Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; SP1: 694-703

Sudip Das
Department of Biochemistry, Punjab Agricultural University, Ludhiana, Punjab, India

Diganggana Talukdar
Department of Plant Pathology, Assam Agricultural University, Jorhat, Assam, India

Manjeet Kaur Sangha
Department of Biochemistry, Punjab Agricultural University, Ludhiana, Punjab, India

Dharam Paul Chaudhary
Department of Biochemistry, Punjab Agricultural University, Ludhiana, Punjab, India

Nilakshi Borah
Medicinal Aromatic & Economic Plants Group, Biological Sciences & Technology Division, CSIR-North East Institute of Science & Technology, Jorhat, Assam, India

Anamika Das
Department of Botany, Tripura University, Agartala, Tripura, India

Subhendu Das
Department of Pharmacy, Tripura University, Agartala, Tripura, India

Siddhartha Proteem Saikia
Medicinal Aromatic & Economic Plants Group, Biological Sciences & Technology Division, CSIR-North East Institute of Science & Technology, Jorhat, Assam, India

Correspondence
Sudip Das
Department of Biochemistry, Punjab Agricultural University, Ludhiana, Punjab, India

Antioxidant enzymes potential in leaves of oats and barley and phytochemistry of stress tolerance

Sudip Das, Diganggana Talukdar, Manjeet Kaur Sangha, Dharam Paul Chaudhary, Nilakshi Borah, Anamika Das, Subhendu Das and Siddhartha Proteem Saikia

Abstract
Activity of antioxidant enzymes viz. superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and peroxidase (POD) was estimated in leaves of barley and oat crops at 30, 60 and 90 days after sowing (DAS) under normal field condition. The antioxidant enzymes scavenge the reactive oxygen species generated during stress conditions. In barley, the APX activity (mmoles of MDA formed/min/g Fresh Weight of tissue) varied from 1742.53 ±92.00 at 30 DAS to 2160.6±42.25 at 90 DAS with highest value of 2917±49.94 at 60 DAS. Whereas the corresponding values recorded in oats were 473.94±615.28, 1923.72±13.16 and 1343.59±18.97 at 30, 60 and 90 DAS. The mean APX activity of all the three stages of barley was 1.84 times higher than the mean values of oat leaves. POD and CAT in barley leaves recorded mean values (three stages) of 116.16 E/min/g FW and 233.86 mmoles of H_2O_2 decomposed/min/g FW. These values were 1.74 and 1.676 fold higher than the corresponding values in oat leaves. Thus the present results are indicative of the fact that barley, a hardy crop is equipped with higher antioxidant enzymes and are thus better adapted to cope with drought.

Keywords: Antioxidant enzymes, Leaves and grains, Barley, Oats, Stress tolerance

Introduction
Oats is important non-legume winter forages of northern India which is grown extensively under irrigated conditions. The crop is an important because of its excellent growth character, quick re-growth and economic source of dietary energy. The positive physiological effects of oat products have been recognized (Pirjo *et al.*, 2003) [41]. Oats are source of several natural antioxidants which contribute to the stability and the taste of food products (Peterson, 2001) [40]. Stress leads to the production of reactive oxygen species (ROS). ROS are produced by cells enzymatically through the action of various soluble and membrane bound enzymes by auto-oxidation reactions. Under optimal conditions the ROS generated are neutralized by antioxidant defense system of the plant comprising enzymatic antioxidants which include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), glutathione reductase (GR), and glutathione (GSH). Therefore an estimation of antioxidant parameters in plant is an indication of its antioxidant potential as well as stress tolerance ability. Searching for stress tolerance mechanism in oat will be of immense importance and can pave way to generation of cultivars with improved stress tolerance and hence leading to sustained productivity.

Materials and methods
The crops (oat genotype *OL-9* and barley genotype *RD-2552*) were grown under normal field condition in the farm of department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana (30° 55' N latitude, 75° 54' E longitude and an altitude of 262 m above the sea level) and the biochemical estimation was carried out in Biochemistry laboratory, Department of Plant Breeding and Genetics. The antioxidant potential of both the crops were studied during the vegetative growth period of the crops as these crops are generally used as fodder in India. Leaf samples were collected randomly at 30, 60, 90 days after sowing (DAS) and kept at -20^{0}C till further used for biochemical estimations. The antioxidant enzymes viz. Superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POD), Ascorbate peroxidase (APX) and Glutathione reductase (GR) were estimated with the following principles:

Estimation of Superoxide dismutase (SOD) (EC 1.15.1.1)
Superoxide dismutase was estimated following the method as described by Marklund and Marklund (1974) [29].

SOD enzyme was extracted from the leaves (1) with 0.1 M potassium phosphate buffer (pH 7.5) containing 1% polyvinyl pyrrolidone (PVP), 1 mM EDTA and 10 mM β-mercaptoethanol. The extract was passed through a muslin cloth and centrifuged at 10,000g for 10 minutes at 4^0C. Reaction mixture consisted of 1.5 ml of 0.1 M Tris HCl buffer (pH 8.2), 0.5 ml of 6 mM EDTA, 1 ml of 6 mM pyrogallol solution and 0.1 ml of enzyme extract was added. Absorbance was recorded at 420 nm after an interval of 30 seconds up to 3 minutes. A unit of enzyme activity is defined as the amount of enzyme causing 50% inhibition of auto-oxidation of pyrogallol observed in blank.

Estimation of Catalase (CAT) (EC 1.11.1.6)

Catalase (CAT) was estimated by following the method developed by Chance and Machly (1955). Fresh leaves (1g) was taken to extract the enzyme with 50 mM sodium phosphate buffer (pH 7.5) containing 1% PVP. To 1.8 ml of 50 mM sodium phosphate buffer (pH 7.5) added 0.2 ml of enzyme extract. The reaction was initiated by adding 1 ml H_2O_2 solution and decomposition of H_2O_2 was recorded at intervals of 30 seconds for 3 minutes by measuring the decrease in absorbance at 240 nm. Catalase activity was expressed as micromoles of H_2O_2 decomposed/min/g FW of tissue. The extinction coefficient of H_2O_2 is 43.6 mM^{-1} cm^{-1}.

Estimation of Peroxidase (POD) (EC 1.11.1.7)

Extraction procedure for peroxidase (POD) was adapted as described by Shannon *et al.* (1966) [44]. The reaction mixture contained 2.8 ml of 0.05 M guaiacol in 0.1 M phosphate buffer (pH 6.5), 0.1 ml of enzyme extracts and 0.1 ml of 0.8 M H_2O_2. The reaction mixture without H_2O_2 was measured as a blank. The reaction was initiated by adding H_2O_2 and rate of change in absorbance was recorded at 470 nm for 3 minutes at an interval of 30 seconds. Peroxidase activity has been defined as change in absorbance/min/g FW of tissue.

Estimation of Ascorbate peroxidase (APX) (EC 1.11.1.1)

Ascorbate peroxidase (APX) was estimated following the method of Nakano and Asada (1987) [34]. The reaction mixture consisted of 1 ml of 50 mM sodium phosphate buffer (pH 7.0), 0.8 ml of 0.5 mM ascorbate, 0.2 ml of enzyme extract and 1 ml of H_2O_2 solution in total volume of 3 ml. The enzyme activity was measured as decrease of absorbance at 290 nm Ascorbate peroxidase was expressed as nmoles of MDA formed/min/g FW of tissue by using extinction coefficient of MDA as 2.8 mM^{-1} cm^{-1}.

Estimation of Glutathione reductase (GR) (EC 1.6.4.2)

The extraction procedure for Glutathione reductase was adapted from the method followed by Sgherri *et al.* (1994) [44]. The reaction was started by adding 0.2 ml of 0.2 M potassium phosphate buffer (pH 7.5), 0.1 ml of 0.2 mM EDTA, 0.1 ml of 1.5 mM $MgCl_2$, 0.2 ml of 0.5 mM NADPH and 0.2 ml of enzyme extract with 0.2 ml of 2 mM glutathione in a quartz cuvette. The enzyme activity was estimated as decrease in absorbance at 340 nm after an interval of 30 seconds up to 3 minutes. Glutathione reductase activity was expressed as nmoles of NADPH oxidized/min/g fresh weight (FW) of tissue by using extinction coefficient of NADPH as 6.2 mM^{-1} cm^{-1}.

Estimation of Ascorbic acid (Vit C)

Ascorbic acid (Vit.C) was estimated using the method of Law *et al (*1983) [27]. 200 mg tissue (grains) was homogenized in 1.5 ml of 5% meta-phosphoric acid and centrifuged at 22,000g for 10 minutes. Supernatant was taken for the estimation of ascorbic acid. To 0.4 ml of supernatant, added 0.4 ml of 5 mM EDTA, 0.4 ml of 16 mM $FeCl_3$ prepared in 0.1 M potassium phosphate buffer (pH 7.5), 0.8 ml of 7.6% O-phosphoric acid and 0.8 ml of 44 mM bipyridyl. After 40 mins of incubation at 40^0C the absorbance was measured at 525 nm. Ascorbic acid concentration was expressed as nmoles/g FW of tissue (standard 0-40 nmoles of ascorbic acid).

Estimation of Hydrogen peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) estimation was done by using the method of Sinha (1971) [46]. 500 mg tissue (grains) was macerated in 3 ml of ice cold 10 mM potassium phosphate buffer (pH 7.0). The homogenate was centrifuged at 10,000g for 20 minutes. The supernatant was collected and used for estimating H_2O_2 content. Supernatant was approximately diluted to 2 ml with 10 mM potassium phosphate buffer (7.0). 2 ml of 5% potassium dichromate and glacial acetic acid (1:3 v/v) was added to the reaction mixture. The OD was read at 570 nm against the reagent blank without sample extract. H_2O_2 content was expressed as µmoles/g FW of tissue (standard 40-200 micromoles of hydrogen peroxide).

Estimation of Malondialdehyde (MDA)

Estimation of malondialdehyde was done using the method of Heath and Packer (1968) [22]. 200 mg tissue (grains) was homogenized in 2 ml of 5% (w/v) trichloroacetic acid (TCA) and the homogenate was centrifuged at 10000xg for 15 min at room temperature. Supernatant was mixed with an equal volume of 20% (w/v) TCA containing 0.5% thiobarbituric acid (TBA). The mixture was heated at 95°C for 30 min, cooled in ice and centrifuged at 10000xg for 10 mins. Absorbance of the supernatant was measured at 532 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. MDA content was calculated using an extinction coefficient of 155 $mM^{-1}cm^{-1}$. The results were expressed as nmol MDA g^{-1} FW.

Statistical analysis

The data recorded during the course of studies was statistically analyzed by analysis of variance (ANOVA) using the completely randomized design (CRD). The least significant difference (LSD) at the 5% (p=0.05) level, calculated from the standard error of the difference (SED) between means to make comparison relevant means.

Result and discussion

Superoxide dismutase

Under unstressed conditions, reactive oxygen species generated in plant cells are detoxified by the antioxidant defense system of the plants. The sources of ROS in plant leaves are chloroplast, mitochondria, endoplasmic reticulum (ER), peroxisomes, plasma membrane and cell wall (Mittler, 2002) [30]. The first ROS generated mostly is superoxide ion, which is dismutated by SOD enzyme to hydrogen peroxide (H_2O_2) (Bowler *et al.*, 1992; Alscher *et al.*, 2002) [7, 2]. In our studies, barley crop showed higher inherent SOD activity under normal field conditions (Table 1, Fig. 1). The SOD activity recorded in leaves of barley crop was 21.33±1.3, 28.06±2.7 and 15.83±3.5 (units/min/g FW) at 30, 60 and 90 DAS respectively. At 60 DAS, leaves exhibited 31.5% higher activity as compared to the activity at 30 DAS but thereafter at 90 DAS the activity went down by 1.7 fold in comparison

to 60 DAS activity. Ehrenbergerova *et al.* (2009) [14] also reported higher SOD activity in young barley as compared to mature barley crop. In oats, the calculated mean SOD values were 13.13±1.8, 19.42±1.8 and 10.79±1.8 units/min/g FW after 30, 60 and 90 DAS respectively. The activity of SOD at all the three intervals of vegetative growth in barley was significantly (CD 5%) higher than respective activities in oat leaves (Table 1, Fig.1). Higher SOD activity might be responsible for better adaptability of barley to environmental variations (Baek *et al.,* 2000) [4]. Demiral and Turkan (2005) [12] reported increased SOD activity in drought-tolerant cultivars of maize. Turkan *et al.* (2005) [48] also reported increased SOD activity in drought-tolerant common bean.

Catalase (CAT)

Catalase represents one of the primary enzymatic mechanisms employed by aerobic organisms to decompose hydrogen peroxide (H_2O_2) generated by SOD, a toxic intermediate of oxygen metabolism. Catalase is abundant in the peroxisomes of green leaves (Corpas *et al.,* 2001) [10]. Data on CAT activity in leaves at different stages of vegetative growth is shown in table 2, Fig. 2. At 30, 60 and 90 DAS, the activity recorded in barley leaves was 194.66±9.7, 299.24±10.5 and 207.69±6.0 (µmoles of H_2O_2 decomposed/min/g FW) respectively. CAT activity differed significantly at all the three stages of vegetative growth at CD 5%.The respective values recorded in oat leaves were127.33±6.9, 204.44±17.1 and 64.85±4.6 (µmoles of H_2O_2 decomposed/min/g FW) which varied significantly (CD 5%) from each other. The level of catalase activity was highest at 60 DAS stage in both barley and oat leaves. Baek *et al.* (2000) [4] reported the presence of substantial amounts of catalase, peroxidase and SODs in overwintering barley healthy leaves under normal field conditions, suggesting the involvement of these enzymes in the tolerance mechanism to the various stresses during winter. Enhanced catalase activity is related with increase in stress tolerance (Foyer *et al.,* 1997; Upadhaya *et al.,* 1990; Olmos *et al.,* 1994; Kraus *et al* 1995) [15, 49, 35, 25]. According to Casano *et al.* (1999) [8] the increased activity of catalase might be due to the enhanced super oxide dismutase activity. In our study also, in both the crops, higher catalase activity is preceeded by higher SOD activity. The increase in catalase activity is useful in dismutating/ disproportionating H_2O_2 that is the key product in reducing senescence under stress. The maintenance of this enzyme at higher level prevents increase in cytosolic H_2O_2, which can prevent creating toxic conditions in the plant cell leading to oxidative stress and cell death (Srivalli and Khanna-Chopra, 2001; Prochazkova *et al.,* 2001) [47, 41]. In the present study, barley leaves exhibited significantly (at CD 5%) higher CAT activity at all the three stages of vegetative growth as compared to oat leaves (Table 2, Fig.2) under normal field conditions. Many studies also reported decline in CAT activity as a general response to many stresses (Herbinger *et al.,* 2002; Bakalova *et al.,* 2004; Jung 2004; Guo *et al.,* 2006; Pan *et al.,* 2006; Liu *et al.,* 2008) [20, 5, 23, 19, 36, 27].

Peroxidase (POD)

Peroxidase is generally involved in plant cell growth by promoting cell wall rigidity through lignin synthesis and cross-linking of polysaccharide components (Fry, 1986; Asada, 1992) [17, 3]. The activity of POD varies considerably depending upon plant species and stress conditions (Gill and Tuteja 2010) [18]. Barley leaves recorded peroxidase value of 95.8±6.9 ΔE/min/g FW at 30 DAS which increased to 146.02±10.0 ΔE/min/g FW at 60 DAS and then declined to 106.65±4.1 at 90 DAS (Table 3, Fig 3). In oats, the POD activity was found to be significantly lower (CD 5%) than barley and recorded a value of 41.48±3.0, 61.36±4.1 and 31.87±2.6 ΔE/min/g FW respectively at 30, 60 and 90 DAS. Data in Table 3, Fig. 3 indicated that maximum POD activity was at 60 DAS in case of both oat and barley leaves. Acar *et al.* (2001) [1] reported higher inherent activities of POD and SOD in seedling leaves of drought tolerant barley variety as compared to sensitive variety. Drought tolerant plants often have higher POD activity than sensitive plants under stress conditions and this is true for drought-tolerant common bean (Turkan *et al.,* 2005) [48] and sorghum (Zhang and Kirkham, 1996) [50]. Higher POD activity has been linked with protection from oxidative damage, lignifications and cross-linking of cell wall to prevent plants from biotic and abiotic stresses (Dalal and Khanna-Chopra, 2001) [11]. Increase in POD activity in both the leaf and root tissues of *V. radiate* (Panda, 2001) and *O.sativa* (Koji *et al.,* 2009) [24] has been reported under salinity stress.

Ascorbate peroxidase (APX)

Ascorbate peroxidase, a component of the ascorbate-glutathione pathway and present mainly in chloroplast, plays a key role in scavenging H_2O_2 (Foyer and Noctor, 2005; Mittler, 2002) [31, 30]. It uses ascorbate as substrate for the reduction of H_2O_2. APX is thought to play the most essential role in scavenging ROS and protecting cells in higher plants. Ascorbate peroxidase (APX) activity was found to be significantly higher in the leaves of barley as compared to oats at all the vegetative stages undertaken for study. In barley, the values of APX activity (nmoles of MDA formed/min/g FW of tissue) recorded at 30, 60 and 90 DAS were 1742.53±92.0, 2917±49.9 and 2160.6±42.2 respectively. In oats also, the minimum APX activity of 1186.26±48.3 was recorded at 30 DAS, which rose to 1923.00± 13.1 at 60 DAS and then.again declined to 1343.59±18.5 at 90 DAS (Table.4, Fig. 4). In barley leaves the APX activity at 30 and 90 DAS was at par while 60 DAS registered the highest activity.

Glutathione reductase (GR)

GR activity was significantly different at all the three vegetative stages in barley and oat leaves. However barley leaves registered higher GR activity as compared to oat leaves which is indicative of better antioxidant potential of barley crop as compared to oat crop and might provide higher ability to combat various stresses. In barley crop 30 day old leaves had 931.8±11.3 nmoles of NADPH oxidized/min/g FW of GR activity. The activity rose to 1259.43±17.8 at 60 DAS and declined to 766.96±21.9 at 90 DAS. In oat leaves GR activity at 30, 60 and 90 DAS registered the value of 410.5±17.3, 847.04±10.5 and 613.24±6.4 respectively (Table 5, Fig. 5).

Ascorbic Acid (Vit-C)

Ascorbic acid acts as a substrate for APX in scavenging H_2O_2 into water. A recent investigation revealed that ascorbate content regulates plant defense gene expression and modulates plant growth and development via phytohormone signaling (Pastori *et al* 2003, Smirnoff 2011) [38, 46]. Ascorbic acid is found in millimolar concentration in leaves and plays an important role in plant tolerance to stresses as a component of the antioxidant system (Noctor and Foyer 1998) [34]. Reduced Ascorbic acid (Vit-C) content was found to be higher in the leaves of barley as compared to oats. In barley, the Vit-C content (nmoles/g FW) varied significantly at all the

three vegetative stages of the crop. The content recorded at 30, 60 and 90 DAS was 1814.8±46.1, 2747.9±53.8 and 2013.89±124.0 respectively (Table 6, Fig. 6), while in oats, the corresponding recorded values were 840.06±28.0, 1579.06±63.9 and 642.3±42.4 respectively. The content in barley leaves was significantly higher (CD 5%) than that in oat leaves.

Hydrogen peroxide (H_2O_2)

H_2O_2 is the product of SOD activity, which is toxic to cells as it causes oxidative stress and must be eliminated by conversion to H_2O in reactions involving APX, POD, and CAT. Therefore, it is important that H_2O_2 be scavenged rapidly by the anti-oxidative defence system to water and oxygen (Guo *et al* 2006) [19]. The over expression of SOD, accompanied by enhanced H_2O_2 scavenging mechanisms like CAT and POD enzyme activities, has been considered as an important anti-drought mechanism to cope with oxidative stress during water deficit conditions (McKersie *et al* 1999) [29]. Hydrogen peroxide (H_2O_2) content was found to be lower in the leaves of barley as compared to oats. In barley leaves, the value of the mean H_2O_2 content (µmoles/g FW of tissue) was 15.86±1.4, 10.11±0.8 and 12.58±0.7 at 30, 60 and 90 DAS respectively. While in oats, the calculated mean values were 25.45±3.6, 16.56±1.0 and 19.54±2.3 after 30, 60 and 90 DAS respectively (Table 7, Fig. 7).

In the present study, the low content of H_2O_2 observed in barley crops and grains as compared to oat crops and grains could be due to higher activities of various H_2O_2 scavenging enzymes in barley as already observed. Hung *et al* (2005) [22] advocated that H_2O_2 seems to serve as a common stress signal in plants which activates transcription factor associated with SOD, APX and catalase. Such findings are indicative of H_2O_2 mediated antioxidant protection against oxidative stress. Devi *et al* (2008) [13] showed that exogenous application of H_2O_2 activates CAT, APX and GR but does not activate SOD in wheat seedlings under salt stress. Moussa and Abdel-Aziz (2008) [32] studied the comparative response of drought tolerant and drought sensitive genotype to water stress. They found lower values of MDA and H_2O_2 in tolerant genotypes indicating that at cellular level this genotype is better equipped with efficient free radical quenching system that offer protection against oxidative stress. Higher levels of SOD, CAT and POD were also observed in the tolerant genotype. H_2O_2 play dual role in plants: at low concentrations, it acts as a signal molecule involved in acclamatory signaling triggering tolerance to various biotic and abiotic stresses and at high concentrations it leads to PCD (Quan *et al* 2008) [42]. H_2O_2 is starting to be accepted as a second messenger for signals generated by means of ROS because of its relatively long life and high permeability across membranes.

Malondialdehyde (MDA)

Lipid peroxidation is considered to be highly damaging process which occurs when above threshold ROS levels are generated. It also aggravates oxidative stress through function of lipid-derived radicals (Montillet *et al* 2005) [31]. It is taken as an indicator of tissue destruction in terms of MDA under various stresses. Malondialdehyde (MDA) content was found to be higher in the leaves of oats as compared to barley. In barley crop, the values of MDA content (nmol MDA g^{-1} FW) recorded were 1.76±0.23, 4.66±1.35 and 8.41±0.77 at 30, 60 and 90 DAS respectively. While in oats, the calculated mean MDA values were 4.17±0.66, 8.48±0.57 and 10.59±0.40 at 30, 60 and 90 DAS respectively (Table 8, Fig. 8). The barley crop registered significantly lower MDA content than oat crop under unstressed conditions. According to Pandey *et al* (2010) [37] *Avena* species which had high level of MDA content, had 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. Lower levels of lipid peroxidation in barley can be associated with higher APX activity (Demiral and Turkan 2005) [12]. In our study also barley genotypes having lower lipid peroxidation are equipped with higher antioxidant enzyme activities i. e. SOD, CAT, POD, APX and GR. Wang *et al* (2010) produced transgenic poplar for SOD, and found that the activity of this enzyme increased in transgenic plants and MDA content was significantly decreased when exposed to NaCl stress. The study thus highlights that barley crop and grains are equipped with better antioxidant potential status as compared to oat crops proving adaptability of barley to environmental variations. The data in the present study could be useful for genotype selection for higher activity of the antioxidant enzymes which could be useful to the breeders in their barley and oat improvement programmes.

Conclusion

The activities of all the antioxidant enzymes registered maximum value at 60 DAS in barley as well as oat crop leaves. The values in barley leaves were, SOD (20.06±2.70 units/min/g FW), CAT (299.24±10.58 µmoles of H_2O_2 decomposed/min/g FW), APX (2917±49.9 µmoles of MDA formed/min/g FW), GR (1259.43±17.86 nmoles of NADP formed/min/g FW) and POD (146.02±10.01 ΔE/min/g FW). The corresponding values found in oat leaves were, SOD (19.42±1.8 units/min/g FW), CAT (204.44±17.15 µmoles of H_2O_2 decomposed/min/g FW), APX (1923±13.16 µmoles of MDA formed/min/g FW), GR (847.04±10.56 nmoles of NADP formed/min/g FW) and POD (61.36±4.15ΔE/min/g FW). The content of Vit.C in the leaves of barley and oat crops at 60 DAS was 2747.9±53.87 and 1579.06±63.92 nmoles/g FW respectively. The activities of all antioxidant enzymes and Vit.C estimated were significantly (CD 5%) higher in barley leaves as compared to oat leaves at all the three vegetative stages viz. 30, 60 and 90 DAS.

The oxidative stress parameter H_2O_2 recorded was minimum at 60 DAS in both barley and oat leaves i.e. 10.11±0.85 and 16.56±1.05 µmoles/g FW respectively and was significantly (CD 5%) lower in barley leaves than in oat leaves. The content of MDA was lowest at 30 DAS in both the crops viz. barley leaves (1.76±0.23 nmoles/g FW) and in oat leaves (4.17±0.66 nmoles/g FW). Like H_2O_2 the level of MDA was significantly lower in barley leaves as compared to oat leaves.

Comparison between two crops showed that barley leaves contains higher antioxidant enzyme activities as compared to oat. This study depicts that barley crop inherently have higher antioxidant potential as compared to oat, suggesting better survival of barley crop under stressful conditions.

Table 1: Superoxide dismutase (SOD) activity in leaves of barley and oat crops at 30, 60 and 90 days after sowing under normal field conditions

DAS (Days after sowing)	SOD activity (*units/min/g FW)	
	Barley (RD-2552)	Oat (OL-9)
30	21.33±1.3	13.13±1.8
60	28.06±2.7	19.42±1.8
90	15.83±3.5	10.79±1.8
CD (5%)	5.41	3.76
RD-2552×OL-9	4.15	

Values are mean ± SD of three replicates

*One unit corresponds to amount of enzyme required for 50% inhibition of autooxidation of pyrogallol.

Table 2: Catalase (CAT) activity in leaves of barley and oat crops at 30, 60 and 90 days after sowing under normal field conditions

DAS (Days after sowing)	CAT activity (µmoles of H_2O_2 decomposed/min/g FW)	
	Barley (RD-2552)	Oat (OL-9)
30	194.66±9.7	127.33±6.9
60	299.24±10.5	204.44±17.1
90	207.69±6.0	64.85±4.6
CD (5%)	18.04	21.99
RD-2552×OL-9	17.91	

Values are mean ± SD of three replicates

Table 3: Peroxidase (POD) activity in leaves of barley and oat crops at 30, 60 and 90 days after sowing under normal field conditions

DAS (Days after sowing)	POD Activity (ΔE/min/g FW)	
	Barley (RD-2552)	Oat (OL-9)
30	95.8±6.9	41.48±3.0
60	146.02±10.0	61.36±4.1
90	106.65±4.1	31.87±2.6
CD (5%)	14.87	6.67
RD-2552×OL-9	10.26	

Values are mean ± SD of three replicates

Table 4: Ascorbate peroxidase (APX) activity in leaves of barley and oat crops at 30, 60 and 90 days after sowing under normal field conditions

DAS (Days after sowing)	APX Activity (nmoles of MDA formed/min/g FW)	
	Barley (RD-2552)	Oat (OL-9)
30	1742.5±92.0	1186.26±48.3
60	2917±49.9	1923.00±13.1
90	2160.6±42.2	1343.59±18.5
CD (5%)	130.23	10.38
RD-2552×OL-9	454.76	

Values are mean ± SD of three replicates

Table 5: Glutathione reductase (GR) activity in leaves of barley and oat crops at 30, 60 and 90 days after sowing under normal field conditions

DAS (Days after sowing)	GR Activity (nmoles of NADPH oxidized/min/g FW)	
	Barley (RD-2552)	Oat (OL-9)
30	931.8±11.3	410.5±17.3
60	1259.43±17.8	847.04±10.5
90	766.96±21.9	13.24±6.4
CD (5%)	35.13	24.62
RD-2552×OL-9	27.01	

Values are mean ± SD of three replicates

Table 6: Ascorbic acid (Vit-C) content in leaves of barley and oat crops at 30, 60 and 90 days after sowing under normal field conditions

DAS (Days after sowing)	Vit-C content (nmoles/g FW)	
	Barley (RD-2552)	Oat (OL-9)
30	1814.8±46.1	840.06±28.0
60	2747.9±53.8	1579.06±63.9
90	2013.89±124.0	642.3±42.4
CD (5%)	186.94	94.27
RD-2552×OL-9	131.83	

Values are mean ± SD of three replicates

Table 7: Hydrogen peroxide (H_2O_2) content in leaves of barley and oat crops at 30, 60 and 90 days after sowing under normal field conditions

DAS (Days after sowing)	H_2O_2 content (µmoles/g FW)	
	Barley (RD-2552)	Oat (OL-9)
30	15.86±1.4	25.45±3.6
60	10.11±0.8	16.56±1.0
90	12.58±0.7	19.54±2.3
CD (5%)	2.50	4.01
RD-2552×OL-9	2.98	

Values are mean ± SD of three replicates

Table 8: Malondialdehyde (MDA) content in leaves of barley and oat crops at 30, 60 and 90 days after sowing under normal field conditions

DAS (Days after sowing)	MDA content (nmoles/g FW)	
	Barley (RD-2552)	Oat (OL-9)
30	1.76±0.2	4.17±0.66
60	4.66±1.3	8.48±0.57
90	8.41±0.7	10.59±0.40
CD (5%)	1.82	1.11
RD-2552×OL-9, 1.34		

Values are mean ± SD of three replicates

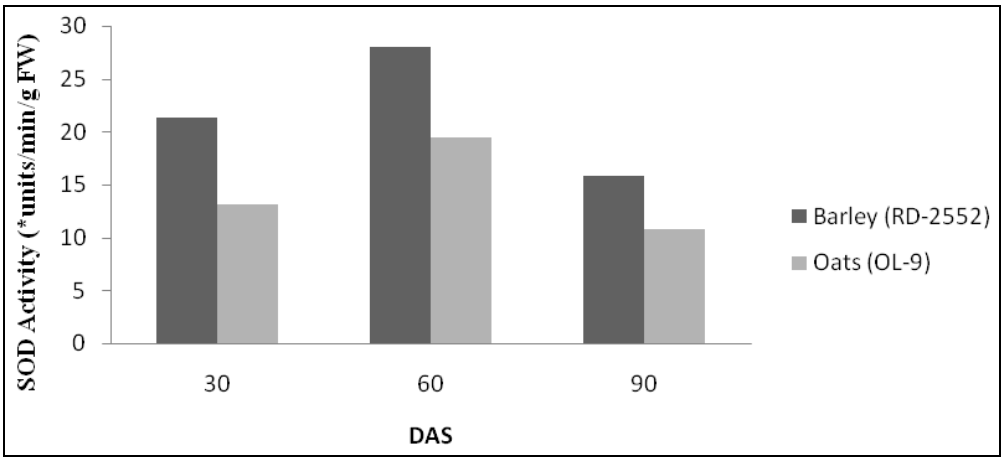

Fig 1: Superoxide dismutase (SOD) activity in leaves of barley and oat crops at 30, 60 and 90 days after sowing (DAS) under normal field conditions

*One unit corresponds to amount of enzyme required for 50% inhibition of autooxidation of pyrogallol.

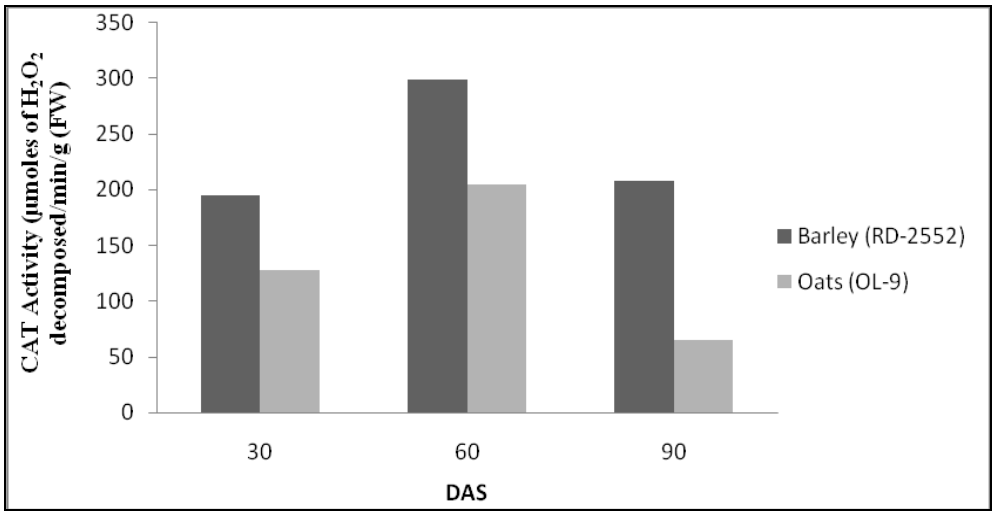

Fig 2: Catalase (CAT) activity in leaves of barley and oat crops at 30, 60 and 90 days after sowing under normal field conditions

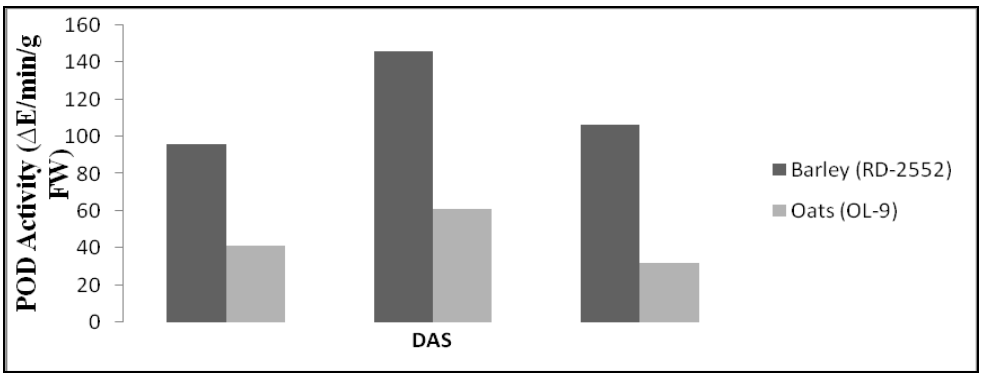

Fig 3: Peroxidase (POD) activity in leaves of barley and oat crops at 30, 60 and 90 days after sowing under normal field conditions

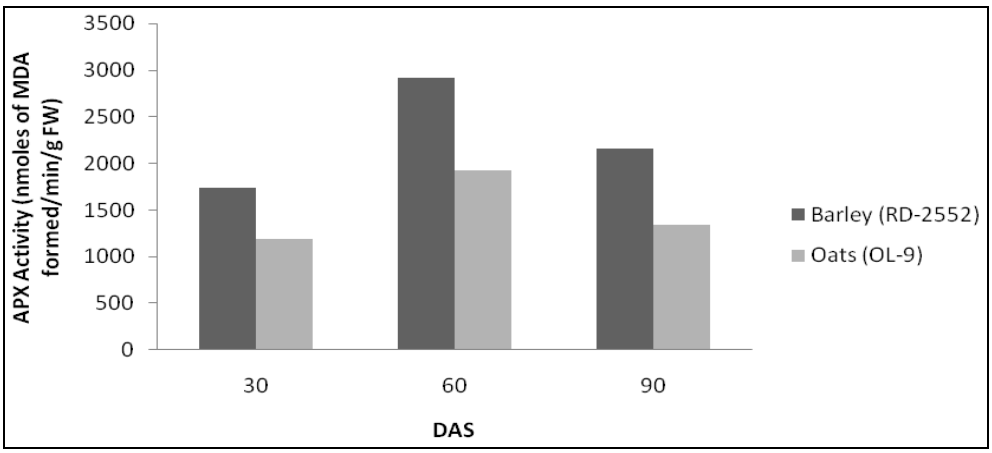

Fig 4: Ascorbate peroxidase (APX) in leaves of barley and oat crops at 30, 60 and 90 days after sowing under normal field conditions

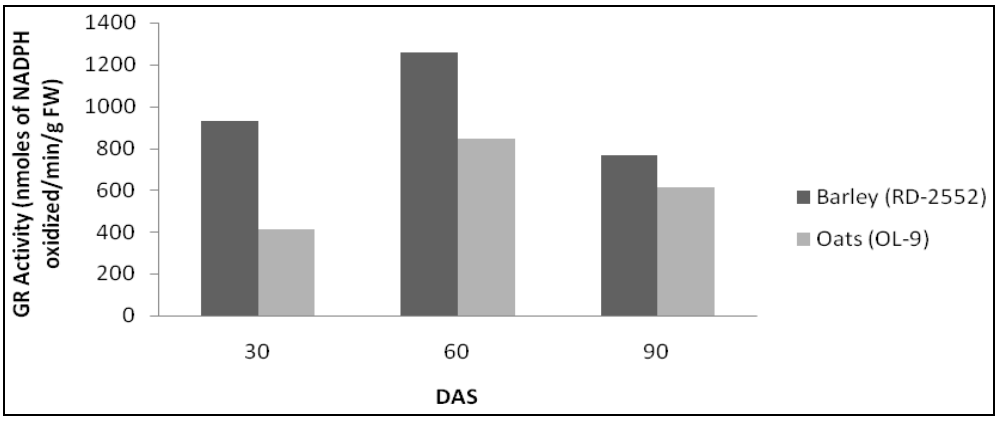

Fig 5: Glutathione reductase (GR) activity in leaves of barley and oat crops at 30, 60 and 90 days after sowing under normal field conditions

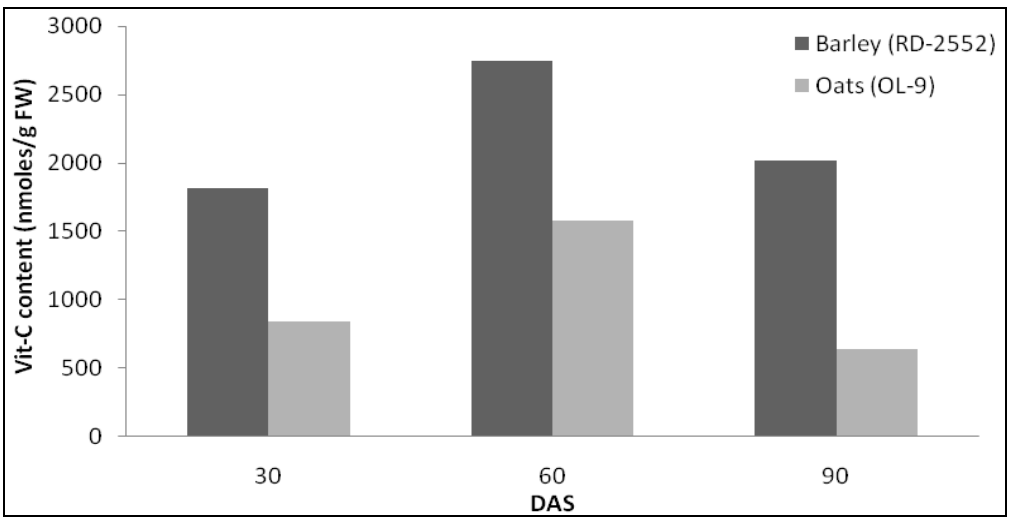

Fig 6: Ascorbic acid (Vit-C) content in leaves of barley and oat crops at 30, 60 and 90 days after sowing under normal field conditions.

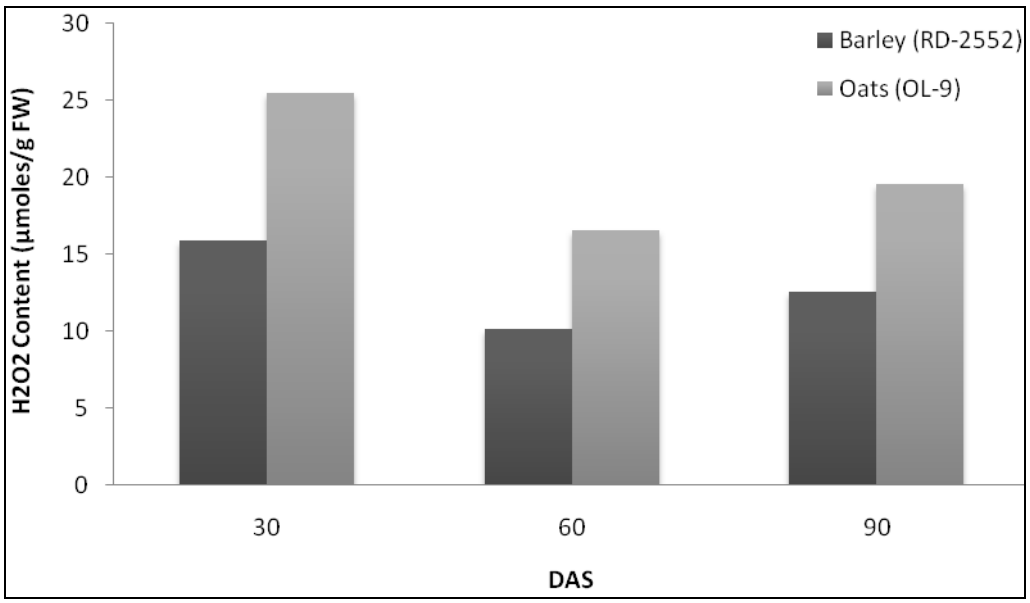

Fig 7: Hydrogen peroxide (H_2O_2) content in leaves of barley and oat crops at 30, 60 and 90 days after sowing under normal field conditions

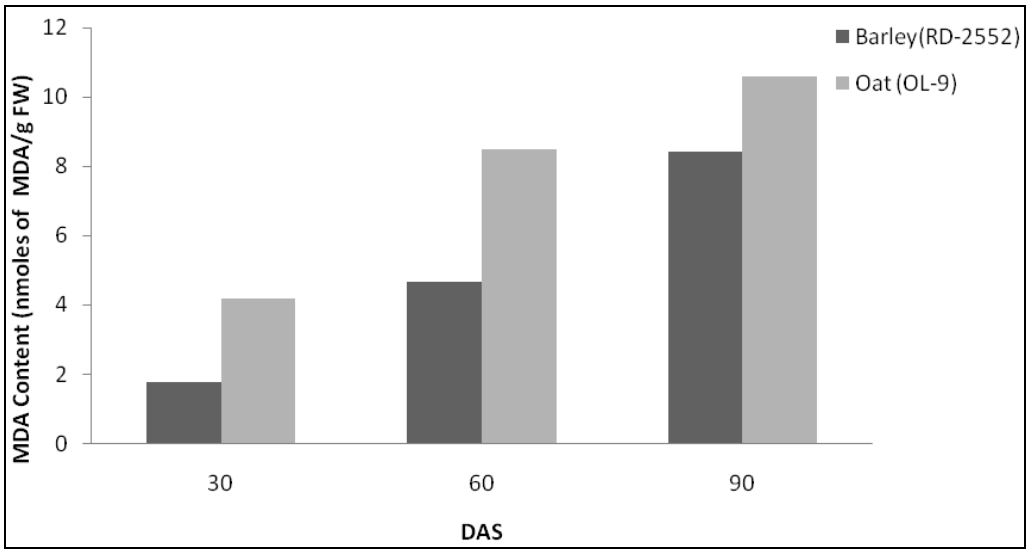

Fig 8: Malondialdehyde (MDA) content in leaves of barley and oat crops at 30, 60 and 90 days after sowing under normal field condition

Acknowledgements
The authors are grateful to the Directorate of Research, Punjab Agricultural University, Ludhiana, India for permission to publish the work. The authors express their deepest sense and gratitude to Directorate of Wheat Research, Karnal, Harayana for providing plant genotypes.

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