

Effect of the Mutagenic Agents and NaCl on the Activity of Antioxidant Enzymes in Two Genotypes of Iraqi Rice (*Oryza sativa* L.)

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Abstract: This study was carried out to determine the effects of NaCl levels and the mutagenic agents, the chemical mutagen ethyl methyl sulphonate (EMS) at 0.5% and the physical mutagen UV-B irradiation for 40 min time exposure on the activity of antioxidant enzymes in two Iraqi rice genotypes Amber 33 (A33) and Amber Baghdad (AB). Different levels of NaCl were (0.00, 50, 100, 150, 180 or 200) mM. NaCl caused increase in antioxidant enzymes (POX, CAT, and APX) activity. Exposure to both mutagens increased the activity of these enzymes significantly compared with non-treated.

Keywords: Rice, EMS, UV-B, NaCl

1. Introduction

Iraq is one of the Asian countries which has suitable agroclimatic conditions for rice cultivation. Rice is the staple food for the greater majority of the Iraqi population. In Iraq, a number of traditional and improved genotypes have been released for cultivation in different regions. Amber genotype is the most important traditional Iraqi rice genotype (Chakravarty 1976). Rice (*Oryza sativa* L.), belongs to the family Poaceae (Graminae), is the staple food for over two billion people over the world (Chakravarthi and Naraveni2006). It is the most economic cereal crop in many parts of the world and considered as a salt sensitive crop (Zeng *et al.*2002). Efforts were made to increase rice productivity, since 70% of the world's poor people depend on rice as the major source of food energy (Zeigler, 2010). Salinity is considered an important physical factor influencing rice production. In fact, salinity can cause severe damage at any stage of rice growth and development, which leads to yield loss (Rajakumar, 2013). Salinity alters a wide array of metabolic processes in growing plants and induces changes in contents and activity of many enzymes (Khan and Panda, 2008). Additionally secondary stresses often occur, such as oxidative stress due to the over production of reactive oxygen species (ROS) in salt stressed plants. ROS are molecules like hydrogen peroxide (H₂O₂), ions like the hypochlorite ion, radicals like the hydroxyl radical (OH[·]) and the superoxide anion (O^{·-}). Against these ROS, plants have evolved a complex antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbat peroxidase (APX) and peroxidase (POD) in the different cell compartments, achieve a balance between the rate of formation and scavenging of ROS, and maintain ROS at suitable levels required for cell signaling (Batels and Sunkar, 2005). Peroxidase enzyme decomposes H₂O₂ by oxidation of co-substrate such as phenolic compounds or antioxidants (Gaspar *et al.*, 1991). Catalase is the major endogenous antioxidants, and catalyzes H₂O₂ decomposition (Wang *et al.*, 2004). Catalase plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells (Abassi *et al.*, 1998). Ascorbate peroxidases (APX) are enzymes that detoxify peroxides such

as hydrogen peroxide using ascorbate as a substrate. The reaction that catalyse is the transfer of electrons from ascorbate to peroxide, producing dehydroascorbate and water as products. APX is an integral component of the glutathione- ascorbate cycle. These enzymes are commonly hemoproteins and the haem cofactor is the site of the oxidation-reduction reaction shown below (Raven *et al.*, 2004).
$$C_6H_8O_6 + H_2O_2 \rightarrow C_6H_6O_6 + 2 H_2O$$

2. Materials and Methods

EMS concentration 0.5% (v/v) in 100 ml phosphate buffer solution at (pH7) was prepared. Seeds of the two rice genotypes Amber 33 and Amber Baghdad were presoaked in distilled water for 16-20 hrs at room temperature. The presoaked seeds were divided into two groups, the first was irradiated with UV-B (280-320 nm) for 40 min and the second was treated with aqueous EMS 0.5% for 3 hrs under the laboratory conditions with intermittent shaking to maintain uniformity (Talebi *et al.*, 2012; Wattoo *et al.*, 2013).

Screening rice genotypes for salinity tolerance

Twenty five seeds of each genotype for each treatment were allowed to germinate on a filter paper in 15 cm diameter Petri dishes. Each filter paper was moistened with 10 ml of 1/10 MS NaCl solution at the concentrations 0.0, 50, 100, 150, 180 or 200 mM according to the treatments, placed in an incubator at 25 °C, for 10 days. The Petri plates were arranged in completely randomized design (CRD) with three replicates for each treatment. Seedlings were transferred to universal test tubes containing 1/2 strength MS salts supplemented with 0.0, 50, 100, 150, 180, or 200 mM NaCl with three replicates. The test tubes were placed in a growth chamber at 16/8 hrs (light/dark) photoperiod at a light intensity of 1000 lux, with ambient temperature of 25±2 °C. Means of shoot height, root length and root number were recorded after three weeks (Anbumalarmathi and Mehta, 2013).

Activity of antioxidative enzymes

Enzyme extraction

Rice roots (100 mg) were homogenized in 3 ml of 10 mM phosphate buffer (pH 7.0) with pestle and mortar. The homogenates were centrifuged at 14000 g for 20 min and the supernatant was saved for analysis (Liu and Ger, 1997). The supernatant was used to estimate antioxidative activities of enzymes.

Peroxidase activity (POX) assay

Peroxidase activity was estimated following the procedure of Putter (1974). Aliquots of 3 ml of 0.1 M Phosphate buffer, 0.05 ml of 20 mM Guaiacol solution, 0.1 ml of tissue extract and 0.03 ml of 12.3 mM hydrogen peroxide (H₂O₂) solution were mixed in a cuvette and placed in a spectrophotometer, and then read at 470 nm. The peroxidase activity was measured as the rate of guaiacol dehydrogenation product formation.

Catalase activity (CAT) assay

The reaction mixture included 0.6 ml of phosphate buffer (0.1M, pH7), 0.1ml of enzyme extract, then 0.3 ml of hydrogen peroxide 75 mM were added to the mixture. The optical density (OD) at 240 nm was read after 2 min (Cakmak and Maschner, 1992).

Ascorbate Peroxidase activity (APX) assay

The mixture used for APX activity assay was containing 3 ml of phosphate buffer (0.1 M, pH7), 0.1 ml of 0.5 mM ascorbic acids plus 0.5 ml of enzyme extract. Aliquot of 0.1 ml (0.5 mM, H₂O₂) was added and immediately was read at 290 nm of OD (Cakmak and Maschner, 1992).

3. Result and Discussion

Effect of genotype, mutagenic agents and NaCl on the activity of antioxidant enzymes.

Effect of genotype, mutagenic agents and NaCl on peroxidase (POX) activity

The peroxidase (POX) activity was estimated after ten days of rice seedlings grown on various NaCl (mM) concentrations. The POX activity of the two genotypes after exposure to mutagens and NaCl is shown in figures 1 and 2. No significant differences between A33 and AB in the mean of POX activity. The POX activity increased significantly at all NaCl concentration excepts 50 and 100 mM, recording 0.151, 0.292, 0.43, 0.906, 1.447 and 2 unit/ g (F.W.) for control, 50, 100, 150, 180 and 200 mM NaCl respectively in A33 genotype. Mutagenesis caused no significant differences in mean of POX activity with mean values 0.726, 1.095 and 0.790 unit/ g (F.W.) for control treatment, 0.5% EMS and UV-B respectively. The interaction between mutagens and NaCl concentrations caused a gradual increase in POX activity. The highest POX activity was recorded in 0.5% EMS treatment at 200 mM NaCl (2.35) unit/ g (F.W.) while the lowest was in non-treated control treatment (0.032) unit/ g (F.W.). In AB genotype the POX activity increased significantly at all NaCl concentrations except 50 and 100 mM NaCl, reached 0.153, 0.372, 0.431, 0.906, 1.442 and 2.075 unit/ g (F.W.) for control, 50, 100, 150, 180 and 200 mM NaCl respectively. No significant differences resulted

by mutagen treatment between POX activities recorded 0.801, 1.141 and 0.805 unit/ g (F.W.) for control treatment, 0.5% EMS and UV-B respectively. The interaction between mutagens and NaCl concentrations resulted in stepwise increase in POX activities. The highest POX activity was recorded in 0.5% EMS treatment at 200 mM NaCl (2.465) unit/ g (F.W.) while the lowest was in non-treated control treatment (0.033) unit/ g (F.W.).

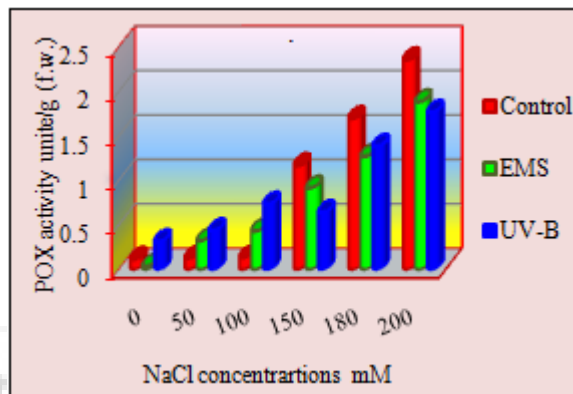


Figure 1 (A33)

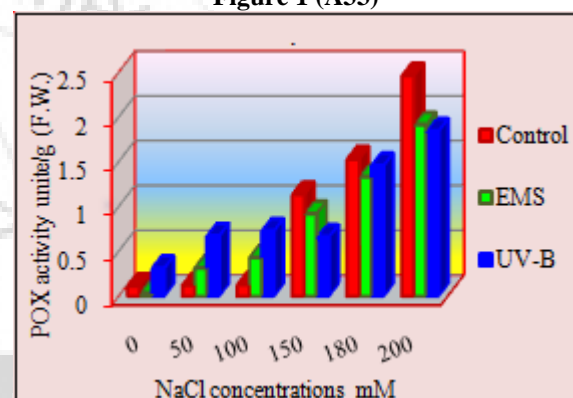


Figure 2 (AB)

Figure 1 and 2: Effect of the interaction between genotype and NaCl on the mean of POX activity unit/g (F.W.) of seedlings, after three weeks of inoculating into half strength MS medium.

The presented results are in line with those of Lin and Kao (1999) indicated a progressive increase in POX activity when NaCl levels elevated from 50 to 150 mM in rice. In other study peroxidase showed increased in activity under salt stress in four rice genotypes during different developmental stages (Swapna, 2003). Similar conclusion was drawn by Daud *et al.* (2012) in cotton callus cultures exposed to elevated NaCl levels.

Effect of genotype, mutagenic agents and NaCl on Catalase (CAT) activity

The catalase (CAT) activities were estimated after ten days seedlings growth on various NaCl concentrations. CAT activity of the two genotypes after exposure to mutagens and NaCl is shown in figures 3 and 4. No significant differences between A33 and AB in mean of CAT activities. CAT activity increased significantly at 180 and 200 mM NaCl recording 0.051 and 0.073 unit/g (F.W.) respectively compared with control (0.016) unit/g (F.W.) in A33. Exposure to 0.5% EMS increased the CAT activity significantly compared with UV-B and non-treated.

Maximum CAT activity occurred at 0.5% EMS was 0.064 unit/g (F.W.), followed by UV-B (0.045) unit/g (F.W.), while the control treatment recorded 0.031 unit/g (F.W.). The interaction between the mutagens and NaCl concentrations caused in a gradual increase in CAT activity. The highest CAT activity value was scored in 0.5% EMS treatment and at 200 mM NaCl (0.085) unit/g (F.W.) while the lowest was in control treatment (0.012) unit/g (F.W.).

In AB genotype CAT activity was increased significantly in all NaCl concentration except 50 and 100 mM NaCl, recording 0.017, 0.026, 0.033, 0.044, 0.052 and 0.075 unit/g (F.W.) for control, 50, 100, 150, 180 and 200 mM NaCl respectively. Mutagenesis caused no significant increasing in CAT activity. The interaction between the mutagens and NaCl concentrations caused in a stepwise increase in CAT activity. The highest CAT activity value was recorded in 0.5% EMS treatment and at 200 mM NaCl (0.087) unit/g (F.W.) while the lowest was in control treatment (0.015) unit/g (F.W.).

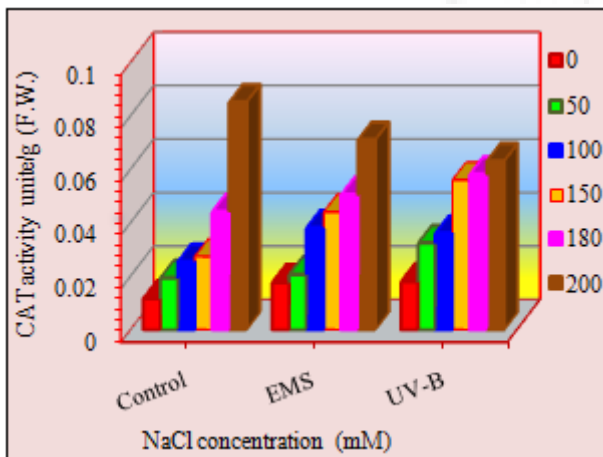


Figure 3 (A33)

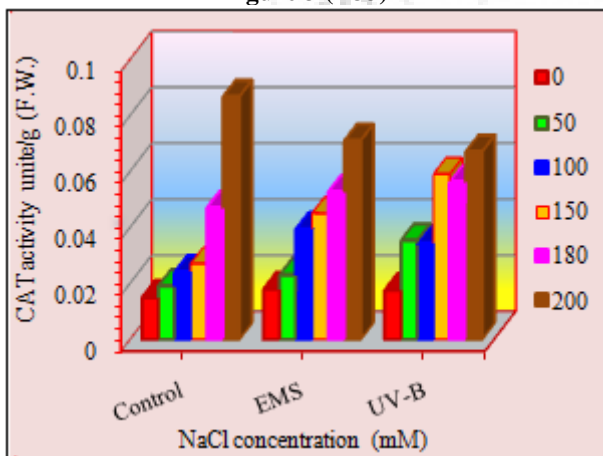


Figure 4 (AB)

Figures 3 and 4: The interaction effect between genotype and NaCl on the activity of CAT (unit/g F.W.) for A33 and AB seedlings

These findings are in line with previous studies which reported that CAT activity is higher at high concentrations of salt stress in two rice genotypes (white ponni and BPT-5204) (Thamodharan and Pillai, 2014). Sevengor (2010) concluded that CAT activity is important for elimination of H₂O₂ under salinity stress. Plant processes a number of

antioxidant enzymes that protect them from potential cytotoxic effects (Chookhamaeng, 2011; Kusvuran *et al.*, 2012). H₂O₂ has also considered an essential molecule of signal transduction in both a biotic and biotic stresses. Matsuda *et al.* (1996) demonstrated that application of H₂O₂ at low concentrations induces stress tolerance in plants due to the induction of the synthesis of certain substances similar to those normally synthesized during chilling stress. The effects of NaCl on CAT activity were studied in diverse groups of plants, such as a unicellular alga, *Chlorella sp.*, an aquatic macrophyte, *Najas graminea*, and a mangrove plant, *Suaeda maritime*. All showed high tolerance to NaCl. Significant accumulation of H₂O₂ was observed in all the tested plants upon their exposure to high levels of NaCl, and CAT activity increased significantly in response to the NaCl treatment (Mallik *et al.*, 2011). Interestingly, the same authors found that growing the plants in the presence of a high degree of salinity also resulted in the synthesis of new isoforms of CAT.

Effect of genotype, mutagenic agents and NaCl (mM) on ascorbate peroxidase (APX) activity

The ascorbate peroxidase (APX) activities were estimated after ten days of seedlings growth on various NaCl concentrations. APX activity of the two genotypes to after exposure to mutagens and NaCl is shown in figures 5 and 6. No significant differences between A33 and AB in mean of APX activities. APX activity increased significantly at all NaCl concentrations recording 0.0131, 0.191, 0.240, 0.297 and 0.424 unit/g (F.W.) for control, 50, 100, 150, 180 and 200 mM NaCl respectively in A33. Exposure to both mutagens increased the APX activity significantly compared with non-treated. Maximum APX activity occurred at 0.5% EMS recorded 0.292 unit/g (F.W.), followed by UV-B which recorded 0.257 unit/g (F.W.), while the control treatment recorded 0.169 unit/g (F.W.). The interaction between the mutagens and NaCl caused in a gradual increase in APX activity. The highest APX activity value was recorded in UV-B treatment and at 200 mM NaCl (0.475) unit/g (F.W.) while the lowest was in control treatment (0.063) unit/g (F.W.).

In AB genotype APX activity increased significantly in all NaCl concentrations recording 0.130, 0.165, 0.195, 0.242, 0.300 and 0.435 unit/g (F.W.) for control, 50, 100, 150, 180 and 200 mM NaCl respectively. Exposure to both mutagens increased the APX activity significantly compared with non-treated. Maximum APX activity occurred at 0.5% EMS recorded 0.294 unit/g (F.W.), followed by UV-B which recorded 0.258 unit/g (F.W.), while the control treatment recorded 0.18 unit/g (F.W.). The interaction between the two mutagens and NaCl increased in APX activity. The highest APX activity value was recorded in UV-B treatment at 200 mM NaCl (0.505) unit/g (F.W.), while the lowest was in control treatment (0.062) unit/g (F.W.).

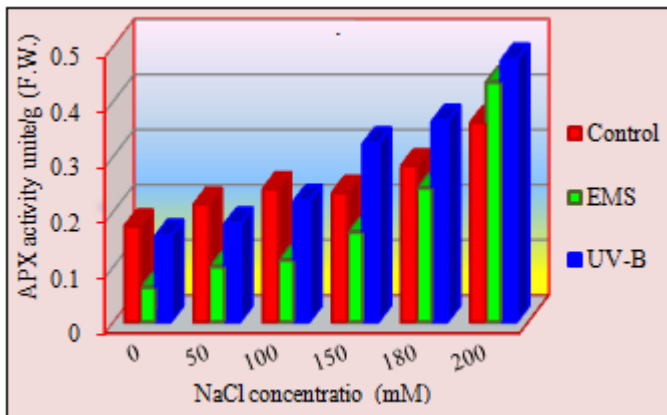


Figure 5 (A33)

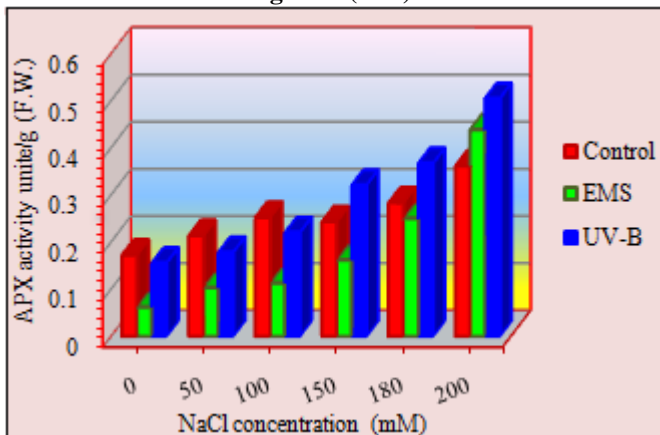


Figure 6 (AB)

Figures 5 and 6: The interaction effect between genotype and NaCl on the APX activity (unit/g F.W.) of A33 and AB genotypes seedlings.

These results are in agreement with Thamodharan and Pillai (2014) who showed that treatment with NaCl raising the activities of APX in rice. Higher antioxidative capacity was reported in rice under salt stress (Nguyen *et al.*, 2005). The antioxidative enzyme activities play a protective role against salt stress. In the plant cells, certain reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydrogen radicals can be responsible for cellular damage under stress conditions. These radicals can damage vital cellular macromolecules as denaturation of protein and peroxidation of lipids (Huang *et al.*, 2009). Ascorbate peroxidase is one of the major members of the ROS scavenging system that plays an important role in improving saline-alkali tolerance in plants, detoxifying H₂O₂ in different cell compartments (Diaz-Vivancos *et al.*, 2013).

Helaly and Hanan El-Hosieny (2011) suggested that the activities of Super Oxide Dismutase (SOD), POX, APX, CAT and Glutathione Reductase (GR) enzymes increased in irradiated shoots compared with non-irradiated under salinized and non-salinized conditions. It could be concluded that, irradiated shoots had a higher hereditary and induced capability under salt stress providing a better protection from oxidative and cellular damage caused by NaCl salinity. Sofo *et al.* (2015) concluded that APX has a higher affinity for H₂O₂ and reduces it to H₂O in chloroplasts, cytosol, mitochondria and peroxisomes, as well as in the apoplasmic space, utilizing ascorbate as specific electron donor. Weisany *et al.* (2012) indicated that under salt stress, the CAT, APX,

polyphenoloxidase (PPO) and peroxidase activity and also proline content increase significantly as a result of salinity stress compared with control.

References

- Chakravarty, H.L. (1976). "Plant Wealth of Iraq" Vol. I. Botany Directorate, Ministry of Agriculture and Agrarian reform, Baghdad, Iraq Pp. 398-432.
- Chakravarthi, B.K. and Naraveni, R. (2006). SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa* L.). *Afric. J. Biotech.*, 5(9), 684-688.
- Zeng, L., Shannon, M.C. and Grieve, C.M. (2002). "Evaluation of salt tolerance in rice genotypes by multiple agronomics parameters" *Euphytica*, 127, 235-245.
- Zeigler, R.S. (2010). "International Rice Research Institute, Los Banos, Philippines", In 5th International Rice Blast Conference, 5.
- Rajakumar, R.A. (2013). "study on effect of salt stress in the seed germination and biochemical parameters of rice (*Oryza sativa* L.) under *in vitro* condition" *Asian J. Plant Sci. Res.*, 3(6), 20-25.
- Khan, M.H. and Panda, S.K. (2008). Alteration in lipid peroxidase and antioxidative responses in two rice cultivars under NaCl- salinity stress. *Act. Physiol. Plant*, 30: 81-89.
- Batels, D. and Sunkar, R. (2005). Drought and salt tolerance in plants. *Crit. Rev. Plant Sci.*, 24:23-58.
- Gaspar, T., Penel, C., Hagega, D. and Greppin, H. (1991). Peroxidases in plant growth, differentiation and development processes. University of Genève, Switzerland. Pp. 249-280.
- Wang, Z.L., Li, P.H., Fredricksen, M., Gong, Z.Z., Kim, C.S., Zhang, C., Bohnert, H.J., Zhu, J.K., Bressan, R.A., Hasegawa, P.M., Zhao, Y.X. and Zhang, H. (2004). Expressed sequence tags from *Thelolungiella halophila*, a new model to study plant salt tolerance. *Plant Sci.*, 166: 609-616.
- Abassi, N.A., Kushad, M.M. and Endress, A.G. (1998). Active oxygen-scavenging enzymes activities in developing apple flowers and fruits. *Sci. Hort.*, 74: 183-194.
- Raven, E.L., Lad, L., Sharp, K.H., Mewies, M. and Moody, P.C. (2004). Defining substrate specificity and catalytic mechanism in ascorbate peroxidase. *Biochem. Soc. Sym.*, 71: 27-38.
- Talebi, A.B., Talebi, A.B. and Shahrokhifar, B. (2012). Ethyl methane sulphonate (EMS) induced mutagenesis in Malaysian rice (cv. MR219) for lethal dose determination. *American Journal of Plant Science*, 3: 1661-1665.
- Wattoo, J.I., Aslam, K., Shah, S.M., Shabir, G., Sabar, M., Naveed, S.A., Waheed, R., Samiullah, Q.H. and Arif, M. (2013). Ethyl methane sulphonate (EMS) induced mutagenic attempts to create genetic variability in Basmati rice. *African Journal of Water Conservation Sustainable*, 1 (3): 45-48.
- Anbumalarmathi, J. and Mehta, P. (2013). Effect of Salt Stress on Germination of *indica* Rice Varieties. *J EJBS* 6 (1): 1-6.
- Liu, Z.H. and Ger, M.J. (1997). Changes of enzyme activity during pollen germination in maize, and

- possible evidence of lignin synthesis. *Aust. J Plant Physiol.*, 24: 329–335.
- [16] Putter, J. (1974). *Methods in Enzymatic Analysis*. 2^{ed} (Ed. Bergmeyer). Academic Press New York, P. 685.
- [17] Cakmak, I. and Maschner, H. (1992). Magnesium deficiency and highlight intensity enhanced activities of super oxide dismutase, ascorbate peroxidase and glutathione reductase in bean leaves. *Plant Physiol.*, 98: 1222-1226.
- [18] Lin and Kao (1999) Lin, C.C. and Kao, C.H. (1999). NaCl induced changes in ionically bound peroxidase activity in roots of rice seedlings. *Plant and Soil*, 216: 147-153.
- [19] Swapna, T.S. (2003). Salt stress induced changes on enzyme activities during different developmental stages of rice (*Oryza sativa* Linn). *Indian J. Biotech.*, 2: 251-258.
- [20] Daud, M.K., Ali, S., Variath, M.T. and Zhu, S.J. (2012). Antioxidative enzymes status in upland cotton callus culture under osmotic stresses. *Int. Conference on Computational Techniques and Artificial Intelligence*. Penang, Malaysia. Pp: 1-4.
- [21] Thamodharan, G. and Pillai, M.A. (2014). Role of antioxidative enzymes activity in salt stress and salinity screening in rice grown under *in vitro* condition. *Electronic J. Plant Breeding*, 5 (3): 371-377.
- [22] Sevengor, S. (2010). Investigation of some antioxidant enzyme activities in pumpkin genotypes under salt stress in term of *in vitro* and *in vivo*. Ph.D. Thesis. Institute of Natural and Applied Science, University of Ankara, Turkey, p.165.
- [23] Chookhamaeng, S. (2011). The effect of salt stress on growth, chlorophyll content, proline content and antioxidative enzymes of pepper (*Capsicum annuum* L.) seedlings. *European J. Sci. and Res.*, 49: 103-109
- [24] Kusvuran, S., Ellialtioglu, S.F., Yasar, F. and Abak, K. (2012). Antioxidative enzyme activities in the leave and callus tissues of salt-tolerant and salt-susceptible melon varieties under salinity. *Afric. J. Biotech.*, 11(3): 635-641.
- [25] Matsuda, Y., Okuda, T., Sagisaka, S. (1994). Regulation of protein synthesis by hydrogen peroxide in crowns of winter wheat. *Biosci. Biotech. Biochem.*, 58: 906–909.
- [26] Mallik, S., Nayak, M., Sahu, B.B., Panigrahi, A.K., and Shaw, B.P. (2011). Response of antioxidant enzymes to high NaCl concentration in different salt-tolerant plants. *Biol. Plant*, 55: 191–195.
- [27] Thamodharan, G. and Pillai, M.A. (2014). Role of antioxidative enzymes activity in salt stress and salinity screening in rice grown under *in vitro* condition. *Electronic J. Plant Breeding*, 5 (3): 371-377.
- [28] Nguyen, H.T.T., Shim, I.S., Kobayashi, K. and Ushi, K. (2005). Effect of salt stress on ion accumulation and antioxidative enzymes activities in *Oryza sativa* L. and *Echinochloa oryzicola* vasing. *Weed Bio. Management*, 5: 1-7.
- [29] Diaz-Vivancos, P., Faize, M., Barba-Espin, G., Faize, L., Petri, C., Hernández, J.A. and Burgos, L. (2013). Ectopic expression of cytosolic superoxide dismutase and ascorbate peroxidase leads to salt stress tolerance in transgenic plums. *Plant Biotech. J.*, 11: 976–895.
- [30] Helaly, M.N.M. and Hanan El-Hosieny, A.M.R. (2011). Effectiveness of gamma irradiated protoplasts on improving salt tolerance of lemon (*Citrus limon* L. Burm.f.). *American J. Plant Physiol.*, 6: 190-208.
- [31] Sofo, A., Scopa, A., Nuzzaci, M., and Vitti, A. (2015). Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. *Inter. J. Mol. Sci.*, 16: 13561-13578.
- [32] Weisany, W., Sohrabi, Y., Heidari, G., Siosemardeh, A. and Golezani, K.G. (2012). Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L.). *PO J.*, 5 (2): 60-67.