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Intervarietal variations in various oxidative stress markers and antioxidant potential of finger millet (*Eleusine coracana*) subjected to drought stress

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Abstract

Drought is a major form of abiotic stress leading to lower crop productivity. Experiment was carried out for selecting the most tolerant genotype among six different genotypes of finger millet under drought stress. Seeds of six finger millet genotypes were sown in pots and grown for 35 days. After this period, drought was induced by withholding watering for stressed plants while control plants were watered regularly for comparison. Among all six different varieties of finger millet screened (PR202, PES400, PRM6107, VL283, VL328 and VL149) under varying intensities of drought stress,PRM6107 and PR202 showed highest stress tolerance by limiting excessive accumulation of reactive oxygen species (ROS) through activation of ROS scavenging antioxidative enzymes. A 200% increase in ascorbate content was recorded in PRM6107 and PR202, while in other varieties limited increase in ascorbate content was observed. Maximum decrease in chlorophyll content was observed in VL328 (83%) while least drop was observed in VL149 (65%). Relative water content indicated that PR202 was able to retain maximum water content under stress, as it recorded least drop in relative water content (55%), contributing to its better survival under stress. In conclusion finger millet genotypes PRM6107 and PR202 possessed maximum drought tolerance potential and thus may be used for allele mining of drought tolerant genes, which can further be employed for the development of more drought stress tolerant staple crops using biotechnological approach.

Key words

Antioxidative enzymes, Finger Millet, Oxidative stress, Reactive oxygen species, Relative water content

Introduction

Drought stress has long been a major limiting factor for crop productivity in various parts of the world. It suppresses the growth and development of most crops, leading to loss in crop quality and productivity (Tester and Langridge, 2010). Prolonged exposure to abiotic stresses such as drought imposes certain common effects on higher plants resulting in accumulation of reactive oxygen species (ROS).The drought-induced stomata closure increases oxidative load on plant tissues, causing perturbations in biochemical pathways. Resulting oxidative stress leads to lipid peroxidation and damage to other important bio-molecules. Apart from morphological adaptations to drought stress, plants have evolved a variety of physiological and biochemical processes to protect cellular and subcellular system from the cytotoxic effects of ROS like lipid peroxidation leading to membrane injury (Marron *et al.*,2006), protein degradation and nucleic acid damage (Fazeli *et al.*, 2007). To withstand drought stress, plants display enzymatic (SOD, APX, GPX, GR etc.) and non-enzymatic (carotene, ascorbic acid, α-tocopherol, reduced glutathione and low molecular weight osmolytes like polyhydroxy alcohols etc.) mechanisms (Gholizadeh, 2010).

In previous studies, low photosynthetic pigment stability and relative water content (RWC) were also used as indicator in representing higher oxidative damage in various plant species (Suriya-arunroj *et al*., 2004).

Finger millet is a hardy crop that can withstand substantial level of drought (Dida et al., 2007). Multiplicity of genes involved in tolerance makes it imperative to look for potent stress tolerant alleles in such plants. As this plant has not been explored much for its potential for withstanding abiotic stresses, antioxidative response of a variety can be used as an important tool for screening its stress tolerance capability. It is important to understand the phenomenon by which different finger millet varieties adopt different biochemical strategies to survive prolonged water deficit condition. The present investigation focuses on estimating the activity of crucial antioxidant enzymes, as well as chlorophyll, ascorbate and relative water content in six different varieties of finger millet with an objective to identify the most tolerant variety among them.

Materials and Methods

Plant material: To study the effect of drought stress on finger millet, six different varieties *viz.* VL283, PRM6107, VL328, PR202, VL149 and PES400 were selected based on their performance on field. Seeds were obtained from Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora and GB Pant University of Agriculture and Technology, Uttarakhand, India.

Stress conditions: Seeds of six finger millet varieties *viz*. PES400, PR202, PRM6107, VL149, VL283 and VL328 were sown in pot containing 2-mm sieved clay loam soil (10 seeds per pot). Sterile water was used regularly to facilitate germination and growth of seedlings. After 14 days, 5 seedlings each for a particular variety were transplanted into earthen pots. After transplanting, seeds were grown in pots containing sand: soil: vermi-compost in 1:2:1 ratio. Seedlings were grown in poly-house under controlled conditions (at 28°C with light intensity of 40µE m^{-2} s^{-1}) for 35 days with regular watering. Thirty five-days-old plants were then subjected to progressive drought stress for 7 days by withholding watering while controls were watered daily. Samples were collected daily from day 1 to 7 and were assessed for various biochemical parameters.

Preparation of enzyme extract: Three leaf sets (replicates) were collected from plants of same treatment and each replicate was analyzed in duplicate. All the sampled leaves were frozen in liquid nitrogen for the estimation of enzyme activity, extracted immediately and supernatant was collected for analysis.

Fresh plant leaves (0.5g) were collected and homogenized in 10ml of 50mM potassium phosphate buffer (pH 7.5) containing 0.1mM EDTA, 0.3% (v/v) Triton-X100 and 2% (w/v) insoluble PVPP. Homogenate was then centrifuged at 13000rpm for 10min at 4°C and supernatant was collected and used for enzyme assays (Grace and Logan, 1996).

Estimation of antioxidative enzyme activity : Ascorbate peroxidase activity was determined according to Nakano and Asada(1981). One unit of enzyme was the amount of enzyme catalyzing the oxidation of 1µM ascorbate per minute. Ascorbate peroxidase activity was expressed in µmol min^{-1} mg^{-1} protein, taking molar extinction coefficient of 2.8mM^{-1} cm^{-1}.

Guaiacol peroxidase activity was assayed as described by Urbanek *et al.* (1991). Increase in absorbance was measured at 470nm for 3min at an interval of 5sec. Guaiacol peroxidase activity was expressed in µmol min^{-1} mg^{-1} protein using molar extinction coefficient 26.6mM^{-1} cm^{-1}.

For determining the activity of glutathione reductase an assay described by Grace and Logan (1996) was followed. One unit of glutathione reductase activity was defined as reduction of 1µM GSSG per minute. Glutathione reductase activity was expressed in µmol min^{-1} mg^{-1} protein using molar extinction coefficient 6.2mM^{-1} cm^{-1}.

Estimation of relative water content, chlorophyll content,and total ascorbate content : Total chlorophyll content of control and drought treated plants was determined by using method described by Hiscox and Israelstam (1979). DMSO was taken as blank. To determine the change in ascorbate content, method of Sadasivam and Manickam (1996) was followed. The amount of dye consumed is equivalent to the amount of ascorbic acid. Relative water content was measured for all the stressed and control plants and expressed as ratio (Du *et al.*, 2011):

[(fresh mass–dry mass)/(water-saturated mass–dry mass)]×100

Statistical analysis : Data presented are mean values ± SE. Measurements were performed on duplicates for each treatment (n=2). Data were submitted to factorial analysis of variance (ANOVA), with treatment (control and stressed) used for analyzing varieties and differences between the means were compared using least significant differences at $p<0.05$.

Results and Discussion

In present study, progressive increase in the level of ascorbate peroxidase activity was observed in all the tested varieties till 5th day of stress. In PR202, this increase continued till 7th day, while it declined after 5th day of stress in remaining varieties. PR202 and PRM6107showed 6.4 (7th day) and 2.6 (5th day) fold higher activity of APX than unstressed plants (fig 1 a). APX activity increased throughout

the stress period (7th day) in PR202, while in other varieties highest activity was observed on 5th day after which it started declining due to excessive ROS accumulation. Ascorbate peroxidase uses ascorbate as a hydrogen donor for reducing H_2O_2 to H_2O and monodehydroascorbate (Asada, 2000). APX has an important role in AsA-GSH pathway and is primary scavenger of H_2O_2 in plant cell thus PR202 was reported with higher APX activity, which is indicative of efficient removal of H_2O_2. Supporting the results, an increased APX activity has been reported to play a crucial role in *R. soongorica* under severe drought stress. Increased APX activity under drought stress has also been reported in genotypes of *Vicia sativa* L (Abbasi *et al.*,2014). Higher activity of APX has also been observed in two rice cultivars under salinity stress. Stress intensity increased the activity of this enzyme (Thamodharan and Pillai, 2014). APX activity increased with progression of drought stress and reached maximum under sever drought condition, as is observed in the present findings (Bai *et al*., 2009).

Sen and Alikamanoglu (2013) reported that increased intensity of drought led to higher APX activity in sugar beet but under severe stress the activity decreased. In another study drought stress significantly lowered the activity of APX enzyme in canola varieties (Omidi, 2010). This suggests that different plant species respond differently to stress in terms of APX activity. Similar observations have also been recorded by Abbasi *et al.* (2014).

Guaiacol peroxidase decomposes indole-3-acetic acid and has a role in the biosynthesis of lignin and defense against oxidative stresses by consuming H_2O_2(Kasote et al., 2015). Activity of guaiacol peroxidase increased under drought. In VL328, PES400 and VL149 varieties the activity was 3.6, 3.2 and 4.0 fold higher than control, while in PRM6107 and PR202 this increase in activity was 2.3 and 2.8 fold respectively. The basal activity of GPX was higher in VL283, PRM6107 and PR202 under normal condition but with onset of drought, activity further increased in PRM6107 and PR202 while least increase was observed inVL283 (1.7 fold). GPX activity drastically decreased after 5th day of drought onset in the varieties, except in PR202 (fig. 1 b). In case of better responsive varieties of finger millet, GPX contributes to elimination of ROS resulting in lesser accumulation as well as preventing membrane from peroxidation. Hence, plant varieties with increased GPX activity showed better survival under drought stress.

Supporting the results, higher GPX activityhas also been reported in different genotypes of sugar beet under drought stress. The activity was highest under severe stress as compared to the control or moderate stress treatment (Habibi, 2014).In another study, four wheat varieties were examined for their drought tolerance ability and it was observed that GPX activity in two drought tolerant wheat varieties was higher as compared to the susceptible ones (Stoilova *et al.*, 2008). The results of the study also showed most tolerant varieties, PR202 and PRM6107,with higher activity of the same enzyme under drought stress as compared to other varieties. Similar results were also reported by Rostami and Rahemi, (2013)where four caprifig varieties were evaluated for their GPX activity under drought stress and the tolerant genotype showed highest activity of same enzyme among all the varieties under stress. Glutathione reductase activity increased in all the tested varieties. The level of enzyme activity was found to be 2.8,2.8 and 3.2 fold higher in VL328, PRM6107 and PR202 respectively as compared to their respective controls, and this activity slowly increased with progression of drought stress till day 6. GR activity recorded was 4.4 fold higher in VL283 and 5.3 fold higher in PES400 than their respective control, followed by a decline in activity after 5th day of stress (Fig. 1 c). The study shows the crucial role of glutathione reductase in protection of finger millet against oxidative stress by maintaining GSH level. Elevated levels of GR also help in maintaining availability of $NADP^+$ to accept electrons from the photosynthetic electron transport chain. The regenerated NADPH can then be used to maintain the activity of anti-oxidative enzymes involved in scavenging ROS. Rahimizadeh *et al.* (2007) reported a rise in GR activity in sunflower plants under drought stress. Drought tolerant variety *P. acutifolius* showed higher GR activity while *P. vulgaris* variety proved to be a sensitive variety, with lower GR activity (Turkan *et al.*, 2005). Similar observations were obtained in the present study as tolerant variety was reported with higher GR activity than the sensitive one. Thus the capability of a plant to withstand oxidative stress is a direct manifestation of its potential to scavenge ROS. Finger millet varieties, PRM6107 and PR202 were found to possess these criterions, and can be categorized as drought tolerant varieties.

Variation in relative water content was used as a measure of stress. The percentage of relative water content was similar in all the six screened varieties which declined with increasing intensity of drought stress as compared to their respective control. Initially, relative water content was least affected under mild stress indicating that finger millet plants have the ability to sustain their water content under mild stress, whereas this ability was ultimately lost under moderate stress treatment. Decrease in relative water content in PRM6107 was 56% and in PR202 was 55% respectively. In VL283 (59%), VL149 (63%), PES400 (65%) and VL328 (60%), the drop in RWC was higher (Fig. 2 b). Among all the screened varieties, least reduction was observed in PRM6107 and PR202. A significant reduction in RWC was observed in leaf disc of two maize varieties under different levels of stress exposure, where the tolerant variety was least effected

(D'souza and Devaraj, 2011). Consistent decrease in RWC in response to water deficit stress has also been reported in *R. soongorica* (Bai *et al.*, 2009) and in wheat seedlings (Ahmad and Haddad, 2011). Thus, higher relative water content in tolerant variety of finger millet could play an important role in drought stress tolerance.

In the present study, reduction in total chlorophyll content with the progression of drought was observed in all the varieties. On 7th day of the cessation of watering, reduction in chlorophyll content was more than 80% in VL283 and VL328. However, comparatively lesser reduction in chlorophyll was recorded in the remaining four varieties *viz*. VL149 (65%), PRM6107 (76%), PR202 (77%) and PES400 (78%), than their respective controls (Fig. 2 a). Reduction in chlorophyll content in tested finger millet varieties could be because of CO_2 limitation, which may arise due to stomata closure under stress, as well as damage to photosynthetic apparatus. This physiological condition may be an outcome of increased oxidative stress and low anti-

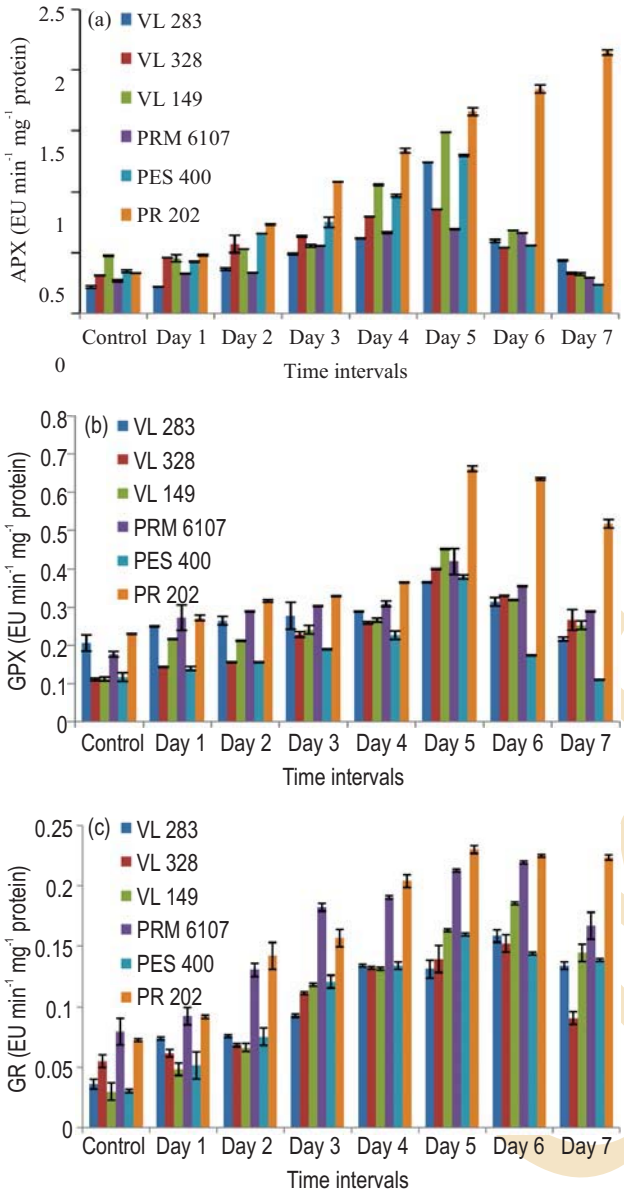

Fig. 1 : Specific activity of (a) ascorbate peroxidase; (b) guaiacol peroxidase and (c) glutathione reductase in different finger millet varieties exposed to progressive drought stress for seven days. Line above bars represents Mean ± SE ($p<0.05$)

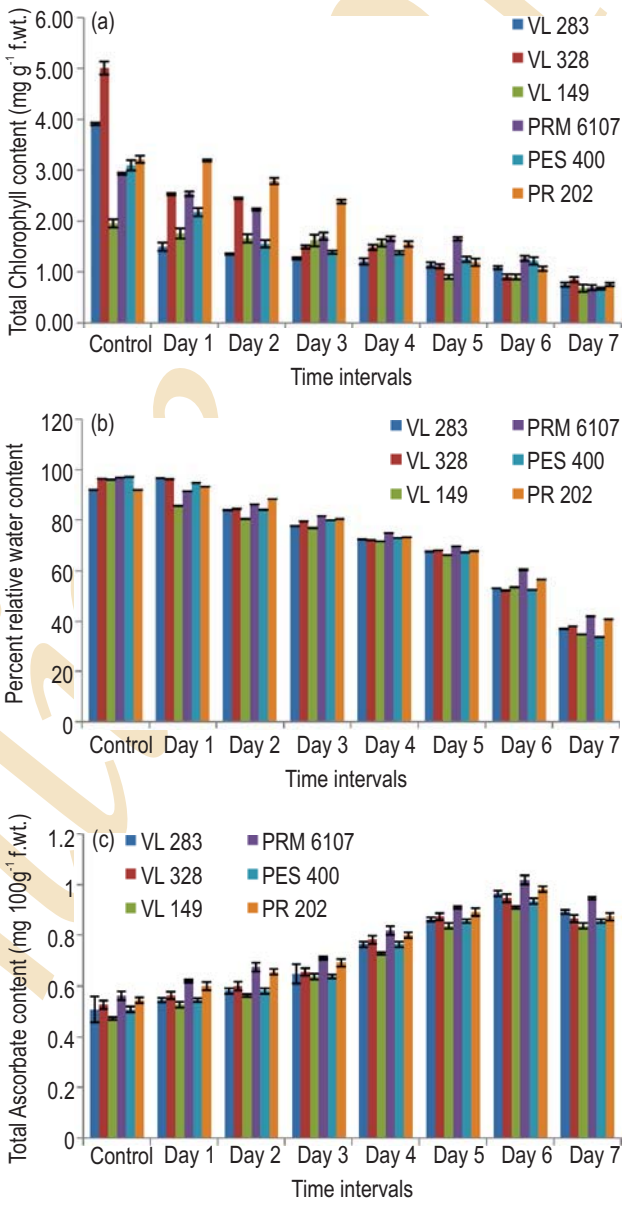

Fig. 2 : (a) Total chlorophyll content; (b) relative water content and total ascorbate content in different finger millet varieties exposed to progressive drought stress for seven days. Line above bars represents Mean ± SE ($p<0.05$)

oxidative protection in later stages of drought (Hanci and Cebeci, 2015). It should be noted that the pigment apparatus in different varieties of the same species may exhibit differential sensitivity to drought. Decrease in chlorophyll content under drought stress is considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation (Xiao *et al.*, 2008). Saglam *et al.*, (2011) reported decrease in total leaf chlorophyll content in different common bean varieties under drought stress. Under heat stress, among different varieties of *Brassica juncea,* the tolerant variety had higher chlorophyll content than the susceptible variety (Wilson *et al.*, 2014).

Ascorbate is required for many key metabolic functions in plant cell's scavenging process and is a major water-soluble antioxidant in plants (Sharma and Dubey, 2005).There was an increase in the level of total ascorbate in drought stressed plants. The level of ascorbate slowly rose with progressive drought stress and was almost double in all the screened varieties by day 6 or 7 of imposition of stress. Maximum recorded level in VL283, VL149, VL328 and PES400 was 89%, 92%, 82% and 83% respectively, as compared to their respective controls.While PRM6107 and PR202 showed increase of about 80% (Fig. 2 c). Thus ascorbate content increased in response to the defense mechanism of plants under stress. Sufficient amount of ascorbate is required to be regenerated for efficient activity of APX which further converts H_2O_2 into H_2O. Therefore, tolerant varieties with higher antioxidative potential ensured higher increase in total ascorbate content compared to sensitive varieties with relatively weaker antioxidative response.

Various studies, on *Zea mays* (Chugh *et al.*, 2011), D. *moldavica* (Halimeh *et al.*, 2013) and *R. soongorica* (Bai *et al.*, 2009) have emphasized the role of ascorbate in regulation of ROS scavenging mechanisms, under abiotic stresses.In the present study also increase in the total ascorbate content correlated with higher APX activity in stress tolerant varieties. It has also been observed that application of ascorbate activates antioxidant mechanisms and this improves tolerance against drought stress (Shalata and Neumann, 2001).

Lesser variation among the parameters other than the antioxidative enzymes *viz.* total chlorophyll content, relative water content and total ascorbate content indicates that all these factors are equally important and affected the finger millet plant under stress. The higher response of antioxidative enzymes in PRM6107 and PR202 might be due to their higher drought responsive gene expression under stress and the cumulative effect of other drought responsive proteins or regulatory switches, which trigger higher activity of APX, GPX and GR.

It could be concluded from the present study that finger millet varieties PRM6107 and PR202 have improved antioxidant potential and are more drought tolerant. Thus these stress tolerant varieties can be explored for allele mining of drought stress responsive genes and used for the development of improved varieties of economically important crops.

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References

Abbasi, A.R., R. Sarvestani, B. Mohammadi and A. Baghery: Drought stress induced changes at physiological and biochemical levels in some Vtech (*Vicia sativa* L.) genotypes. *J. Agri. Sci. Tech.,* **16,** 505-516 (2014).

Ahmad, S.T. and R. Haddad: Study of silicon effects on antioxidant enzyme activities and osmotic adjustment of wheat under drought stress. *Czech. J. Genet. Plant. Breed.*, **47,** 17- 27 (2011).

Asada, K.: The water-water cycle as alternative photon and electron sinks. *Phil. Trans. R. Soc. Lond. B. Biol. Sci.*, **355,** 1419-1431 (2000).

Bai, J., C.M. Gong, K. Chen, H.M. Kang and G. Wang: Examination of antioxidative system's responses in the different phases of drought stress and during recovery in desert plant *Reaumuria soongorica* (Pall.) Maxim. *J. Plant. Biol.*, **52,** 417- 425 (2009).

Chugh, V., N. Kaur and A.K. Gupta: Evaluation of oxidative stress tolerance in maize (*Zea mays L.*) seedlings in response to drought. *Indian. J. Biochem. Biophys*, **48,** 47- 53(2011).

D'souza, M.R. and V.R. Devaraj: Specific and non-specific responses of Hyacinth bean (*Dolichos lablab*) to drought stress. *Indian. J. Biotechnol.*, **10,** 130- 139 (2011).

Dida, M.M., Srinivasachary, S. Ramakrishnan, J.L. Bennentzen, M.D. Gale and K.M. Devos: The genetic map of finger millet (*Eleusine coracana*). *Theor. App. Genet.*, **144,** 321- 332 (2007).

Du, J.B., S. Yuan, Y.E. Chen, Sun X, Z.W Zhang, F. Xu, M. Yuan, J. Shang and H.H. Lin: Comparative expression analysis of dehydrins between two barley varieties, wild barley and Tibetan hulless barley associated with different stress resistance. *Acta. Physiol. Plant*, **33,** 567- 574 (2011).

Fazeli, F., M. Ghorbanli and V. Niknam: Effect of drought on biomass, protein content, lipid peroxidation and antioxidant enzymes in two sesame cultivars. *Biol. Plant.*, **51,** 98-103 (2007).

Gholizadeh, A.: Anti-oxidation profile in the leaves of maize inbreds: Elevation in the activity of phenylalanine ammonia lyase under drought stress. *J. Plant. Sci.*, **5,**137- 145 (2010).

Grace, S.C. and B.A. Logan: Acclimation of foliar antioxidant systems to growth irradiance in three broad-leaved evergreen species. *Plant. Physiol*, **112,** 1631-1640 (1996).

Habibi, D.: Evaluation of antioxidant enzymes activity in sugar beet genotypes under drought stress. *MAGNT. Res. Rep.*, **2,** 225-238 (2014).

Halimeh, R., G. Mahlagh, P. Maryam and A. Pazoki: Effect of drought

interactions with ascorbate on some biochemical parameters and antioxidant enzymes activities in *Dracocephalum moldavica* L. *Middle. East. J. Sci. Res.*, **13,** 522-531 (2013).

Hanci, F. and E. Cebeci: Comparison of salinity and drought stress effects on some morphological and physiological parameters in onion (*Allium cepaL.*) during early growth phase. *Bulgarian Journal of Agricultural Science*, **21,** 1204-1210 (2015).

Hiscox, A.D. and G.S. Israelstam: A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.*, **57,**1332- 1334 (1979).

Jajic, I., T. Sarna, K., Strzalka: Senescence, stress, and reactive oxygen species. *Plants,* **4**, 393-411(2015).

Kasote, D., M., Katyare, S.,S., Hegde, M., V., Bae H: Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International journal of biological sciences*. **11**, 982 (2015)

Marron, N., S. Maury, C. Rinaldi and F. Brignolas: Impact of drought and leaf development stage on enzymatic antioxidant system of two *Populus deltoids X nigra clones*. *Ann. For. Sci.*, **63,** 323-327 (2006).

Nakano, Y. and K. Asada: Hydrogen peroxide is scavenged by ascorbate-specific peroxidases in spinach chloroplasts. *Plant. Cell.Physiol.*, **22,** 867- 880 (1981).

Omidi, H.: Changes of proline content and activity of antioxidative enzymes in two canola genotype under drought stress. *Am. J. Plant. Physiol.*, **5,** 338-349 (2010).

Rahimizadeh, M., D. Habibi, H. Madani, G.N. Mohammadi, A. Mehraban and A.M. Sabet: The effect of micronutrients on antioxidant enzymes metabolism in sunflower (*Helianthus annus* L.) under drought stress. *Helia*, **30,** 167-174 (2007).

Rostami, A.A. and M. Rahemi: Screening drought tolerance in capriFig. varieties in accordance to responses of antioxidant enzymes. *World. Appl. Sci. J.*, **21,**1213- 1219 (2013).

Sadasivam, S. and A. Manickam: Estimation of ascorbic acid. Biochemical Methods. (Ed.2.) New Age International (P) Limited, Publishers, New Delhi , pp .184-186 (1996).

Saglam, A., N. Saruhan, R. Terzi and A. Kadioglu: The relations between antioxidant enzymes and chlorophyll fluorescence parameters in common bean cultivars differing in sensitivity to drought stress. *Russ. J. Plant. Physiol.*, **58,** 60- 68 (2011).

Sen, A. and S. Alikamanoglu: Antioxidant enzyme activities, malondialdehyde, and total phenolic content of PEG-induced hyperhydric leaves in sugar beet tissue culture. *In Vitro. Cell. Dev. Biol. Plant.*, **49**, 396-404 (2013).

Shalata, A. and P.M. Neumann: Exogenous ascorbic acid (Vitamin C) increase resistance to salt stress and reduces lipid peroxidation. *J. Exp. Bot.*, **52,** 2207-2211 (2001).

Sharma, P. and R.SDubey: Drought induces oxidative stress enhances the activities of antioxidant enzymes in growing rice seedlings. *Plant. Growth. Regul.*, **46,** 209- 221 (2005).

Stoilova, L.S., K. Demirevska, T. Petrova, N. Tsenov and U. Feller: Antioxidative protection in wheat varieties under severe recoverable drought at seedling stage. *Plant. Soil. Environ.*, **54,** 529- 536 (2008).

Suriya-arunroj, D., N. Supapoj, T. Toojinda and A. Vanavichit: Relative leaf water content as an efficient method for evaluating rice cultivars for tolerance to salt stress. *Sci. Asia*, **30,** 411- 415 (2004).

Tester, M. and P. Langridge: Breeding technologies to increase crop production in a changing world. *Science.*, **327**, 818-822 (2010).

Thamodharan, G. and M.A. Pillai: Role of antioxidative enzymes activity in salt stress and salinity screening in rice grown under *in vitro* condition. *Electronic. J. Plant. Breed*, **5,** 371-377 (2014).

Tu¨rkan, I., M. Bor, F. Ozdemir and H. Koca: Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius*gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. *Plant. Sci.*, **168,** 223- 231 (2005).

Urbanek, H., E. Kuzniak-Gebarowska and K. Herka: Elicitation of defense responses in bean leaves by *Botrytis cinerea* polygalacturonase. *Acta. Phys. Plant*, **13,** 43- 50 (1991).

Wilson, R.A., M.K. Sangha, S.S. Banga, A.K. Atwal and S. Gupta: Heat stress tolerance in relation to oxidative stress and antioxidants in *Brassica juncea* . *J. Environ. Biol.,* **35,** 383-387 (2014).

Xiao, X., X. Xu and F. Yang: Adaptive responses to progressive drought stress in two *populuscathayana* populations. *Silva. Fennica*, **42,** 705- 719 (2008).