understanding of salt stress.

ACKNOWLEDGEMENT

We wish to thank Dr. R. P. Joshi, Programme Coordinator, Krishi Vigyan Kendra, Jeolikot, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttra Khand, India for providing runners of strawberry cv**.** Selva. We also extend our heartfelt gratitude to Dr. S.B. Lal, Pro-Vice chancellor SHIATS, Allahabad (UP), India for providing necessary facilities during research to accomplish present work.

REFERENCES

1. Sharma, V.P., Sharma, R.R. 2003. The Strawberry. Indian Council of Agricultural Research.pp.22-23.
2. Greenway, H., Munns, R. 1980. Mechanisms of salt tolerance in non halophytes. *Annual Reviewof Plant Physiology* **31**:149-190
3. Martinez, B.M.C., Alvarez, C.E. 1997. Toxicity symptoms and tolerance of strawberry to salinity in irrigation water. *Scientia Horticulture* **71**:177-188.

4. Gabrijel, O.A., Davor, R., Marija, R., Boris, D., Ivan, M. 2006. Strawberry growth and yield in saline environment. *Agriculturae Conspectus Scientificus*. **71**(4):155- 158.
5. Noctor, G., Foyer, C.H. 1998. Ascorbate and glutathione: keeping active oxygen under control. *Plant Molecular Biology* **49**:249-279.
6. Flowers, T.J. 1999. Salinization and Horticultural production. *Scientia Horticulture* **78**:1-4
7. Nakano, Y., Asada, K. 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplast, *Plant and Cell Physiology* **22:** 867-880.
8. Lowery, O.H, Rosebrough, N,J., Farr, A.L, Randall, R.J. 1951. *Journal of Biological Chemistry* **193***:*265
9. Giannopolitis, C. N., Ries, S.K. 1977. Superoxide dismutases: I. Occurrence in Higher Plants. *Plant Physiology* **59** (2):309–314.
10. Aebi, H. 1984. Catalase in *vitro- Method of Enzymology* **105**: 121-126.
11. Bates, L.S., Waldeen, R.P., Teare, I.D.1973. *Plant Soil* **39**:205.
12. Mohamed, D., Houda, G., Akiira, S., Mohamed, H.G. 2005. NaCl Stress effects on enzymes involved in nitrogen Assimilation path way in tomato "*Lycopersicon esculentum*" seedlings**.** *Journal of Plant Physiology* **163**:1247-1258.
13. Hernandez, J.A., DelRio, L.A., Sevilla, F. 1994**.** Salt stress induced changes in super oxide dismutae iso enzymes in leaves and mesophyll protoplast from vigna anguiculatea L. Walp. *New Phytol*ogist **126**:37-42.
14. Fridovic, I. 1986. Biological effects of the super oxide radical. *Archives Biochemistry and Biophy*sics **247**:1-11.
15. Dhindsa, R. S., Plumb, D.P., Thorpe, T.A. 1981. Leaf senescence correlated with increased levels of membrane permeability, Lipid peroxidation and decreased levels of superoxide dismitase and catalase. *Journal of Experimental Biology* **32**:93-101.
16. Bor, M. Ozdemir, F., Turkan, I. 2003. The effect of Salt stress on lipid peroxidation and antioxidants in leaves of sugar beet Beta vulgaris L. and wild bet Beta maritima L. *Plant Science* **164**:77-84.
17. Shangguan, Z.P., Shao, M.A., Ren, S.J., Zhang, L.M., Xue, Q. 2004. Effect of Nitrogen on root and shoot relations and gas exchange in winter wheat. *Botanical Bulletin of Academia Sinica* **45**:49-54.
18. Schutzendubel, A., Nikolova, P., Rudolf, C., Pole, A. 2002. Cadmium and H_2O_2 induced oxidative stress in *Populus canescens* roots. *Plant Physiology and Biochem*istry **40**:577-584.
19. Gossett, D.R., Millhollon, E.P., Lucas, M.C. 1994. Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton, *Crop Science* **34:** 706-714.
20. Wang, J., Zhang, H., Allen, R.D. 1999. Over expression of an Arabidopsis peroxisomal ascorbate gene increases protection against oxidative stress. *Plant cell physiology* **40**:725-732.
21. Shalata, A., Al, M. 1998. The effect of salt stress on lipid peroxidation and antioxidants in the cultivated tomato and its wild salt tolerant relative *lycopersican pennellii*. *Physiol.plant* **104**: 169-174.
22. Sandalio,L.M., Dalurzo,H.C., Gomez,M., romero-puertas, M.C. and Delrio.L.A. 2001. Cadmium induced changes in the growth and oxidative metabolism of pea plants. *Journal of Experimental Botany* **52**:2115-2126.
23. Alia, P., Saradhi, P. 1993. Suppression in mitochondrial electron transport is the prime cause behind stress induced proline accumulation, *Biochemical and Biophysical Research Communications* **193**: 54-58.