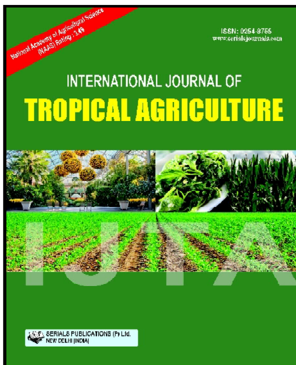

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Activities of antioxidant enzymes and phytochemistry of stress tolerance in grains of oats and barley

Sudip Das[1], Nilakshi Borah[2], Manjeet Kaur Sangha[1], Anamika Das[3] and Siddhartha Proteem Saikia[2*]

[1] *Department of Biochemistry, Punjab Agricultural University, Ludhiana, Pumjab, India*
[2] *Medicinal Aromatic & Economic Plants Division, CSIR-North East Institute of Science & Technology, Jorhat, Assam, India*
[3] *Department of Botany, Tripura University, Agartala, Tripura, India*
* *Corresponding author E-mail: spsaikia@gmail.com*

Abstract: Oat and barley are two important non-legume winter forages of northern India. Barley is hardier in respect to water deficient situation as compare to oats. The genotype of oat and barley grain was analyzed to estimate the antioxidant enzyme potential by following the standard principles and methods. In order to understand the difference between the hardiness of oat and barley crop, comparison of antioxidant potential between this two crop, genotypes (oat genotypes: OL-9, OS-6, OS-7, HJ-8 and HFO-114; and barley genotypes: RD-2552, PL-172, PL-807, PL-426, DWRUB-52 and VJM-201) were studied. Activity of antioxidant enzymes viz. superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and peroxidase (POD) was estimated in grains of barley and oat genotypes under normal field condition. Among different barley grains, PL-172, RD-2552, PL-807 and DWRUB-52 had higher and VJM-201 and PL-426 lower antioxidant enzyme activities. There seemed to be no association between two rowed and six rowed barley genotypes and antioxidant enzyme activities. In oats, OL-9, OS-6 and OS-7 grains had high antioxidant enzyme activities whereas HJ-8 and HFO-114 grains registered lower values.

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

INTRODUCTION

Oats and barley are two important crops in India which are grown extensively because of its excellent growth character and economic source of dietary energy and are largely used in cattle breeding, have occurred in human diet for a long time. The positive

physiological effects of oat products are recognized (Pirjo *et al* 2003). It is a rich source of soluble fibre, well-balanced proteins, several vitamins and minerals essential for the human health (Demirbas 2005). It also contain relatively high amount of lipids compared with other cereal grains, with a substantial level of essential linoleic acid (Hareland and Manthey 2003), important component of diet for hyper-cholesterolemic patients (Czerwinski *et al* 2004). Oats are source of several natural antioxidants which contribute to the stability and the taste of food products (Peterson 2001). On the other hand, barley (*Hordeum vulgare* L.) is one of the first domesticated cereals of the world. It is a temperate as well as tropical crop and widely adaptable to various climatic conditions. It is staple food diet of many countries and is largely required for making breads and beer. Animal food and livestock is the primary use of barley crop. Malt is another important use of this crop. It is nutritious cereal grain that supplies many bioactive compounds, dietary fibre, antioxidants, vitamins, minerals (calcium, magnesium potassium, phosphorous), sphingolipids and unsaturated fatty acids. In many studies, eating whole grains has been linked to protection against atherosclerosis, ischemic stroke, diabetes, insulin resistance, obesity and cancer (Behall *et al* 2004). In semi arid zones of India, farmers cultivate barley as a dual purpose crop. During the scarcity period farmers harvest it for fodder and then leave the crop for grain purposes.

MATERIALS AND METHODS

The crops (oat and barley genotypes) 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 for the antioxidant enzymes viz. Superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POD), Ascorbate peroxidase (APX), Glutathione reductase (GR) and non-antioxidant enzymes viz. Ascorbic acid (Vit-C), Hydrogen Peroxide (H_2O_2) and Malondialdehyde (MDA) using 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). 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 40C. 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). 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). 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). 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). 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 FeCl3 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 400C 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). 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). 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.

RESULTS AND DISCUSSION

Superoxide dismutase (SOD)

SOD activity was estimated in grains of different barley genotypes viz. RD-2552, PL-172, PL-807, DWRUB52, PL-426 and VJM-201. The highest activity of SOD was recorded in PL-172 genotype (40.71±3.5 units/min/g FW) and lowest in VJM-201 (23.12±3.1 units/min/g FW) (Table 1). The genotypes PL-172, RD-2552, PL-807 and DWRUB-52 were at par with respect to SOD activity and they varied significantly (CD 5%) from the other two genotypes of barley i.e. PL-426 and VJM-201. Among the barley genotype DWRUB-52 and VJM-201 are two rowed and RD-2552, PL-172, PL-807, PL-426 are six rowed. There seemed to be no association between SOD activity and different barley types i.e. two rowed and six rowed. All the barley genotypes are recommended for sowing under irrigated conditions, but they harboured differential SOD activity, indicating that there was variation in their antioxidant potential. Turkan *et al* (2005) also reported increased SOD activity in drought-tolerant common bean. Ehrenbergerova *et al* (2009) reported significant varietal variability in SOD activity in barley green biomass. In their study variety *Sebastian* had significantly higher SOD activity compared to the variety *Malz* and line *KM1910*. However, the variety *Malz* and line *KM1910* did not show any significant difference. They also reported that samplings, locations, years and their interactions significantly affected the variability of SOD activity. The varietal differences in SOD activity and other enzymes under stress conditions were also detected in barley leaves (Wu *et al* 2003) and in sweet potatoes (Wang *et al* 1999). Similarly oat genotypes varied in their SOD activity (Table 1). OL-9 harboured highest SOD activity (20.56±2.9) and HJ-8 registered the lowest (7.06±1.4). Like barley genotypes, none of the oat genotypes taken is recommended for sowing under rainfed conditions. All oat genotype grains were significantly (CD 5%) different with respect to SOD activity except for the genotypes HJ-8, OS-7 and HFO-114 which are at par for the enzyme activity. Pandey *et al* (2010) also reported variation in SOD activity in seven different *Avena* species in relation to drought stress and found lower SOD activity in drought tolerant species than in drought susceptible ones.

Table 1
Superoxide dismutase (SOD) activity in grains of different barley and oats genotypes

Barley		*Oats*	
Genotypes	*SOD Activity (*units/min/g FW)*	*Genotypes*	*SOD Activity (*units/min/g FW)*
PL-172	40.71±3.5	OL-9	20.56±2.9
RD-2552	40.24±1.3	OS-6	18.12±2.2
DWRUB-52	39.44±3.5	OS-7	10.74±2.7
PL-807	38.05±2.8	HFO-114	10.19±1.0
PL-426	26.55±2.7	HJ-8	7.06±1.4
VJM-201	23.12±3.1		
CD (5%)	5.26	CD (5%)	4.01

Values are mean ± SD of three replicates
*One unit corresponds to amount of enzyme required for 50% inhibition of auto oxidation of pyrogallol.

Catalase (CAT)

Data on CAT activity in grains of barley genotype displayed in Table 2, showed the comparative catalase

activity in grains of six genotypes of barley. The activity was found to be highest in grains of PL-172 and the lowest in PL-426. The catalase activities in different grains of barley genotypes viz. PL-172, RD-2552, PL-807, DWRUB52, PL-426 and VJM-201 exhibited values of 235.15±3.9, 230.81±3.9, 228.43±2.7, 227.58±7.8, 200.08±9.2 and 185.26±11.7 µmoles of H_2O_2 decomposed/min/g FW respectively. The genotypes PL-172, RD-2552, PL-807, DWRUB52 were at par with respect to CAT activity which varied significantly (CD 5%) from PL-426 and VJM-201. In oats**,** the catalase activity in grains was found the highest in OL-9 (177.54 ±9.6 ìmoles of H_2O_2 decomposed/min/g FW) and the lowest in HJ-8 (122.15±4.9 µmoles of H_2O_2 decomposed/min/g FW). The catalase activity in rest of the genotypes was 128.63±8.5, 129.67±6.1, and 124.69±4.5 for OS-6, OS-7 and HFO-114 respectively. Overall the grains of barley genotypes had higher activity as compared to oat grains supporting the inherent hardyness of barley than oats. Variable CAT activity was recorded in different oat species (Pandey *et al* 2010) suggestive of variable drought tolerance. 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). Baek *et al* (2000) reported the presence of substantial amounts of catalase, peroxidase and SODs in over wintering 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). According to Casano *et al* (1999) 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). Many studies also reported decline in CAT activity as a general response to many stresses (Guo *et al* 2006, Pan *et al* 2006, Liu *et al* 2008). Data displayed in Table 2 showed the comparative catalase activity in the grains of six genotypes of barley. The activity was found to be highest in grains of PL-172 and the lowest in PL-426. The catalase activities in different grains of barley genotypes viz. PL-172, RD-2552, PL-807, DWRUB52, PL-426 and VJM-201 exhibited values of 235.15±3.9, 230.81±3.9, 228.43±2.7, 227.58±7.8, 200.08±9.2 and 185.26±11.7 µmoles of H_2O_2 decomposed/min/g FW respectively. The genotypes PL-172, RD-2552, PL-807, DWRUB52 were at par with respect to CAT activity which varied significantly (CD 5%) from PL-426 and VJM-201. In oats**,** the catalase activity in grains was found the highest in OL-9 (177.54 ±9.6 ìmoles of H_2O_2 decomposed/min/g FW) and the lowest in HJ-8 (122.15±4.9 µmoles of H_2O_2

Table 2
Catalase (CAT) activity in grains of different barley and oats genotypes

Barley		*Oats*	
Genotypes	*CAT Activity (µmoles of H_2O_2 decomposed/ min/g FW*	*Genotypes*	*CAT Activity (µmoles of H_2O_2 decomposed/ min/g FW*
PL-172	235.92±3.9	OL-9	177.54±9.6
RD-2552	230.81± 3.9	OS-6	129.67±6.1
DWRUB-52	228.43±2.7	OS-7	128.63±8.5
PL-807	227.58±7.8	HFO-114	124.69±4.5
PL-426	200.06±9.2	HJ-8	122.15±4.9
VJM-201	185.26±11.7		
CD (5%)	13.10	CD (5%)	12.85

Values are mean ± SD of three replicates

decomposed/min/g FW). The catalase activity in rest of the genotypes was 128.63±8.5, 129.67±6.1, and 124.69±4.5 for OS-6, OS-7 and HFO-114 respectively. Overall the grains of barley genotypes had higher activity as compared to oat grains supporting the inherent hardyness of barley than oats. Variable CAT activity was recorded in different oat species (Pandey *et al* 2010) suggestive of variable drought tolerance.

Peroxidase (POD)

POD activity was compared in grains of different barley genotypes, RD-2552 registered maximum activity of 1.24±0.06 units (Table 3). The other genotypes harbored POD value of 1.22±0.06, 1.01±0.06, 0.76±0.11, 0.43±0.10 and 0.37±0.04 ΔE/min/g FW for PL-172, PL-807, DWRUB-52, PL-426 and VJM-201 respectively. In oat grains OL-9 recorded the highest POD activity (0.59±0.05) followed by that in OS-6 (0.37±0.02), OS-7 (0.27±0.02), HFO-114 (0.20±0.01) and HJ-8 (0.16±0.03) (Table 3). Like the above enzymes POD also did not show any relation with two rowed and six rowed barley types. The activity showed differential variance in the grains of both the crops. Peroxidase is generally involved in plant cell growth by promoting cell wall rigidity through lignin synthesis and cross-linking of polysaccharide components (Asada 1992). The activity of POD varies considerably depending upon plant species and stress conditions (Gill and Tuteja 2010). 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) and sorghum (Zhang and Kirkham 1996). 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). Increase in POD activity in both the leaf and root tissues of *V. radiate* (Panda 2001) and *O.sativa* (Koji *et al* 2009) has been reported under salinity stress.

Table 3
Peroxidase (POD) activity in grains of different barley and oats genotypes

Barley		*Oats*	
Genotypes	*POD Activity (ΔE/min/g FW)*	*Genotypes*	*POD Activity (ΔE/min/g FW)*
PL-172	1.24±0.06	OL-9	0.59±0.05
RD-2552	1.22±0.06	OS-6	0.37±0.02
DWRUB-52	1.01±0.06	OS-7	0.27±0.02
PL-807	0.76±0.11	HFO-114	0.20±0.01
PL-426	0.43±0.10	HJ-8	0.16±0.03
VJM-201	0.37±0.04		
CD (5%)	0.14	CD (5%)	0.07

Values are mean ± SD of three replicates

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). Increase in POD activity in both the leaf and root tissues of *V. radiate* (Panda 2001) and *O. sativa* (Koji *et al* 2009) has been reported under salinity stress.

Ascorbate peroxidase (APX)

The APX activity was recorded highest in the grains of barley genotypes PL-172 (661.19±6.5) and the lowest in VJM-201 (571.36±10.8) (Table 4). The activity of PL-172, RD-2552, PL-807 and DWRUB-52 varied significantly (CD 5%) from PL-426 and VJM-201. APX activity did not show any relation with different barley types (two rowed and six rowed). The data for various oat genotypes (Table 4) demonstrated that OL-9 exhibited the highest APX activity (401.67±18.6) which was significantly higher (CD 5%) than other four genotypes i.e. OS-6, OS-7, HJ-8 and HFO-114 respectively. Data shows that barley crop and barley grains have higher APX activity as compared to oats crop and oat grains indicating barley to be better equipped to combat various stresses. The findings of Koussevitzky *et al* (2008) suggested that higher cytosolic APX play key

Table 4
Ascorbate peroxidase (APX) activity in grains of different barley and oats genotypes

Barley		*Oats*	
Genotypes	*APX activity (nmoles of MDA formed/ min/ g FW)*	*Genotypes*	*APX activity (nmoles of MDA formed/ min/ g FW)*
PL-172	661.19±6.5	OL-9	401.67±18.6
RD-2552	655.53±11.7	OS-6	344.08±6.0
DWRUB-52	655.02±11.2	OS-7	333.11±7.0
PL-807	645.51±5. 3	HFO-114	319.33±7.9
PL-426	576.21±10.8	HJ-8	313.46±6.0
VJM-201	571.36±10.4		
CD (5%)	17.12	CD (5%)	18.77

Values are mean ± SD of three replicates

role in protection of plants to a combination of drought and heat stress. Simonovicova *et al* (2004) also reported increase in APX activity in *Hordeum vulgare* L. cv. Al for root tips under Al stress at 72 hrs. Over expression of *C. annuum* APX like gene in transgenic tobacco plants exhibited increased tolerance to oxidative stress and also enhanced resistance to *Phytophthora nicotianae*. However transgenic plants were not found to be resistant to bacterial pathogen, *Pseudomonas syringae*. Generally overexpression of APX in transgenic plants conferred abiotic stress tolerance (Gill and Tuteja 2010).

Glutathione reductase (GR)

The GR activity value of 267.78±4.5, 260.82±7.3, 255.04±5.0, 249.59±6.2, 200.61±7.6 and 186.05±8.0 nmoles of NADPH oxidized/min/g FW was found in grains of PL-172, RD-2552, PL-807, DWRUB52, PL-426 and VJM-201 respectively (Table 5). PL-172 grains had the highest GR activity. In oat grains, GR activity was highest in OL-9 (197.44±12.4) followed by that in OS-6 (142.32±7.7), HJ-8 (130.74±2.8), OS-7 (116.06±3.3) and HFO-114 (109.02±12.4) (Table 5). Esfandiari *et al* (2007a) showed variation

Table 5
Glutathione Reductase (GR) activity in grains of different barley and oats genotypes

Barley		*Oats*	
Genotypes	*Glutathione Reductase (GR) activity (nmoles of NADPH oxidized/g FW)*	*Genotypes*	*Glutathione Reductase (GR) activity (nmoles of NADPH oxidized/g FW)*
PL-172	267.78±4.5	OL-9	197.44±12.4
RD-2552	260.82±7.3	OS-6	142.32±7.7
DWRUB-52	255.04±5.0	OS-7	130.74±2.8
PL-807	249.59±6.2	HFO-114	116.06±3.3
PL-426	200.61±7.6	HJ-8	109.20±12.4
VJM-201	186.05±8.0		
CD (5%)	11.74	CD (5%)	13.18

Values are mean ± SD of three replicates

in SOD, CAT and GR activity among two wheat cultivars having different sensitivity to salt stress. The tolerant cultivar had higher SOD, CAT and GR activity and lower damage to membranes as compared to the sensitive cultivar. In another study (Esfandiari *et al* 2007b) the effect of water stress on wheat seedlings was undertaken and it was demonstrated that higher levels of antioxidants render the cells more enduring against drought. In our study barley grains had higher GR activity than oat grains supporting the hardiness of barley than oats.

Ascorbic Acid (Vit-C)

Ascorbic acid acts as a substrate for APX in scavenging H_2O_2 into water. Investigation revealed that ascorbate content regulates plant defense gene expression and modulates plant growth and development via phytohormone signaling (Smirnoff 2011). 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). The content of Vit-C in grains of barley and oat crops are depicted in Table 6. Among barley grains PL-172, RD-2552, PL-807, and DWRUB52 had significantly higher Vit-C value than PL-426 and VJM-201. The highest content of Vit-C was recorded in PL-172 (898.29±15.0) and minimum was recorded in VJM-201 (828.60±8.4). The Vit-C content in oat grains varied from 491.08±10.0 in HJ-8 to 682.6±11.08 in OL-9. The Vit-C content recorded in OS-6, HFO-114 and OS-7 was 543.59±7.6, 531.95±3.5 and 519.82±4.7 respectively (Table 6). The content in OL-9 was significantly higher from rest of the genotypes.

Table 6
Ascorbic acid (Vit-C) content in grains of different barley and oats genotypes

Barley		*Oats*	
Genotypes	*Vit-C content (nmoles /g FW)*	*Genotypes*	*Vit-C content (nmoles /g FW)*
PL-172	898.29±15.0	OL-9	682.6±11.08
RD-2552	895.24±7.1	OS-6	543.59±7.6
DWRUB-52	885.58±6.0	OS-7	531.95±3.5
PL-807	869.94±5.9	HFO-114	519.82±4.7
PL-426	833.21±8.2	HJ-8	491.08±10.0
VJM-201	828.60±8.4		
CD (5%)	16.02	CD (5%)	90.86

Values are mean ± SD of three replicates

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). 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). The hydrogen peroxide contents were evaluated in different grains of barley genotypes viz. PL-172, RD-2552, PL-807, DWRUB-52, PL-426 and VJM-201. All the genotypes varied significantly (CD 5%) in H_2O_2 content (Table 7). In oat grains, the hydrogen peroxide content recorded was 21.23±1.3, 16.63±1.4, 12.82±1.1, 11.51±0.9 and 9.19±1.1 in HJ-8, HFO-114, OS-7, OS-6, and OL-9 respectively (Table 7). The lowest H_2O_2 content was in OL-9 and the highest in HJ-8. All the barley and oat grains differed significantly with respect to H_2O_2 content. Furthermore oat grain registered higher H_2O_2 content than barley. In the present study, the low content of H_2O_2 observed in barley crops and grains

Table 7
H_2O_2 content in grains of different barley and oat genotypes

Barley		*Oats*	
Genotypes	*H_2O_2 content (µmoles/g FW)*	*Genotypes*	*H_2O_2 content (µmoles/g FW)*
PL-172	7.05±0.12	OL-9	21.23±1.3
RD-2552	6.07±0.19	OS-6	16.63±1.4
DWRUB-52	4.93±0.06	OS-7	12.82±1.1
PL-807	3.81±0.03	HFO-114	11.51±0.9
PL-426	2.64±0.04	HJ-8	9.19±1.1
VJM-201	2.32±0.03		
CD (5%)	0.17	CD (5%)	2.24

Values are mean ± SD of three replicates

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) 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) 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) 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). 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). It is taken as an indicator of tissue destruction in terms of MDA under various stresses. The MDA content was also estimated in grains of different genotypes of oat and barley. Comparison between the grains of two crops did not show marked difference in MDA content. Among barley genotypes, no lipid peroxidation was detected in PL-172 and RD-2552 grains and highest MDA content was recorded in VJM-201(2.27±0.18) (Table 8). The other genotypes exhibited the MDA content of 1.90±0.12 (PL-426), 0.61±0.55 (DWRUB-52) and 1.4±0.16 (PL-807). In oats no lipid peroxidation was detected in grains of genotypes OL-9 and OS-6 (Table 8). While other genotypes recorded lipid peroxidation value of 0.76±0.12 (OS-7), 1.43±0.12 (HJ-8) and 1.91±0.09 (HFO-114). According to Pandey *et al* (2010) *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). In our study barley genotypes were also 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.

Table 8
Malondialdehyde (MDA) content in grains of different barley and oat genotypes

Barley		*Oats*	
Genotypes	*MDA content (nmoles/g FW)*	*Genotypes*	*MDA content (nmoles/g FW)*
PL-172	2.27±0.1	OL-9	1.91±0.0
RD-2552	1.90±0.1	OS-6	1.43±0.1
DWRUB-52	1.4±0.1	OS-7	0.76±0.1
PL-807	0.61±0.5	HFO-114	0.00±0.0
PL-426	0.00±0.0	HJ-8	0.00±0.0
VJM-201	0.00±0.00		
CD (5%)	0.45	CD (5%)	0.15

Values are mean ± SD of three replicates

CONCLUSION

Antioxidant enzyme activities were estimated in the grains of six barley genotypes viz. six rowed; RD-2552, PL-172, PL-807, PL-426 and two rowed; DWRUB-52 and VJM-201 and were highest in PL-172 and lowest in VJM201. However no association was found between barley genotypes and antioxidant enzyme activities. The other genotypes varied differentially with respect to the enzyme activities. Ascorbic acid also registered highest value in PL-

172 and lowest in VJM-201. The content of H_2O_2 recorded was lowest in PL-807 (23.12±3.13 μmoles/g FW) and highest in VJM-201 (40.71±3.59 μmoles/g FW). The MDA content was undetected in PL-172 and highest in VJM-201 (2.27±0.18 nmoles/g FW). Among five oat genotypes selected for estimation of antioxidant potential in grains viz. OL-9, OS-6, OS-7, HJ-8 and HFO-114, the activities of antioxidant enzymes registered highest values in OL-9 which were at par with generally OS-6 and OS-7, whereas grains of genotypes HJ-8 and HFO-114 generally registered lower antioxidant enzyme activities. However these genotypes had higher H_2O_2 and MDA level in their grains. The study depicts that barley crop and grains inherently have higher antioxidant potential as compared to oat crop and grains, suggesting better survival of barley crop under stressful conditions. The data in the present study could also 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.

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