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Drought stress induced changes in lipid peroxidation and antioxidant system in genus *Avena*

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Abstract: *Seven species of genus Avena viz., Avena sativa, Avena strigosa, Avena brevis, Avena vaviloviana, Avena abyssinica, Avena marocana and Avena sterilis were used to study the impact of drought stress on lipid peroxidation and other antioxidant enzymes. Maximum increase in the catalase activity was recorded in A. vaviloviana (129.97%) followed by A. sativa (122.82%) and A. brevis (83.38%) at vegetative stage; however at flowering stage the maximum increase was reported in A. sativa (25.62%) followed by A. sterilis (20.46%) and A. brevis (18.53%). At vegetative stage drought, maximum increase in peroxidase activity was recorded in A. sativa (122.82%) followed by A. brevis (83.38%) and A. sterilis (49.78%). Flowering stage drought, showed maximum increase in A. sativa (27.09%) followed by A. marocana (23.50%) and A. sterilis (20.46%). A. sativa and A. sterilis showed stress tolerance at both the stages by accumulating higher percentage of peroxidase followed by A. brevis at vegetative and A. marocana at flowering stage. Level of lipid peroxidation in terms of Malondialdehyde (MDA) content was increased in the leaves when plants were subjected to moisture stress. The rate of increase in lipid peroxidation occurs irrespective of stage however; maximum increase was recorded in A. strigosa at both the stages. Avena species which showed high level of MDA content, indicates more lipid peroxidation and more membrane permeability and are comparatively more susceptible for water stress than those which produce less Malondialdehyde (MDA) content at higher magnitude of water stress such species have better capability for moisture stress tolerance.*

Key words: *Drought, Antioxidant system, Lipid peroxidation, Avena sativa*
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Introduction

Many environmental stresses including drought, chilling and salt stress impair electron transport system lead to the formation of activated oxygen (Smirnoff, 1993; Zhang and Kirkhan, 1996; Chandra *et al*., 1998). Activated oxygen compound such as H_2O_2, O_2^- and OH^- may accumulate during water deficit stress and damage the photosynthetic apparatus. Super oxide dismutase (SOD) and ascorbate peroxidase along with antioxidant ascorbic acid and glutathione act to prevent oxidative damage in plants (Allen, 1995). Water deficit reduced both photosynthesis and transpiration; however, transpiration was reduced more relative to photosynthesis. Activity of SOD is maintained during water deficit, whereas catalase activity varied inconsistently as water deficit increased. Peroxidase activity increased with increasing stress. Oxygen free radicals have little direct effect on the photosynthetic apparatus of severely stressed alfalfa leaves, whereas in several grasses as reported by Price and Hendry (1991), oxidative molecules initiate damage in the chloroplast and cause a cascade of damaging effect including chlorophyll destruction, lipid peroxidation and protein loss. Water stress alters the equilibrium between the free radical production and enzymatic defense reactions in wheat species and hexaploid wheat have less efficient antioxidant systems than tetraploid and diploid wheat (Zhang and Kirkham, 1994). Reddy *et al.* (1998) studied effect of water stress on seven rice genotypes (CTH-1, Doddi, Halubbalu. IR-30864, Prabhavathi, Rasi and Valya) and reported that selected tissues of all cultivars exhibited lower levels of lipid per oxidation (MDA content) and higher SOD and CAT activities and soluble proteins under stress. Halubbalu and Rasi were superior to other, as they have accumulated low MDA and greater activities of SOD and CAT. Zhang and Kirkham *et al.* (1994) studied the SOD, POD and CAT activities as well as MDA content and solute potential in seedling of seven wheat species (nine genotypes representing three ploidy levels: hexaploid, tetraploid, diploid) subjected to water stress for different days by withholding water. Solute potential of all genotypes were lowered by water stress. The POD activity and MDA content greatly increase in response to water stress. Oat (*Aven sativa* L.) is widely recognized as one of the major cultivated C_3 fodder as well as grain crop which are nutritive as well as highly palatable. The crude protein percent in oat genotypes varies from 7.4 -16.4% and the dry matter digestibility ranges from 7.6 to 8.4%. Keping in view the importance of oat in this investigation attempt has been made to quantify the effect of moisture stress on the enzyme activity of seven *avena* species for further use of these species in the breeding programme.

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Materials and Methods

Seven species of *Avena viz*. *A. strigosa*, *A. brevis*, *A. vaviloviana*, *A. abyssinica*, *A. sativa*, *A. marocana* and *A. sterilis* were sown in porcelain pots (30x20 cm) at pot culture experimental site of IGFRI, Jhansi (25°N and 78°E, 275 msl). Final plant population of 3 plants in each pot was maintained. The crop was grown as per

recommended agronomic practices. Withholding the irrigation created the water stress. Physiological changes were studied at two growth stages of the crop i.e. vegetative and flowering under well watered (controlled), water stressed (by withholding water) and re-watered environment. One set of each variety was maintained at 100% field capacity irrigation and another set at moisture stress by holding water. After extreme stress, water stressed plants were re-watered and the study was also conducted after the recovery of the crop.

Fresh and young leaves were collected in ice box (4°C) for enzyme extraction. Plant samples were homogenized in three-fold volume of cold extraction buffer in pre chilled pestle mortar to very fine slurry. The homogenate was centrifuged at 12,000 rpm for 20 min. The supernatant obtained was referred as enzyme extracts and used as enzyme source. It was kept in ice till the assay was carried out. An aliquot of the extract was used for protein determination (Lowry *et al*., 1951) and used to determine the specific activity of the enzymes by dividing the total obtained units in one gram fresh weight by total milligram protein in one gram fresh weight.

Superoxide dismutase (SOD) activity was determined by measuring its ability to inhibit photochemical reduction of Nitroblue tetrazolium (NBT) according to the method of Giannopolitis and Ries (1977) with suitable modifications. One enzyme unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of NBT reduction measured at 560nm. Activities of catalase (CAT) and peroxidase (POD) were measured by the method of Chance and Machly (1955). For peroxidase, the oxidation of guaiacol was measured by the increase in absorbance at 470 nm. For catalase, the decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. Activities for POD and CAT were expressed in enzyme units mg^{-1} protein, where one enzyme units was defined as a change of 0.01 absorbance min^{-1}caused by the enzyme aliquot (Zhang *et al*., 1990).

Malondialdehyde (MDA) was estimated in dry leaf tissues by following the procedure of Heath and Packer (1968). The molar extinction coefficient used to calculate the amount of MDA was 155 mM^{-1} cm^{-1}. The data were statistically analyzed by following standard statistical methods (Gomez and Gomez, 1984)

Results and Discussion

At vegetative stage significant change in SOD activity was observed in all the species by imposing moisture stress. The activity ranged from 0.112±0.002 (*A. marocana*) to 0.167±0.003 (*A. sterilis*). When water stress was imposed by withholding the water a drastic decrease in SOD activity was recorded and the value ranged from 0.049±0.002 (*A. marocana*) to 0.104±0.004 (*A. sativa*). However the decrease varies from species to species. Minimum percent decrease over control was recorded in *A. sativa* (32.47) followed by *A. brevis* (36.42) and *A. vaviloviana* (37.58). On re-watering the SOD activity again increased to the tune of well irrigated situation. At the flowering stage SOD activity showed a decreasing trend in all the species of *Avena* when subjected to water stress and again increased to the tune of well watered environment. However the rate of decrease in the enzyme activity varies differently in different species. Under control condition the minimum activity was recorded in *A. brevis* (0.168±0.000) and the maximum was recorded as 1.203±0.024 units mg $protein^{-1}$ min^{-1} in *A. vaviloviana.* Among the species the rate of decrease in SOD activity varies significantly. Minimum percent of decrease in the enzyme activity was recorded in *A. abyssinica* (07.04%) followed by *A. brevis* (18.45%) and *A. sativa* (20%). The maximum decrease was recorded in *A. vaviloviana* (89.19%) (Table 1).

Higher activity of catalase was recorded under water stressed situation irrespective of the stage of the crop. At vegetative stage the activity was recorded under controlled environment ranges from 0.377±0.009 in *A. strigosa* to 0.776±0.019 in *A. brevis.* On imposing stress the catalase activity increased in all the species of *Avena*, however the percent increase in the activity varies from species wise. Maximum increase in the activity was recorded in *A. vaviloviana* (129.97%) followed by *A. sativa* (122.82%) and *A. brevis* (83.38%). On re-water the activity in all the species decreased. At flowering stage under well watered environment the activity ranged from 0.349±0.017 (*A. marocana*) to 0.923±0.017 (*A. brevis*). Under extreme stress environment the activity increased in all the species of *Avena*, however the rate of increase was different in the different species. The maximum percent increase in the catalase activity over control was recorded in A. sativa (25.62%) followed by *A. sterilis* (20.46%) and *A. brevis* (18.53%) (Table 2).

Peroxidase enzyme activity in stressed plants was measured at highest magnitude of water stress (at the lowest water potential). At vegetative stage peroxidase enzyme activity measured at control condition indicated species wise variation in activity. It ranged from 0.410±0.023 (*A. marocana*) to 0.776±0.019 (*A.brevis*). By imposing water stress peroxidase enzyme activity increased in all the species and it ranged from 0.446±0.043 (*A. marocana*) to 1.425±0.023 (*A.brevis*) but the rate of increases varies species to species. The maximum percent increase in peroxidase activity was recorded in *A. sativa* (122.82%) followed by *A. brevis* (83.38%) and *A. sterilis* (49.78%). At the flowering stage peroxidase enzyme activity measured at control condition ranged from 0.349±0.017 (*A. marocana*) to 0.892±0.024 (*A. strigosa*) When water stress imposed by withholding the water peroxidase enzyme activity increased and the value ranged from 0.431±0.004 (*A. marocana*) to 1.048±0.027 (*A.sterilis*). The rate of increase varies among the species of *Avena*. The maximum percent of increase was recorded in *A. sativa* (27.09%) followed by *A. marocana* (23.50%) and *A. sterelis* (20.46%). Finally after re-watering the activity of almost all species reached to the level of control. (Table 3).

Malondialdehyde (MDA) was recorded in the leaf sample of seven *Avena* species grown under well water controlled, extreme stress (by withholding water) and re-watered environment at two growth stages *i.e.* vegetative and flowering. There was a significant change in the MDA content of the plants subjected to extreme stress environment. At vegetative stage maximum and minimum value

Table - 1: Change in Superoxide Dismutase (SOD) enzyme activity (units mg protein^{-1} min^{-1}) in *Avena* species under different conditions at vegetative and flowering stage

Species	Vegetative stage			Flowering stage		
	I (Control)	II (Extreme stress)	III (Re-watered)	I (Control)	II (Extreme stress)	III (Re-watered)
A. strigosa	0.156±0.009	0.080±0.002 (48.72)	0.092±0.000	0.251±0.017	0.198±0.004 (21.12)	0.210±0.004
A. brevis	0.151±0.003	0.096±0.000 (36.42)	0.114±0.001	0.168±0.000	0.137±0.003 (18.45)	0.151±0.003
A. vaviloviana	0.165±0.020	0.103±0.001 (37.58)	0.141±0.002	1.203±0.024	0.130±0.001 (89.19)	0.141±0.002
A. abyssinica	0.144±0.001	0.089±0.007 (38.19)	0.101±0.001	0.199±0.002	0.185±0.003 (07.04)	0.196±0.003
A. sativa	0.154±0.001	0.104±0.004 (32.47)	0.126±0.000	0.270±0.004	0.216±0.003 (20.00)	0.226±0.002
A. marocana	0.112±0.002	0.049±0.002 (56.25)	0..056±0.001	0.203±0.000	0.158±0.007 (22.17)	0.169±0.007
A. sterilis	0.167±0.003	0..076±0.000 (54.49)	0.113±0.001	0.341±0.004	0.223±0.025 (34.060)	0.257±0.000

Values in parentheses represent percent decrease in the SOD activity over control. ± = standard deviation (n=3) (level of significance: p<0.05)

Table - 2: Change in catalase activity (CAT) (units mg^{-1} protein^{1} min^{-1}) in *Avena* species under different conditions at vegetative and flowering stage

Species	Vegetative stage			Flowering stage		
	I (Control)	II (Extreme stress)	III (Re-watered)	I (Control)	II (Extreme stress)	III (Re-watered)
A. strigosa	0.377±0.009	0.532±0.093 (41.11)	0.514±0.064	0.892±0.024	1.020±0.079 (14.35)	1.000±0.014
A. brevis	0.776±0.019	1.423±0.023 (83.38)	0.889±0.328	0.923±0.017	1.094±0.031 (18.53)	0.962±0.016
A. vaviloviana	0.387±0.004	0.890±0.216 (129.97)	0.826±0.040	0.829±0.022	0.914±0.000 (10.25)	0.896±0.015
A. abyssinica	0.619±0.022	0.837±0.254 (35.22)	0.696±0.007	0.649±0.002	0.763±0.010 (17.57)	0.738±0.014
A. sativa	0.447±0.020	0.996±0.007 (122.82)	0.608±0.003	0.683±0.001	0.858±0.012 (25.62)	0.768±0.001
A. marocana	0.410±0.023	0.446±0.043 (08.78)	0.430±0.048	0.349±0.017	0.410±0.028 (17.48)	0.378±0.047
A. sterilis	0.456±0.008	0.683±0.030 (49.78)	0.485±0.009	0.870±0.192	1.048±0.027 (20.46)	0.941±0.035

Values in parentheses represent percent increase in the catalase activity over control. ± = standard deviation (n=3) (level of significance: p<0.05)

Table - 3: Change in Peroxidase enzyme (POD) activity (units mg protein^{-1} min^{-1}) in *Avena* species under different conditions at vegetative and flowering stage

Species	Vegetative stage			Flowering stage		
	I (Control)	II (Extreme stress)	III (Re-watered)	I (Control)	II (Extreme stress)	III (Re-watered)
A. strigosa	0.514±0.064	0.745±0.030 (44.94)	0.532±0.093	0.892±0.024	1.020±0.079 (14.35)	1.000±0.014
A. brevis	0.776±0.019	1.423±0.023 (83.38)	0.889±0.32	0.889±0.004	0.962±0.016 (08.21)	0.923±0.017
A. vaviloviana	0.690±0.048	0.890±0.216 (28.99)	0.826±0.040	0.829±0.022	0.914±0.000 (10.25)	0.896±0.015
A. abyssinica	0.619±0.022	0.837±0.254 (35.22)	0.696±0.007	0.649±0.002	0.738±0.014 (13.71)	0.718±0.005
A. sativa	0.447±0.020	0.996±0.007 (122.82)	0.608±0.003	0.683±0.001	0.868±0.001 (27.09)	0.858±0.012
A. marocana	0.410±0.023	0.446±0.043 (08.78)	0.430±0.048	0.349±0.017	0.431±0.004 (23.50)	0.410±0.028
A. sterilis	0.456±0.008	0.683±0.030 (49.78)	0.485±0.009	0.870±0.192	1.048±0.027 (20.46)	0.841±0.035

Values in parentheses represent percent increase in the peroxidase activity over control. ± = standard deviation (n=3) (level of significance: p<0.05)

was recorded in *A. strigosa* (114.25±1.395) nano moles g^{-1}dwt. and *A. vaviloviana* (167.86±0.215) nano moles g^{-1}dw respectively. However over extreme stress environment the value ranges from 161.64±0.00 nano moles g^{-1}dw. in *A. brevis* to *A. sterilis* (199.64± 0.644) nano moles g^{-1}dw. The percent of increase in the MDA content varies significantly among the species tested. The maximum percent of increase over control was recorded in *A. strigosa* (54.86%) followed by *A. sterilis* (25.51%) and *A. sativa* (25.27%) and re-watering the MDA content again decreased to the tune of controlled environment. At flowering stage under controlled environment maximum MDA accumulation was recorded in *A. abyssinica* (202.37±0.48) nano moles g^{-1}dw followed by *A. vaviloviana* (200.28±0.644) nano moles g^{-1}dw and *A. sterilis* (199.3±1.932) nano moles g^{-1}dw. Under extreme stress the MDA content increased and the maximum value recorded was 334.39±0.483 nano moles g^{-1}dwt. in *A. strigosa*, followed by *A. brevis* (282.23±) nano moles g^{-1}d wt. and *A. sterilis* (265.16±1.449) nano moles g^{-1}dw. The increase in the MDA content varies from species to species. Maximum percent of increase in the MDA content over control was recorded in *A. strigosa* (68.31%) followed by *A. brevis* (56.52%) and *A. sativa* (38.87%). On re-watering the value of MDA again decreased to tune of the plants grown under controlled environment (Table 4).

At vegetative stage increase in the soluble protein accumulation was recorded under extreme stress environment over the well watered controlled environment. Under controlled conditions the maximum value of protein was recorded in *A. vaviloviana* (35.921±0.410) mg g^{-1} fw followed by *A. marocana* (30.953±1.361) mg g^{-1} fw and *A. brevis* (30.434±0.756) mg g^{-1} fw. Under extreme stress environment the protein accumulation ranges from a minimum value of 33.847±0.410 mg g^{-1} fw in *A. sterilis* to a maximum value of

Table - 4: Change in Malondialdehyde (MDA) content (nano moles g^{-1} dw) in *Avena* species under different conditions at vegetative and flowering stage

Species	Vegetative stage			Flowering stage		
	I (Control)	II (Extreme stress)	III (Re-watered)	I (Control)	II (Extreme stress)	III (Re-watered)
A. strigosa	114.25±1.395	176.93±1.449 (54.86)	139.90±0.805	198.67±1.610	334.39±0.483 (68.31)	289.31±0.483
A. brevis	139.15±1.020	161.64±0.000 (16.16)	154.88±0.322	180.32±0.000	282.23±1.449 (56.52)	225.61±0.751
A. vaviloviana	167.86±0.215	192.55±1.610 (14.71)	183.70±0.483	200.28±0.644	246.97±0.000 (23.31)	213.32±3.381
A. abyssinica	161.26±0.698	172.10±0.483 (06.72)	167.60±0.161	202.37±0.48	240.85±0.966 (19.01)	208.49±11.32
A. sativa	154.23±1.181	193.20±0.000 (25.27)	155.20±0.000	162.77±1.020	226.04±1.288 (38.87)	168.24±1.127
A. marocana	160.78±1.288	176.13±0.966 (09.55)	169.53±1.127	192.12±0.000	257.11±0.483 (33.83)	211.23±0.000
A. sterilis	159.06±1.288	199.64±0.644 (25.51)	185.74±0.483	199.31±1.932	265.16±1.449 (33.04)	251.16±0.000

Values in parentheses represent percent increase in MDA over control. ± is standard deviation (n=3) (level of significance: $p<0.05$)

Table - 5: Change in Total soluble protein (mg g^{-1} fw) in *Avena* species under different conditions at vegetative and flowering stage

Species	Vegetative stage			Flowering stage		
	I (Control)	II (Extreme stress)	III (Re-watered)	I (Control)	II (Extreme stress)	III (Re-watered)
A. strigosa	27.259±0.389	49.226±5.292 (80.59)	28.224±0.158	25.646±0.288	40.781±3.024 (59.02)	30.478±1.145
A. brevis	30.434±0.756	40.586±2.311 (33.36)	33.286±0.281	33.372±0.626	39.247±0.583 (17.60)	34.927±0.238
A. vaviloviana	35.921±0.410	62.359±14.53 (73.60)	39.834±1.238	26.784±0.158	42.250±0.216 (57.74)	30.737±0.756
A. abyssinica	28.728±0.130	64.303±17.60 (123.83)	35.834±1.246	29.095±0.238	37.195±0.130 (27.84)	30.413±0.000
A. sativa	27.907±0.259	52.315±2.678 (87.46)	39.881±0.986	23.566±0.151	27.972±0.022 (18.70)	25.466±0..367
A. marocana	30.953±1.361	40.694±4.277 (31.47)	34.229±1.166	19.483±0.115	24.386±0.194 (25.17)	22.896±0.043
A. sterilis	26.568±0.259	33.847±0.410 (27.40)	28.015±1.102	15.473±0.641	25.985±0.022 (67.94)	20.952±0.259

Values in parentheses represent percent increase in soluble protein content over control. ± is standard deviation (n=3) (level of significance: $p<0.05$)

64.303±17.60 mg g^{-1} fw. The increase in the protein accumulation varies from species to species and the maximum percent of increase in soluble protein accumulation over control was recorded in *A. abyssinica* (123.83%) followed by *A. sativa* (87.46%) and *A. strigosa* (80.59). By re-watering the protein value decreased to the tune of controlled environment (Table 5).

SOD are metallo-protein catalyzes dismutation of the superoxide free radicals (O^-_2) to molecular oxygen and H_2O_2. The depression of SOD activity in plants reported in may crops (Chowdhury and Chowdhury, 1985; Quartacci and Navarilzzo, 1992; Zhang *et al.,* 1990). As SOD catalyzes the dismutation of superoxide anion radical (O^-_2) with great efficiency, resulting in the production of H_2O_2 and O_2 (Smimoff, 1993; Winston, 1990), the decrease in SOD activity could improve the O^-_2 scaverging system of cells and favor accumulation of O^-_2 which mainly contribute in damaging the membrane. Because measured enzyme activity is a result of both synthesis and degradation, any decrease in net SOD activity under drought can be ascribed to either reduced synthesis or enhanced degradation of enzyme. Our result showed that minimum decrease in SOD activity was recorded in *A. sativa* followed by *A. brevis*, *A. vaviloviana* and *A. abyssinica* at vegetative stage. All these species showed an at par value in the decrease in SOD activity over control. However at flowering stage the minimum decrease was recorded in *A. abbysinica* followed by *A. brevis* and *A. sativa* over control. The decrease in SOD activity under extreme water stress indicated that the scavenger ability in the cells of leaves was inhibited under extreme water stress. It is also indicated that the species which showed less extent of decrease in SOD activity as compared to the species which showed higher decrease in enzyme activity could be better able to tolerate water stress. Liu and Huang (2000) reported similar findings in Bent grass. The high SOD activity has been associated with stress tolerance in plants because it neutralizes the activity of O^-_2 which over produced under stress (Bowler *et al*., 1992). In our study result indicated that the species *A. brevis*, *A. vaviloviana*, *A. abyssinica* and *A. sativa* with lesser decrease in SOD activity at vegetative stage and *A. abyssinica*, *A. brevis* and *A. sativa* at flowering stage sustain a higher active O^-_2 scavenging ability during water stress, therefore such species were indication of more water stress tolerant in comparision to other species in respective stages of growth.

The increased activity of catalase might be due to the enhance super oxide dismutase activity (Casano *et al.,* 1999). The increase in catalase activity might be useful in dismuting/ disproportionating H_2O_2 that is the key product in reducing senescence under extreme moisture stress. In peroxisome the catalase have an essential role in the removal of toxic H_2O_2, which is continuously formed during photorespiration by the dismutation of the superoxide radicals goverated in the NADH dependent electron transport system of the peroxisomal membrane (Corpas *et al.,* 2001). The maintenance of this enzyme at higher level prevents an increase in cytosolic H_2O_2, which can prevents in creating toxic conditions in the plant cell leading to oxidative stress and cell death (Srivulli and Khanna Chopra, 2001; Prochazkova *et al.,* 2001).

Peroxidase activity in *Avena* species under extreme stress environment increased and the percent increase over control varies from species to species. Peroxidase constitutes a class of haeme containing enzymes ubiquitously present in prokaryotic and

eukaryotic organisms. This enzyme catalyzes the dehydrogenation of structurally diverse phenolic and endolic substances by H_2O_2 and thus often regarded as antoxidant enzyme, protecting cells from the destructive influence of H_2O_2 and derived oxygen species (Shigeoka *et al.,* 2002). Plant peroxidases are commonly known for their capability to reduce H_2O_2 to water at the expense of hydrogen donors. In our experiment at vegetative stage drought maximum increase in peroxidase activity was recorded in *A. sativa* followed by *A. brevis* and *A. sterilis*. However the flowering stage drought showed the maximum increase in *A. sativa* followed by *A. marocana* and *A. sterilis.* Many researchers have reported the increase in peroxidase activity under water stress. (Dwivedi *et al.,* 1979; Badiani *et al.,* 1997; Zhang and Kirkham, 1994) indicating the formation of large part of H_2O_2 during water stress. Water stress could increase the accumulation of peroxidase substrate which in turn are scavenger of activated oxygen species (Elstner, 1982; Winston 1990). The accumulation of these matabolism, could lead to an increase in peroxidase activity in the presence of enhanced level of H_2O_2 (Zhang and Kirkham, 1994). The important role of peroxidase in relation to oxidative tolerance has been reported in many species (Gupta *et al.,* 1993; Lee and Lee 2000; O'Kane *et al.,* 1996). The result of the present study indicated that among the *Avena* species tested, *A. sativa* and *A. sterilis* showed stress tolerance at both the stage by accumulating higher percentage of peroxidase followed by *A. brevis* at vegetative *and A. marocana* at flowering stage.

Malondialdehyde (MDA) content was assessed in seven *Avena* species at well watered, extreme stress and re-watering environment both at vegetative and flowering stage. The present study showed the increase in MDA level when plants are subjected to moisture stress. However the rate of increase in lipid peroxidation irrespective of stage but maximum increase was recorded in *A. strigosa* at both the stages. The different rate in increase of MDA content has been reported in two species of Jute resulted the difference in membrane permeability as a result of increased lipid peroxidation (Chowdhury and Choudhuri, 1985). The level of MDA content increased with increase in the magnitude of the stress (Dhindsa and Motowe, 1981). *Avena* species which showed high level of Malondialdehyde (MDA) content, indicates more lipid peroxidation and more membrane permeability and are comparatively more susceptible for water stress than those which produce less Malondialdehyde (MDA) content at higher magnitude of water stress such species have better capability for moisture stress tolerance. Reddy *et al. (*1998). observed rice lines showed less accumulation of Malondialdehyde (MDA) were found superior to other lines in terms of water stress tolerance.

Soluble Protein was estimated in the fresh leaves of *Avena* species grown under well watered controlled, extreme stress (by withholding water) and re-watered environment. There was an increase in accumulation of protein in the leaves of the *Avena* species grown under water stressed environment irrespective of the stage of the crop. However the rate of increase differed in different species. At vegetative stage maximum increased in soluble protein was recorded in *A. abyssinica* followed by *A. sativa* and *A. strigosa* over control. However at flowering stage the maximum increase was recorded in *A. sterilis* followed by *A. strigosa* and *A. vaviloviana* over the plants grown under well watered environment.This increase in total soluble protein under stress over control indicated that these species showed better ability in comparison to other species to maintain the osmotic level as it has been suggested that in general increase in amino aid level is one of the major attributes of water stress to maintain the osmotic level (Good and Zaplachinski, 1994). Reddy *et al.,* 1998 concluded that better performing lines of rice also showed higher level of protein under stress.

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References

Allen, R.D.: Dissection of oxidative stress tolerance using transgenic plants. *Plant Physiol.*, **107**, 1049-1054 (1995).

Badiani, M., A.R. Paolacci, A. Fusari, R. D'Ovidio, E. Scandalios, Porceddu, and G. Giovannozzi-Sermanni: Non optimal growth temperature and antioxidants in the leaves of *Sorghum bicolor* (L) Moench, II. short-term acclimation. *J. Plant Physiol.,* **151**, 409-421 (1997).

Bowler, C.M., Van. Montagu and D. Inze: Superoxide dismutase and stress tolerance. *Annu. Rev. Plant Physiol.,* **43**, 83-116 (1992).

Casano, L.M., M. Martin, J.M. Zapauta and B. Sabater: Leaf age and paraquate concentration dependent effects on the level of enzymes protecting against photo-oxidative stress. *Plant Sci.*, **149**, 13-22 (1999).

Chance, B. and A.C. Machly: Assay of catalase and peroxidase. *Methods of Enzymol.,* **2**, 764-775 (1955).

Chandra, A., R.K. Bhatt and L.P. Misra: Effect of water stress on biochemical and physiological characteristics of oat genotypes. *J. Agric. Crop Sci.,* **181**, 45-48 (1998).

Chowdhury, S.R. and M.A. Choudhuri: Hydrogen peroxide metabolism as an index of water stress tolerance in Jute. *Physiologia Plantarum,* **65**, 503-507 (1985).

Corpas, F.J., J.B. Barrosa and L.A. del Rio: Perosomes as a source of reactive oxygen species and nitric oxide signal molecules in plant cell. *Trends in Plant Sci*., **6**, 145-150 (2001).

Dhindsa, R.S. and W. Motowe: Drought tolerance in two mosses: Correlated with enzymatic defense against lipid peroxidation. *J. Exp. Bot.,* **32**, 79-91 (1981).

Dwivedi, S., M. Kar and D. Mishra: Biochemical changes in excised leaves of *Oryza sativa* subjected to water stress. *Physiol. Plant,* **45**, 35-40 (1979).

Elstner, E.F.: Oxygen activation and oxygen toxicity. *Ann. Rev. Plant Physiol.,* **33**, 73-96 (1982).

Giannopolitis, C.N. and S.K. Ries: Superoxide dismutase: occurrence in higher plants. *Plant Physiol.,* **59**, 309-314 (1977).

Gomez, K.A. and A.A. Gomez: Statistical procedures for agricultural research. J. Willey and Sons, New York (1984).

Good, A.G. and S.T. Zaplachinski: The effect of drought stress on free amino acid accumulation and protein synthesis in *Brassica napus*. *Physiol. Plants*, **90**, 9-14 (1994).

Gupta, S.R. and S. Gupta: Diversity in marvel grass germplasm. Proceedings of the ISPGR dialogue on PGR: *Developing Nation Policy*. New Delhi (1993).

Heath, R.L. and L. Packer: Photoperoxidation in isolated chloroplast. I. kinetics and stoichiometry of fatty acids peroxidation. *Archives of Biochem. Biophy.,* **125**, 189-198 (1968).

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall: Protein measurement with the Folin phenol regent. *J. Biol. Chem.,* **193**, 265-275 (1951).

Lee, D.H. and C.B. Lee: Chilling stress induced changes of antioxidant enzymes in the leaves of cucumber: In gel enzyme activity assay. *Plant Sci.,* **159**, 75-85 (2000).

Liu, X. and B. Huang: Heat stress injury in relation to membrane lipid peroxidation in creeping bent grass. *Crop Sci.,* **40**, 503-510 (2000).

O' Kane, D., V. Gill, P. Boyd and R. Burdon: Chilling oxidative stress and antioxidant responses in *Arabidopsis thaliana* callus. *Planta.,* **198**, 371-377 (1996).

Price, A.H. and G.A.F. Hendry: Iron catalase oxygen radical formation and its possible contribution to drought damaged in nine native grasses three cereals. *Plant Cell and Environ.,* **14**, 477-484 (1991).

Prochakova, D., R.K. Sairam, G.C. Srivastava and D.V. Singh: Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. *Plant Sci.*, **161**, 765-771 (2001).

Quartacci, M.F. and F. Navarri-Izzo: Water stress and free radicals mediated changes in sunflower seedlings. *J. Plant Physiol.*, **139**, 621-625 (1992).

Reddy, C.P., S.N. Vajranabhaian and M. Udaya Kumar: Lipid peroxidation as a mechanism of stress tolerance in upland rice (*Oryza sativa* L.) calli. *Ind. J. Plant Physiol.*, **3**, 68-70 (1998).

Shigeoka, S., T. Ishikawa, M. Tamoi, Y. Miyagawa, T. Takeda, Y. Yabuta and K. Yoshimura: Regulation and function of ascorbate peroxidase isozymes. *J. Exp. Bot.*, **53**, 1305-1319 (2002).

Smirnoff, N.: The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytol.*, **125**, 27-58 (1993).

Srivalli, B. and R. Khanna Chopra: Induction of new isoforms of superoxide dismutase and catalase enzyme in flag leaf of wheat during monocropic scenescence. *Biochem. Biophys. Res. Commun.*, **288**, 1037-1042 (2001).

Winston, G.W.: Physiochemical basis for free radical formation in cell: production and defenses. *In*: Stress responses in plants: Adaptation and Acclimation mechanism (*Eds.*: R.G. Alscher and J.R. Cumming). Wiley-Liss. (1990).

Zhang, J. and M.B. Kirkham: Enzymatic responses of the ascorbate-glutathione cycle to drought in sorghum and sunflower plants. *Plant Sci.*, **113**,139-147 (1996).

Zhang, J., S. Cui, J. Li and J. Wei: Response of cell protective enzymes in corn leaf to water stress at seedling stage. *Acta Agric. Boreali-Sinica (Suppl.)*, **5**, 19-23 (1990).

Zhang, J.X. and M.B. Kirkham: Drought stress induced changes in activities of superoxide dismutase, catalase, and peroxidase in wheat species. *Plant Cell Physiol.,* **35**, 785-791 (1994).