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ORIGINAL ARTICLE

Variability in enzymatic and non enzymatic antioxidants in wheat (*Triticum aestivum* L.) genotypes

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Abstract Variability in enzymatic and non enzymatic antioxidants could be useful for breeding genotypes tolerant to different abiotic stresses. The objective of present study was to determine the variability in enzymatic and non enzymatic antioxidants in wheat at three different stages of development including leaves of vegetative stage, flag leaf stage after 5 days of anthesis and in mature grains. Forty wheat genotypes including 10 commercial cultivars, 5 rainfed cultivars, 17 advanced breeding lines and 8 Australian cultivars were raised under irrigated conditions. At vegetative stage, high activity of superoxide dismutase (SOD), peroxidase (POX), glutathione reductase (GR) and ascorbate peroxidase (APX) and low hydrogen peroxide (H_2O_2) content was observed in many of the advanced breeding lines, while high proline and low malondialdehyde (MDA) content was observed in many commercial cultivars. In flag leaf after 5 days of anthesis higher activity of SOD and APX was observed in many of rain-fed cultivars; many commercial cultivars showed high activity of POX and GR while low H_2O_2 content was observed in many of Australian cultivars. Ruby, Binnu and Datatine have low H_2O_2 and MDA content so they could be used for studying tolerance towards different types of abiotic stresses. PBW 550 showed high antioxidant activity in leaves during vegetative and flag leaf stage, it could be worthwhile to study the performance of this cultivar under different abiotic stresses. Variability was also observed in mature grains of different wheat genotypes. In mature grains high proline content was observed in many of rainfed cultivars while less GR, CAT and APX activity was observed in many of Australian genotypes. Mature grains of wheat genotypes PBW 644, PBW542, DBW 16, DBW 17, WH 1021, PBW 676, BWL 73 and PBW 175 have high activity of APX, GR and some have high proline content. In general genotypes with high enzymatic antioxidants and low H_2O_2 and MDA content may be useful for studying tolerance towards different abiotic stresses. Genotypes with high antioxidants were identified for possible use in wheat breeding programme.

Keywords Antioxidants · Ascorbate peroxidase · Glutathione reductase · Flag leaf · Proline · Superoxide dismutase · Wheat

Introduction

Wheat (*Triticum aestivum* L.) is recognized as a staple food crop globally and it typically grown over 200 Mha throughout the world. India ranks second on the list of producers of wheat all over the world. The advent of green revolution witnessed a steady increase in wheat productivity which has been associated with genetic improvements in yield potential, resistance to diseases, adaptation to abiotic stresses (Reynolds and Borlaugh 2006) as well as better agronomic practices (Evenson & Gollin, 2003). Ever-increasing global demand for wheat and limited availability of land is placing pressure on breeding programs to provide elite cultivars that can adapt to a range of environments without compromising agronomic performance, grain quality, stress tolerance and disease resistance.

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Crop cultivation in open fields is seen to be dependent on various biotic and abiotic factors. Abiotic stresses lead to high leakage of electrons towards oxygen during photosynthetic and respiratory processes leading to enhancement in Reactive oxygen species (ROS) generation (Asada, 1999). ROS are ubiquitous molecules produced as a consequence of normal cellular metabolism (Kotchoni, 2004). Several environmental factors such as cold, high light, ozone, drought, salt, pathogen and UV radiations can cause stress in plants and may lead to the over production of reactive oxygen species (ROS) in plants which are highly reactive and toxic and cause damage to proteins. This excessive accumulation of ROS necessitates the activation of additional defenses (Doke & Scandalios, 1997). The antioxidant defense machinery protects plants from oxidative stress damage. Plants possess very efficient enzymatic (Superoxide Dismutase, Ascorbate Peroxidase, Glutathione Reductase, Guaiacol Peroxidase and Catalase) and non-enzymatic (ascorbic acid, glutathione and alpha tocopherol) antioxidant defense systems to protect the plants from oxidative damage (Gill & Tuteja, 2010). Many of the genes are shared between abiotic and biotic stresses. This highlights the complexity of stress response and adaptation to plants (Mantri et al., 2012).

The ROS such as O_2, H_2O_2 and $OH^•$ radicals can directly attack membrane lipids, inactivate metabolic enzymes, and damage the nucleic acid leading to cell death. It has been reported that high antioxidant enzymes in wheat are related with various kinds of abiotic and biotic stresses (Sairam et al., 1998; Valifard et al., 2012). Therefore, a genotype which has higher status of antioxidant enzymes could behave differently as compared to a genotype which has lower status of these enzymes. In plant cells chloroplast, mitochondria and peroxisomes are important intracellular generators of reactive oxygen species.

$O_2^•$ is the primary ROS formed in the cell which initiates a cascade of reactions to generate "secondary" ROS, either directly or through enzyme or metal catalyzed processes depending on the cell type or cellular compartment (Valko et al., 2006). By comparison, $O_2^•$ and H_2O_2 are weaker oxidizing agents. Under normal condition, the half-life of H_2O_2 is probably 1 ms, and other forms of ROS, including superoxide anion ($O_2^•$), hydroxyl radicals ($OH^•$) and singlet oxygen ($^1O_2$), have very short half-life, about 2–4 µs (Gill & Tuteja, 2010). H_2O_2 plays a dual role in plants at low concentrations, it acts as a signal molecule involved in signaling tolerance to various biotic and abiotic stresses and at high concentrations it leads to programmed cell death (Quan et al., 2008). $OH^•$ is among the most highly reactive ROS known. In the presence of suitable transition metals, especially Fe, $OH^•$ can also be produced from $O_2^•$ and H_2O_2 at neutral pH and ambient temperatures by the iron catalyzed, $O_2^•$ driven Fenton reaction (Gill & Tuteja, 2010).

Reactive oxygen species produced as a result of various abiotic stresses needs to be scavenged for maintenance of normal growth. The primary scavenger is superoxide dismutase (SOD; EC 1.15.1.1), which converts $O_2^•$ to H_2O_2. This toxic product of SOD reaction is eliminated by ascorbate peroxidase (APX; EC 1.11.1.11) in association with dehydro-ascorbate reductase (EC1.8.5.1) and glutathione reductase (GR; EC 1.6.4.2), the latter two help in regeneration of ascorbic acid (AA). H_2O_2 is also scavenged by catalase (EC 1.11.1.6), though the enzyme is less efficient than APX-GR system (Quan et al., 2008).

Drought poses critical environmental constraints to plant survival and crop productivity (Chaves et al., 2003). Dynamic changes in the antioxidative enzyme activities have been attributed as an important anti-drought mechanism to cope with oxidative stress during drought conditions (Shao et al., 2005). Better resistance and acclimation to drought is experimentally correlated with enhanced antioxidative protection (Khanna-Chopra & Selote, 2007). Similar to water deficient stress, reactive oxygen species are also produced during salinity stress, and are responsible for the damage to membranes and other essential macromolecules such as photosynthetic pigments, proteins, DNA and lipids.

Esfandiari et al. (2007) observed that high SOD, CAT and GR activity in leaves of Sardari a normal growing wheat cultivar is associated with salt tolerance while the less SOD, CAT and GR activity in leaves of Alvand a normal growing wheat cultivar is associated with salt sensitive nature. Variability in enzymatic and non enzymatic antioxidants activity has been associated with salt tolerance. The activitites of antioxidant enzymes such as catalase, peroxidase, glutathione reductase and superoxide dismutase differently change in wheat genotypes (Huseynova et al., 2010). Kavir a normal growing wheat cultivar having high SOD, CAT and APX is associated with drought tolerance (Hasheminasab et al., 2012). Thus, the variability in enzymatic and non enzymatic antioxidants could be useful for breeding genotypes tolerant to a number of factors that can cause stress to the plant cultivation.

Material and methods

Plant material

Crop was raised in the fields of Punjab Agricultural University, Ludhiana, India. Activities of antioxidant enzymes and non-enzymatic antioxidants were determined in leaves after 45 days of sowing and in flag leaf at 5 days after anthesis. These crops were raised in the fields of

Punjab Agricultural University, Ludhiana, India. Activities of antioxidant enzymes and non-enzymatic antioxidants were determined in leaves after 45 days of sowing and in flag leaf at 5 days after anthesis.

Extraction and estimation of antioxidative enzymes

Antioxidative enzymes assay

Enzymes were extracted from fresh plant tissues at 4 °C as described by Kaur et al. (2009). SOD, POX and GR activities were determined spectrophotometrically by the methods of Marklund and Marklund (1974), Shannon et al. (1966), respectively. APX and CAT activities were determined following the procedure of Nakano and Asada (1987) and Chance and Maehly (1955), respectively. Protein concentration was measured by the method of Lowry et al. (1951).

Ascorbate and H_2O_2 measurement

Ascorbate (Asc) measurement was based on the reduction of ferric to ferrous ion with ascorbate in acid solution followed by the formation of the pink complex between ferrous ion and bipyridyl that absorbs at 525 nm. Fresh tissue (0.1 g) was homogenized in 1.5 mL of 5% ice cold metaphosphoric acid and centrifuged at 10,000 g for 10 min. Supernatant was collected and used for the estimation of ascorbate according to Law et al. (1983). For H_2O_2 extraction, fresh tissue (0.3 g) was homogenized in 2.0 mL of ice-cold 10 mm sodium phosphate buffer (pH 7.0) and centrifuged at 10 000 g for 20 min. Supernatant was collected, and H_2O_2 content was estimated by reaction with 5% potassium dichromate and acetic acid (1: 3 V/V) as described by Sinha (1971).

Malondialdehde (MDA) content measurement

Malondialdehde (MDA) equivalent content as thiobarbituric acid reactive substances (TBARS) was measured as described by Ohkawa et al. (1979). Fresh tissue (0.2 g) was homogenized in 1.0 mL of 5% trichloroacetic acid (TCA) and centrifuged at 13,500 g for 15 min at room temperature. The supernatant of tissue was mixed with an equal volume of 20% (w/v) TCA containing 0.5% (w/v) thiobarbituric acid. The mixture was heated to 96 °C for 30 min, cooled quickly in ice and centrifuged at 9500 g for 10 min. The absorbance of the supernatant was measured at 532 nm. Correction of nonspecific turbidity was obtained by subtracting the absorbance value taken at 600 nm. The extinction coefficient used for this assay was 155 mm^{-1} cm^{-1}.

Proline content measurement

Proline content was measured as described by Bates (1973). 100 mg of sample was homogenized with 4 mL of 3% aqueous sulfosalicylic acid and the homogenate was filtered through Whatman filter paper and filterate was used for proline estimation. To 2 mL of supernatant, 2 mL of acidic ninhydrin solution and 2 mL of glacial acetic acid were added. The tubes were kept in a water bath at 100 °C for 1 h. Thereafter, the reaction was terminated in an ice bath. The reaction mixture was extracted with 4 mL toluene, mixed vigorously with a test tube stirrer for 15–20 s. The chromophore containing upper toluene layer was aspirated from aqueous phase and absorbance was read at 520 nm using toluene as blank. The concentration of proline was determined using proline standards (0.02 to 0.1 µmol) run simultaneously.

DPPH radical scavenging activity

The free radical scavenging capacity of wheat extracts was determined using the stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH.) as outlined by Blois (1958). The required tissue (100 mg) was homogenized in 2 mL of methanol and centrifuged at 10,000$\times g$ for 10 min. To 1 mL of supernatant, 3 mL of DPPH were added. After 30 min of incubation at room temperature in dark, the absorbance was measured at 517 nm.

Statistical analysis

The results are analyzed as means ± S.D ($n \geq 3$). Tukey's test (SPSS 16.0 software) was used to determine the difference between the genotypes ($P \leq 0.05$).

Result and discussion

Variability of enzymatic and non enzymatic antioxidants was studied in forty different wheat genotypes at different stages of crop development.

Variability of enzymatic and non enzymatic antioxidants in leaves of wheat after 45 days of sowing

Tukey's comparison indicated significant difference in superoxide dismutase (SOD) activity in the wheat cultivars (Table 1). Mean comparison of the cultivars showed that highest activity of SOD was observed in advanced breeding lines. Within advanced breeding lines wheat cultivars BW 4101and PBW 687 had maximum SOD activity. High specific activity of SOD was observed in PBW 687 and

Table 1 Variability of enzymatic antioxidants in leaves of wheat after 45 days of sowing

	Enzymatic antioxidants				
	SOD (units min^{-1} $g^{-1}$of FW)	POX (ΔA min^{-1} g^{-1} FW)	GR (n moles of $NADP^{+}$ formed min^{-1} g^{-1} FW)	CAT (μ moles of H_2O_2 decomposed min^{-1} g^{-1} FW)	APX (n moles of MDA formed min^{-1} g^{-1} FW)
Commercial cultivars					
PBW 343	189.1 ± 15.4^{efghijk}	75.90 ± 3.25^{m}	1455 ± 52^{bcdefgh}	438 ± 48^{hij}	5786 ± 218^{lmn}
PBW 502	151.2 ± 21.5^{k}	120.5 ± 5.16^{ijkl}	1375 ± 32^{defgh}	319 ± 4.9^{k}	4628 ± 135no
WH 542	167.2 ± 1.13^{jk}	113.6 ± 11.3^{jkl}	1497 ± 72^{abcdefgh}	644 ± 29^{bcd}	7666 ± 296^{fghijk}
PBW 550	222.2 ± 8.70^{abcdef}	131.4 ± 7.64^{hij}	1555 ± 31^{abcdefgh}	490 ± 15^{fghi}	9209 ± 68^{cdef}
PBW 621	212.4 ± 6.15^{abcdefgh}	140.4 ± 12.7^{fghi}	1186 ± 16^{hi}	370 ± 39^{jk}	9642 ± 272^{c}
DBW 17	236.1 ± 5.52^{abc}	86.40 ± 5.09^{mn}	1461 ± 41^{bcdefgh}	569 ± 49^{cdef}	8341 ± 159^{cdefghi}
PBW 373	208.4 ± 11.8^{abcdefghi}	99.80 ± 24.0^{lm}	1309 ± 17^{fgh}	654 ± 54^{bcd}	7184 ± 68^{hijkl}
RAJ 3765	224.1 ± 11.5^{abcde}	108.8 ± 11.3^{jklm}	1237 ± 52^{ghi}	555 ± 68^{defg}	9642 ± 272^{c}
DBW 16	198.4 ± 26.0^{cdefghij}	104.0 ± 4.45^{klm}	1555 ± 31^{abcdefg}	497 ± 4.9^{fghi}	8823 ± 204^{cdef}
WH 1021	182.6 ± 31.9^{ghijk}	122.4 ± 5.09^{ijkl}	1816 ± 11^{a}	497 ± 12^{fghi}	9209 ± 277^{cdef}
Mean	199	110	1444	502.7	8012
Rainfed cultivars					
PBW 175	176.8 ± 23.7^{hijk}	140.4 ± 5.09^{fghi}	1512 ± 23^{abcdefgh}	418 ± 19^{ijk}	5159 ± 177^{mno}
PBW 527	218.1 ± 19.9^{abcdefg}	127.8 ± 5.16^{ij}	1338 ± 51^{efgh}	456 ± 53^{ghij}	3905 ± 204^{o}
PBW 596	198.6 ± 9.26^{bcdefghij}	137.7 ± 8.91^{ghi}	1418 ± 18^{cdefgh}	644 ± 39^{bcd}	4628 ± 135no
WH 1080	192.4 ± 34.4^{defghij}	138.0 ± 17.0^{ghi}	1404 ± 21^{defgh}	486 ± 10^{fghi}	8726 ± 67^{cdefgh}
PBW 644	212.4 ± 6.15^{abcdefgh}	124.0 ± 22.6^{ijk}	1288 ± 36^{fgh}	486 ± 10^{fghi}	8967 ± 272^{cdefg}
Mean	199	128	1392	497.5	6276
Advanced breeding line					
BW 6866	222.1 ± 14.3^{abcdef}	161.8 ± 11.6^{cde}	1338 ± 21^{efgh}	788 ± 15^{a}	7521 ± 136^{ghijk}
BW 4101	245.9 ± 2.97^{a}	183.5 ± 4.95^{abc}	1317 ± 36^{fgh}	432 ± 19^{hij}	14,416 ± 286^{a}
PBW 676	208.3 ± 17.4^{abcdefghi}	128.7 ± 3.82^{ij}	1476 ± 62^{abcdefgh}	480 ± 39^{ijk}	6268 ± 136^{klm}
BWL 931	186.6 ± 26.2^{efghijk}	166.0 ± 5.66^{bcde}	1469 ± 11^{abcdefgh}	421 ± 4.2^{fghi}	8919 ± 286^{cdef}
BWL 932	224.2 ± 5.87^{abcde}	175.0 ± 7.07^{bcde}	1562 ± 16^{abcdefg}	415 ± 4.9^{ijk}	8968 ± 136^{cdefg}
BWL 934	208.3 ± 17.4^{abcdefghi}	140.0 ± 5.66^{fghi}	1686 ± 19^{abcde}	510 ± 44^{efghi}	7907 ± 136^{defghij}
BWL 83	214.4 ± 3.32^{abcdefgh}	188.1 ± 21.6^{ab}	1758 ± 11^{abc}	489 ± 34^{fghi}	8919 ± 240^{cdefg}
BW 6280	234.2 ± 8.27^{abc}	166.0 ± 5.66^{abcd}	1374 ± 24^{defgh}	479 ± 20^{fghi}	8678 ± 218^{cdefgh}
BWL 936	220.1 ± 17.1^{abcdefg}	168.0 ± 6.79^{bcde}	1244 ± 18^{ghi}	476 ± 4.9^{fghi}	7714 ± 272^{efghijk}
RAJ 4134	218.1 ± 19.9^{abcdefg}	173.7 ± 8.91^{bcde}	1324 ± 33^{fgh}	527 ± 9.9^{efgh}	7955 ± 286^{defghij}
BWL 927	202.5 ± 14.8^{bcdefghij}	164.1 ± 8.41^{cde}	1374 ± 41^{defgh}	562 ± 29^{cdef}	9257 ± 272^{cde}
PBW 668	230.1 ± 8.56^{abcd}	200.6 ± 12.1^{a}	1410 ± 51^{cdefgh}	486 ± 9.9^{fghi}	8003 ± 110^{defghij}
PBW 687	237.9 ± 8.34^{ab}	177.8 ± 11.0^{abcd}	1454 ± 17^{bcdefgh}	531 ± 15^{efgh}	4628 ± 135no
BW 7197	210.3 ± 14.6^{abcdefghi}	164.7 ± 3.82^{cde}	1772 ± 31^{ab}	486 ± 30^{fghi}	9112 ± 277^{cdef}
BWL 924	214.6 ± 13.4^{abcdefgh}	164.7 ± 3.82^{cde}	1374 ± 42^{defgh}	517 ± 63^{efghi}	11,282 ± 136^{b}
BWL 73	183.2 ± 23.8^{fghijk}	154.4 ± 7.92^{efgh}	1374 ± 43^{defgh}	486 ± 38^{fghi}	6846 ± 236^{ijkl}
BW 7296	171.4 ± 23.5^{ijk}	138.8 ± 10.5^{fghi}	1599 ± 30^{abcdef}	531 ± 15^{efgh}	7762 ± 205^{efghijk}
Mean	213	165.6	1465	506.7	8479
Australian cultivars					
Cook	206.6 ± 2.0^{abcdefghij}	160.7 ± 3.61^{cdef}	1309 ± 33^{abcd}	685 ± 29^{ab}	6894 ± 205^{ijkl}
Sunco	196.8 ± 4.6^{bcdefghij}	166.0 ± 5.66^{bcde}	1490 ± 62^{abcdefgh}	664 ± 9.9^{bc}	8534 ± 68^{cdefgh}
Sunmist	206.4 ± 14^{bcdefghij}	156.3 ± 9.76^{cdefg}	1700 ± 53^{abcd}	476 ± 4.9^{fghi}	5930 ± 277^{lmn}
Carnamah	190.6 ± 20^{efghijk}	120.3 ± 1.84^{ijkl}	1323 ± 74^{fgh}	637 ± 68^{bcd}	9739 ± 272^{bc}
Stretton	206.6 ± 2.0^{abcdefghij}	125.1 ± 1.34^{ijk}	1237 ± 30^{ghi}	654 ± 24^{bcd}	7714 ± 245^{efghijk}
Binnu	216.1 ± 22^{abcdefgh}	137.7 ± 8.91^{ghi}	896 ± 21^{hi}	613 ± 4.2^{bcde}	9450 ± 136^{cd}
Ruby	204.4 ± 17^{bcdefghij}	87.7 ± 10.9^{mn}	629 ± 72^{i}	507 ± 49^{fghi}	8823 ± 159^{cdefg}
Datatine	198.4 ± 26^{cdefghij}	118.9 ± 0.14^{ijkl}	1295 ± 34^{fgh}	514 ± 9.2^{efghi}	6508 ± 295^{jklm}
Mean	203	134.1	1235	593.5	7948

Values are mean ± SD of three replicates

Mean values with different letters in the same column are significantly different ($p < 0.05$)

Table 2 Variability of non-enzymatic antioxidants in leaves of wheat after 45 days of sowing

	Non-enzymatic antioxidants				
	DPPH percentage activity	H_2O_2 content (μmol g^{-1} of FW)	MDA content (n moles g^{-1} of FW)	Proline content (μmol g^{-1} of FW)	Ascorbic acid content (μmol g^{-1} of FW)
Commercial cultivars					
PBW 343	53 ± 1.4^{fgh}	111 ± 35^{mno}	15.8 ± 0.99^{abcd}	0.93 ± 0.01^{lmno}	21.4 ± 0.9^{jklm}
PBW 502	46 ± 1.4^{kl}	181 ± 37^{hijkl}	13.8 ± 0.14^{bcde}	0.90 ± 0.03^{nopqr}	21.3 ± 1.2^{ijklm}
WH 542	55 ± 1.4^{efg}	233 ± 12^{efgh}	14.7 ± 2.05^{ab}	1.05 ± 0.01^{nopqr}	19.9 ± 0.2^{klmn}
PBW 550	50 ± 2.8^{ghij}	252 ± 34^{defg}	16.2 ± 0.92^{abc}	0.96 ± 0.06st	17.0 ± 0.1^{qrst}
PBW 621	64 ± 2.1^{cd}	134 ± 18^{klmno}	9.70 ± 2.83^{abcd}	0.92 ± 0.06^{klmno}	21.6 ± 0.7^{ijkl}
DBW 17	72 ± 2.8^{b}	372 ± 37^{a}	21.8 ± 0.64^{cdefgh}	0.79 ± 0.02^{efghi}	15.1 ± 0.3^{t}
PBW 373	26 ± 2.1^{o}	199 ± 37^{ghijklm}	22.7 ± 3.11^{cdefgh}	0.80 ± 0.02^{def}	19.1 ± 0.7^{mnopq}
RAJ 3765	34 ± 1.4^{n}	327 ± 1.4^{b}	23.6 ± 0.28^{fghijk}	0.68 ± 0.06^{de}	21.1 ± 0.4^{ijklm}
DBW 16	57 ± 2.1^{ef}	171 ± 35^{hijklm}	13.8 ± 1.91^{hijk}	0.56 ± 0.03^{mnopqr}	20.9 ± 1.7^{ijklmn}
WH 1021	52 ± 2.1^{efgh}	252 ± 9.9^{cdef}	9.20 ± 1.27^{efghijk}	0.71 ± 0.02^{t}	18.8 ± 0.5^{nopqr}
Mean	51.1	223	16.1	0.83	19.6
Rainfed cultivars					
PBW 175	60 ± 2.8^{de}	181 ± 37^{hijklm}	11.3 ± 0.78^{bcdef}	0.89 ± 0.08^{rst}	27.5 ± 0.9^{abc}
PBW 527	73 ± 2.1^{b}	337 ± 37^{b}	22.2 ± 1.41^{defghijk}	0.72 ± 0.08^{efgh}	28.1 ± 1.2^{ab}
PBW 596	60 ± 2.8^{de}	159 ± 5.7^{ijklmn}	15.1 ± 2.26^{ghijkl}	0.64 ± 0.01^{mnopq}	33.5 ± 0.9^{a}
WH 1080	68 ± 2.1^{bc}	261 ± 23^{cdef}	18.8 ± 1.91^{fghijk}	0.68 ± 0.02^{hijkl}	24.7 ± 1.2^{defg}
PBW 644	46 ± 2.8^{kl}	218 ± 9.2^{fghij}	19.7 ± 1.06^{ijk}	0.51 ± 0.07^{ghijk}	19.9 ± 1.7^{klmno}
Mean	61.6	231	17.4	0.69	26.7
Advanced breeding line					
BW 6866	52 ± 2.1^{efgh}	171 ± 11^{hijklm}	17.2 ± 2.76^{ijk}	0.52 ± 0.06^{jklmn}	16.8 ± 0.3^{rst}
BW 4101	60 ± 2.8^{de}	212 ± 18^{fghijk}	11.7 ± 1.06^{efghijk}	0.69 ± 0.07^{pqrst}	18.6 ± 1.6^{opqr}
PBW 676	80 ± 3.5^{a}	176 ± 33^{hijklm}	10.0 ± 0.71^{cdefgh}	0.79 ± 0.03st	15.8 ± 0.7st
BWL 931	52 ± 2.1^{efgh}	353 ± 22^{a}	16.2 ± 2.47^{defghij}	0.72 ± 0.04^{klmno}	19.8 ± 1.1^{lmnop}
BWL 932	49 ± 6.3^{ghij}	319 ± 12^{bc}	9.20 ± 0.57^{hijkl}	0.62 ± 0.08^{t}	20.8 ± 0.6^{jklmn}
BWL 934	35 ± 4.9^{mn}	161 ± 33^{ijklmn}	11.5 ± 0.28^{bcdefghij}	0.85 ± 0.05^{qrst}	26.5 ± 0.1^{abcde}
BWL 83	37 ± 3.5^{lmn}	94 ± 12^{o}	16.0 ± 0.85^{cdefgh}	0.79 ± 0.02^{lmno}	22.9 ± 1.9^{ghij}
BW 6280	50 ± 2.8^{ghij}	157 ± 38^{ijklmn}	9.45 ± 2.62^{hijkl}	0.63 ± 0.04st	24.5 ± 0.6^{defg}
BWL 936	82 ± 0.7^{a}	100 ± 3.5^{no}	14.6 ± 0.78^{cdefghij}	0.77 ± 0.01^{nopqr}	28.2 ± 0.1^{ab}
RAJ 4134	82 ± 1.4^{a}	224 ± 25^{efghi}	35.5 ± 0.21^{cdefgh}	0.81 ± 0.08^{a}	26.4 ± 0.2^{bcde}
BWL 927	41 ± 2.1^{mno}	145 ± 11^{ijklmno}	15.4 ± 1.41^{cdefgh}	0.78 ± 0.08^{lmnop}	25.4 ± 0.6^{cdef}
PBW 668	80 ± 2.1^{a}	160 ± 42^{ijklmn}	20.0 ± 0.64^{bcdef}	0.89 ± 0.05^{fghij}	16.7 ± 0.5^{rst}
PBW 687	46 ± 2.1^{jkl}	302 ± 12^{bcd}	12.9 ± 2.62^{ghijkl}	0.65 ± 0.01^{opqrs}	17.5 ± 1.3^{pqrs}
BW 7197	80 ± 2.1^{a}	198 ± 20^{ghijk}	20.4 ± 0.14^{efghijk}	0.69 ± 0.03^{efghi}	16.8 ± 0.3^{rst}
BWL 924	73 ± 2.1^{b}	138 ± 24^{jklmno}	21.9 ± 0.92^{hijk}	0.56 ± 0.08^{efghi}	24.4 ± 0.7^{efgh}
BWL 73	82 ± 0.7^{a}	193 ± 4.2^{ghijkl}	18.4 ± 1.56^{cdefgh}	0.80 ± 0.08^{ijklm}	24.7 ± 0.5^{defg}
BW 7296	73 ± 2.1^{b}	235 ± 9.9^{efgh}	17.4 ± 0.14^{a}	1.14 ± 0.03^{jklmn}	26.8 ± 0.6^{abcd}
Mean	62	196	16.3	0.74	21.9
Australian cultivars					
Cook	37 ± 3.4^{lmn}	328 ± 25^{b}	34.5 ± 2.19^{cdefghi}	0.77 ± 0.08^{a}	24.1 ± 1.2^{fgh}
Sunco	44 ± 1.4^{kl}	328 ± 49^{b}	37.7 ± 0.14^{jk}	0.51 ± 0.01^{a}	17.9 ± 0.2^{opqrs}
Sunmist	46 ± 2.1^{jkl}	173 ± 15^{hijklm}	30.8 ± 0.14^{k}	0.45 ± 0.01^{bc}	18.7 ± 1.4^{nopqr}
Carnamah	42 ± 2.8^{lm}	141 ± 16^{jklmno}	27.2 ± 2.26^{defghi}	0.74 ± 0.01^{c}	27.0 ± 0.6^{abc}
Stretton	52 ± 2.1^{efgh}	244 ± 23^{defg}	25.1 ± 1.20^{bcdefg}	0.66 ± 0.05^{de}	28.1 ± 1.2^{ab}
Binnu	50 ± 2.8^{ghij}	109 ± 40^{defg}	29.7 ± 0.57^{bcdefg}	0.85 ± 0.06^{cd}	22.2 ± 0.5^{hijk}
Ruby	47 ± 3.5^{ijkl}	116 ± 19^{lmno}	33.4 ± 0.07^{efghijk}	0.69 ± 0.07^{b}	23.2 ± 0.9^{ghi}

Table 2 continued

	Non-enzymatic antioxidants				
	DPPH percentage activity	H_2O_2 content (µmol g^{-1} of FW)	MDA content (n moles g^{-1} of FW)	Proline content (µmol g^{-1} of FW)	Ascorbic acid content (µmol g^{-1} of FW)
Datatine	68 ± 2.1^{bc}	284 ± 11^{bcde}	34.0 ± 2.97^{defghi}	0.74 ± 0.06^{b}	16.0 ± 0.4^{st}
Mean	48.5	215	31.5	0.67	22.1

Values are mean ± SD of three replicates

Mean values with different letters in the same column are significantly different ($p < 0.05$)

Australian cultivar Cook. High SOD activity in leaves of wheat has been related with salt tolerance (Kahrizi et al. 2012) and with drought stress tolerance (Hasheminasab et al., 2012). So wheat genotypes having high SOD activity could be tolerant to salt/drought stress. Minimum SOD activity was observed in PBW 502 and WH 542. High activity of SOD in normally growing seedlings has also been related with high temperature stress tolerance (Sairam et al., 1998). High activity of SOD therefore appears to be good trait for abiotic stress tolerance.

Peroxidase (POX) activity in wheat cultivar has been illustrated in Table 1. Almeselmani et al. (2006) observed that high POX activity in leaves was associated with high temperature tolerance. Mean comparison of the cultivars showed that highest activity of POX was observed in advanced breeding lines while the lowest POX activity was observed in commercial cultivars. Highest POX activity was observed in PBW 668 and BWL 83 whereas specific activity of POX was high in PBW 687 and Australian cultivar Cook. These cultivars may be associated with high temperature tolerance but validation in the field is necessary.

Activity of glutathione reductase (GR) is depicted in Table 1. Mean comparison of commercial, rain-fed and advanced breeding lines did not show significant differences among themselves. Minimum GR activity was observed in Australian cultivars. Within Australian cultivars Ruby and Binnu has the minimum GR activity. Valifard et al. (2012) observed that in the wheat high GR activity was related to drought tolerance. WH 1021, BWL 83 and BW 7197 have highest activity of GR hence they could be good candidate for studying drought tolerance in the field.

Mean comparison showed that highest catalase (CAT) activity was present in Australian cultivars while there were no significant differences between commercial and advanced breeding lines (Table 1). Advanced breeding line BW 6866 has highest CAT activity. Huseynova et al. (2010) reported that high CAT activity was associated with drought tolerance. However, the cultivars recommended for sowing under rain-fed condition have lower CAT activity in leaves.

Tukey's comparison indicated significant difference in ascorbate peroxidase (APX) activity in the wheat cultivars (Table 1). Higher APX activity was observed in advanced breeding lines. Within advanced breeding lines BW 4101 and BWL 924 have the highest APX activity. Esfandiari et al. (2007) showed that Egypt 449 a wheat cultivar having high APX activity was related with drought tolerance hence BW 4101 and BWL 924 could be drought tolerant.

Variability was also observed in non enzymatic antioxidants in different wheat cultivars. DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity showed variability in different wheat genotype (Table 2). Highest DPPH radical scavenging activity was observed in advanced breeding lines while the lowest DPPH radical scavenging activity was observed in Australian cultivars. Among advanced breeding lines BWL 73, BW 7197, RAJ

Table 3 Status in terms of highest/lowest ratio of enzymatic antioxidants in leaves after 45 days of sowing

Parameter	Enzymatic antioxidants				
	SOD	POX	GR	CAT	APX
Highest/lowest ratio	1.62	2.64	2.88	2.47	3.69
Genotype					
Highest	BW 4101	PBW 668	WH 1021	BW 6866	BW 4101
Lowest	PBW 502	PBW 343	RUBY	PBW 502	PBW 527

Table 4 Status in terms of highest/lowest ratio of non enzymatic antioxidants in leaves after 45 days of sowing

Parameter	Non enzymatic antioxidants				
	DPPH	H_2O_2	MDA	Proline	Ascorbic acid
Highest/lowest ratio	3.11	3.45	4.09	2.53	2.21
Genotype					
Highest	BWL 936	DBW 17	SUNCO	BW 7296	PBW 596
Lowest	PBW 373	BWL 83	BWL 932	SUNMIST	DBW 17

4134 and BWL 936 have high DPPH radical scavenging activity. High DPPH activity could be positive trait for scavenging superoxide radicals produced during abiotic stresses.

Sairam et al. (1998) observed that C 306 a wheat cultivar having less H_2O_2 content was tolerant to drought stress while HD 2329 a wheat cultivar having high H_2O_2 content was sensitive to drought stress. On this basis genotypes BWL 83, BWL 936, PBW 343, PBW 621 having less H_2O_2 content could be an ideal material for studying drought stress tolerance in the field. Lower H_2O_2 content was observed in advanced breeding lines while the highest H_2O_2 content was observed in rain-fed cultivars (Table 2).

Data on malondialdehyde (MDA) content in different wheat genotype has been presented in Table 2. Maximum MDA content was observed in Australian cultivars while the minimum MDA content was observed in commercial cultivars. Asharaf et al. (2010) observed that less MDA was associated with salt tolerance.

Mean comparison of proline content showed no significant variability between rainfed, Australian and advanced breeding lines (Table 2). Commercial cultivars have maximum proline content while the Australian cultivars have minimum proline content. BW 7296, WH 542 and PBW 621 have maximum proline content. Khan et al. (2009) observed that Lu-26s wheat cultivar having high proline content was associated with salt tolerance. Wheat cultivars BW 7296, WH 542 and PBW 621 have relatively high proline content.

Table 2 depicts ascorbic acid content in different wheat genotypes. Tuekey's comparison showed significant variability between different wheat cultivars. Highest ascorbic acid content was observed in rainfed cultivars while the lowest ascorbic acid content was observed in commercial cultivars. Among rainfed cultivars PBW 596, PBW 175 and PBW 175 have high ascorbic acid content. DBW 17, PBW 550, DATATINE have low ascorbic acid content. High ascorbic acid content could be useful trait as an antioxidant. However, no reports are available in the literature showing correlation between leaf ascorbic acid content and abiotic stresses, though Mandhania et al. (2010) have reported high ascorbate and proline content in wheat seedlings and correlated it with salt stress tolerance.

After 45 days of sowing, the leaves showed maximum variability of 3.69 folds in APX with BW 4101 showing highest activity (Table 3). BW 4101 also has highest SOD activity. MDA showed maximum variability of 4.09 folds among non enzymatic antioxidants. Wheat genotypes BWL 83 and BWL 932 showed lowest H_2O_2 and MDA content (Table 4).

Variability of enzymatic and non enzymatic antioxidants in wheat in flag leaf stage after 5 days of anthesis

Activity of Superoxide Dismutase (SOD) is depicted in Table 5. Mean comparison of SOD activity between commercial, Australian cultivars and advanced breeding lines did not show significant differences. However, Australian cultivars have high specific activity of SOD. Minimum SOD activity was observed in commercial cultivars. Within commercial cultivars PBW 502, WH 542 and PBW 621 have low SOD activity. Valifard et al. (2012) observed that in the wheat high SOD activity was related to drought tolerance. Rainfed genotype PBW 644, Advanced breeding line PBW 668 and BWL 73 and Australian cultivar Binnu have high SOD activity.

Tukey's comparison indicated significant difference in peroxidase (POX) activity in the wheat cultivars (Table 5). Mean comparison of the cultivars showed that highest activity of POX was observed in commercial cultivars. Within commercial cultivars wheat cultivars PBW 343 and PBW 502 have maximum POX activity. High POX activity in leaves of wheat has been related with salt tolerance (Kahrizi et al. 2012) and with drought stress (Hasheminasab et al., 2012). So wheat cultivar having high POX activity could be studied for tolerance to these stresses. Minimum POX activity was observed in Stretton and Binnu so these cultivars could be susceptible to salt stress. High activity of POX in the roots of normally growing wheat has also been related with drought stress (Csiszar et al., 2008).

Data on glutathione reductase (GR) activity in wheat cultivar has been given in Table 5. Almeselmani et al. (2006) observed that high GR activity in leaves was associated with high temperature tolerance. Mean comparison of the cultivars showed that highest activity of GR

Table 5 Variability of enzymatic antioxidants in flag leaf stage after 5 days of anthesis

	Enzymatic Antioxidants				
	SOD (units min^{-1} g^{-1} FW)	POX (ΔA min^{-1} g^{-1} FW)	GR (n moles of $NADP^{+}$ formed min^{-1} g^{-1} FW)	CAT (µ moles of H_2O_2 decomposed min^{-1} g^{-1} FW)	APX (n moles of MDA formed min^{-1} g^{-1} FW)
Commercial cultivars					
PBW 343	169 ± 7.78^{hijklmn}	408 ± 11^{a}	4775 ± 204^{b}	627 ± 15^{fghijk}	$21,535 \pm 455^{bc}$
PBW 502	126 ± 15.6^{no}	413 ± 14^{a}	5874 ± 182^{a}	671 ± 38^{efghij}	$18,482 \pm 273^{efgh}$
WH 542	132 ± 7.78^{mno}	306 ± 24^{bc}	3416 ± 193^{ef}	764 ± 28^{cde}	$16,392 \pm 682^{ijkl}$
PBW 550	145 ± 10.6^{lmno}	339 ± 26^{a}	3758 ± 96.9^{de}	673 ± 33^{efghij}	$27,803 \pm 682^{a}$
PBW 621	182 ± 10.6^{efghijkl}	247 ± 13^{fghijk}	3345 ± 42.4^{efg}	494 ± 27^{lmno}	$21,776 \pm 113^{b}$
DBW 17	213 ± 8.4^{abcdefg}	245 ± 16^{hijk}	4280 ± 85.6^{c}	572 ± 57^{hijklm}	$12,401 \pm 780^{nopqrs}$
PBW 373	230 ± 4.95^{abcde}	206 ± 2.8^{klmno}	3689 ± 194^{de}	751 ± 32^{cdef}	$10,564 \pm 515^{st}$
RAJ 3765	194 ± 7.07^{defghijk}	288 ± 23^{bcdef}	4051 ± 205^{cd}	477 ± 31^{lmno}	$12,294 \pm 643^{nopqrs}$
DBW 16	225 ± 14.1^{abcdef}	363 ± 7.8^{a}	3922 ± 90.5^{cd}	543 ± 58^{jklm}	$20,491 \pm 705^{bcd}$
WH 1021	213 ± 19.1^{abcdefg}	349 ± 12^{a}	3729 ± 363^{de}	366 ± 44^{opqrs}	$19,607 \pm 682^{cdef}$
Mean	182	316	4384	593	18,134
Rainfed cultivars					
PBW 175	212 ± 17.7^{abcdefg}	305 ± 1.4^{bcd}	3416 ± 387^{ef}	459 ± 19^{mnop}	$19,773 \pm 448^{cdef}$
PBW 527	182 ± 10.6^{efghijkl}	264 ± 11^{defghij}	3416 ± 387^{ef}	383 ± 40^{opqr}	$14,223 \pm 477^{lmn}$
PBW 596	187 ± 17.7^{defghijkl}	305 ± 1.4^{bcd}	3143 ± 193^{fgh}	689 ± 36^{efghi}	$21,824 \pm 45.3^{b}$
WH 1080	156 ± 10.6^{ijklmno}	227 ± 16^{jklmn}	2801 ± 96.2^{hij}	571 ± 6.4^{hijklm}	$20,303 \pm 379^{bcde}$
PBW 644	255 ± 12.0^{a}	247 ± 12^{ghijk}	4272 ± 107^{c}	521 ± 14^{klmn}	$21,123 \pm 780^{bc}$
Mean	197	269	3409	524	19,448
Advanced breeding line					
BW 6866	235 ± 15.6^{abcd}	189 ± 27^{nopq}	2405 ± 54.4^{ijkl}	308 ± 20^{qrs}	$23,089 \pm 834^{a}$
BW 4101	212 ± 17.7^{abcdefg}	253 ± 18^{fghij}	2769 ± 278^{hij}	266 ± 30^{rs}	$21,144 \pm 280^{bc}$
PBW 676	150 ± 18.4^{jklmno}	194 ± 20^{rs}	2528 ± 96.9^{ijk}	564 ± 45^{ijklm}	$16,848 \pm 644^{hij}$
BWL 931	200 ± 17.1^{cdefghi}	127 ± 17^{mnopq}	2451 ± 11.3^{ijkl}	294 ± 6.4^{qrs}	$12,241 \pm 265^{nopqrs}$
BWL 932	194 ± 7.07^{defghijk}	297 ± 13^{bcde}	2487 ± 62.2^{ijkl}	1078 ± 32^{a}	$22,071 \pm 303^{b}$
BWL 934	203 ± 5.66^{bcdefhijk}	233 ± 11^{jklm}	2966 ± 307^{gh}	697 ± 33^{defgh}	$13,580 \pm 114^{mnop}$
BWL 83	191 ± 13.3^{defghijkl}	263 ± 11^{efghij}	2453 ± 59.4^{ijkl}	876 ± 12^{abc}	$13,001 \pm 932^{mnopqr}$
BW 6280	156 ± 10.6^{o}	203 ± 11^{lmnop}	2798 ± 101^{hij}	274 ± 68^{qrs}	$12,830 \pm 175^{mnopqr}$
BWL 936	113 ± 18.4^{ijklmno}	282 ± 17^{cdefgh}	2801 ± 96.2^{hij}	247 ± 50^{s}	$20,303 \pm 379^{bcde}$
RAJ 4134	202 ± 13.9^{cdefghi}	287 ± 21^{bcdefg}	2170 ± 170^{kl}	333 ± 50^{pqrs}	9449 ± 272^{t}
BWL 927	129 ± 9.19^{mno}	296 ± 15^{bcde}	2058 ± 182^{lm}	927 ± 37^{ab}	$11,742 \pm 363^{pqrs}$
PBW 668	244 ± 17.6^{abc}	330 ± 21^{a}	2829 ± 182^{hi}	592 ± 29^{hijkl}	$15,728 \pm 788^{jkl}$
PBW 687	148 ± 16.8^{klmno}	328 ± 11^{b}	2801 ± 96.2^{hij}	402 ± 60^{nopq}	$11,491 \pm 795^{qrs}$
BW 7197	231 ± 15.3^{abcd}	239 ± 1.4^{ijkl}	2254 ± 290^{kl}	763 ± 32^{cde}	$14,769 \pm 508^{klm}$
BWL 924	173 ± 15.7^{ghijklmn}	280 ± 11^{cdefghi}	2182 ± 187^{kl}	1016 ± 18^{a}	$19,071 \pm 303^{defg}$
BWL 73	252 ± 14.7^{ab}	313 ± 9.9^{bc}	2749 ± 204^{hij}	488 ± 9.9^{lmno}	$14,737 \pm 750^{klm}$
BW 7296	199 ± 17.1^{cdefghij}	306 ± 25^{bc}	2532 ± 103^{ijk}	783 ± 38^{cde}	$17,946 \pm 379^{fghi}$
Mean	190	260	2543	583	15,884
Australian cultivars					
Cook	221 ± 19.1^{abcdefg}	281 ± 12^{cdefgh}	2387 ± 102^{jkl}	533 ± 52^{klm}	$13,551 \pm 231^{mnop}$
Sunco	217 ± 9.9^{abcdefgh}	125 ± 27^{rs}	983 ± 245^{p}	344 ± 64^{pqrs}	$11,828 \pm 243^{opqrs}$
Sunmist	205 ± 7.07^{bcdefg}	163 ± 18^{pqr}	1519 ± 103^{no}	970 ± 8.5^{a}	$13,797 \pm 579^{lmno}$
Carnamah	169 ± 7.78^{hijklmn}	156 ± 5.7^{qr}	1125 ± 228^{op}	734 ± 31^{defg}	$13,205 \pm 644^{mnopqr}$
Stretton	150 ± 18.4^{jklmno}	104 ± 11^{s}	811.5 ± 125^{p}	667 ± 32^{efghij}	$13,419 \pm 341^{mnop}$
Binnu	252 ± 16.3^{ab}	128 ± 5.7^{rs}	1100 ± 48.8^{op}	824 ± 4.9^{bcd}	$15,728 \pm 788^{jkl}$
Ruby	182 ± 10.6^{efghijkl}	175 ± 9.9^{opq}	1507 ± 85.6^{no}	597 ± 37^{hijkl}	$17,571 \pm 818^{ghij}$

Table 5 continued

	Enzymatic Antioxidants				
	SOD (units min^{-1} g^{-1} FW)	POX (ΔA min^{-1} g^{-1} FW)	GR (n moles of $NADP^+$ formed min^{-1} g^{-1} FW)	CAT (μ moles of H_2O_2 decomposed min^{-1} g^{-1} FW)	APX (n moles of MDA formed min^{-1} g^{-1} FW)
Datatine	176 ± 18.4[fghijklm]	192 ± 23[mnopq]	1638 ± 99.7[mn]	606 ± 32[hijkl]	11,384 ± 644[rst]
Mean	196	165	1384	659	13,810

Values are mean ± SD of three replicates

Mean values with different letters in the same column are significantly different ($p < 0.05$)

was observed in commercial cultivars while the lowest GR activity was observed in Australian cultivars. Highest GR activity was observed in PBW 343 and PBW 502 however, these cultivars have been recommended for timely sowing under irrigated condition.

Tukey's comparison indicated significant difference in catalase (CAT) activity in the wheat cultivars (Table 5). High CAT activity was observed BWL 932, BWL 924 BWL 927 and Sunmist. High CAT activity could be responsible for reducing the H_2O_2 content of leaves. The genotype WH 542 has the high specific activity of CAT while the low specific activity was observed in BWL 936.

Mean comparison showed that ascorbate peroxidase (APX) activity was more in rainfed cultivars and less in Australian cultivars (Table 5). Tukey's comparison showed significant difference among wheat cultivars. PBW 550 which is a commercial cultivar has the highest APX activity. Esfandiari et al. (2007) showed that Egypt 449 a wheat cultivar having high APX activity was related with drought tolerance. PBW 550 a high yielding cultivar can also be tried under water deficient condition.

High DPPH radical scavenging activity was observed in advanced breeding lines while the low DPPH radical scavenging activity was observed in Australian cultivars (Table 6). DBW 17, PBW 373, BWL 932, BWL 934 and DT BWL 0927 have high DPPH radical scavenging activity. Though no report could be found in literature showing correlation between abiotic stress and DPPH radical scavenging activity but better radical scavenging activity could be a desirable trait during different stress conditions.

Data on hydrogen peroxide (H_2O_2) content in different wheat genotypes has been presented in Table 6. High H_2O_2 content was observed in rainfed cultivars while the low H_2O_2 content was observed in Australian cultivars. Sairam et al. (1998) observed that less H_2O_2 was associated with drought tolerance. Among Australian cultivars Binnu, Datatine and Carnamah have low H_2O_2 content hence these cultivars could be studied for tolerance to drought stress.

Asharf et al. (2010) observed that S-24 a wheat cultivar having less malondialdehyde (MDA) content was tolerant to salt stress while MH-97 a wheat cultivar having high MDA content was sensitive to drought stress. On this basis genotypes BWL 932, BWL 931, PBW 373, PBW 621 having less MDA content could be an ideal material for studying salt stress tolerance in the field. Lower MDA content was observed in rain-fed cultivars while the higher H_2O_2 content was observed in advanced breeding lines (Table 6).

Tuekey's comparison showed significant variability between different wheat cultivars. Higher proline content was observed in rain-fed cultivars while the lower proline content was observed in Australian cultivars (Table 6). Among rainfed cultivars PBW 527 and PBW 175 have maximum proline content. Cook and Carnamah have minimum proline content. Proline could act as protective osmolyte during stress condition (Ashraf et al. 2010).

Mean comparison of ascorbic acid content showed no significant variability between rainfed, Australian, advanced breeding lines and commercial cultivars (Table 6). Commercial cultivars have maximum ascorbic acid content while the advanced breeding lines have minimum ascorbic acid content. PBW 343, PBW 550 and PBW 621 have maximum ascorbic acid content. High ascorbic acid content could be useful trait as an antioxidant. However reports linking ascorbic acid content with abiotic stresses are lacking in literature.

In the flag leaf maximum variability of 10.9 folds was observed with GR. Cultivar PBW 502 has the highest GR activity. This cultivar also showed maximum POX activity (Table 7). In non enzymatic antioxidants, variability in DPPH and ascorbic acid was more as compared to H_2O_2, MDA and proline (Table 8). Many of the Australian cultivars showed lower non enzymatic antioxidants.

Variability of enzymatic and non enzymatic antioxidants in mature grains of wheat

Tukey's comparison indicated significant difference in superoxide dismutase (SOD) activity in the mature grains of wheat cultivars (Table 9). Mean comparison of the cultivars showed that higher activity of SOD in Australian

Table 6 Variability of non-enzymatic antioxidants in flag leaf stage after 5 days of anthesis

	Non-enzymatic antioxidants				
	DPPH percentage activity	H_2O_2 content (μ moles g^{-1} FW)	MDA content (n moles g^{-1} FW)	Proline content (μ moles g^{-1} FW)	Ascorbic acid content (μ moles g^{-1} FW)
Commercial cultivars					
PBW 343	51.3 ± 1.0^{ghi}	173 ± 25^{jklmn}	23.6 ± 1.1^{cdef}	0.56 ± 0.04^{bc}	15.1 ± 0.49^{defg}
PBW 502	44.6 ± 2.8^{lmno}	185 ± 16^{hijklm}	21.3 ± 0.7^{bc}	0.63 ± 0.06^{cd}	13.3 ± 0.99^{ghij}
WH 542	51.3 ± 2.8^{ghi}	204 ± 11^{fghijk}	16.9 ± 0.8^{cde}	0.58 ± 0.02^{de}	12.2 ± 0.49^{lmnop}
PBW 550	50.1 ± 0.9^{ghijk}	143 ± 6.4^{nopqrs}	19.1 ± 0.8^{efghi}	0.52 ± 0.03^{bcd}	13.7 ± 0.49^{ijklmn}
PBW 621	46.1 ± 0.9^{jklmn}	208 ± 12^{efghijk}	26.7 ± 1.3^{cdefgh}	0.54 ± 0.01^{bc}	14.4 ± 0.28^{cde}
DBW 17	75.3 ± 1.1^{a}	171 ± 2.8^{klmno}	30.5 ± 1.1^{a}	0.74 ± 0.02^{cd}	13.4 ± 0.71^{b}
PBW 373	70.7 ± 1.9^{ab}	185 ± 16^{hijklm}	27.2 ± 1.6^{jklmno}	0.42 ± 0.03^{fghi}	9.10 ± 0.71^{bcd}
RAJ 3765	53.3 ± 1.8^{fgh}	229 ± 1.4^{cdefg}	16.9 ± 0.8^{ijklmn}	0.45 ± 0.04^{cd}	13.4 ± 0.28^{lmnop}
DBW 16	46.0 ± 0.9^{jklmn}	188 ± 12^{hijklm}	17.5 ± 1.5^{ghijklm}	0.46 ± 0.06^{cd}	13.6 ± 0.49^{klmnop}
WH 1021	48.7 ± 0.9^{hijkl}	253 ± 11^{bcd}	11.1 ± 1.8^{defghi}	0.53 ± 0.06^{a}	16.1 ± 0.49^{rs}
Mean	53.6	193	21	0.54	13.3
Rainfed cultivars					
PBW 175	53.3 ± 1.8^{fgh}	214 ± 2.8^{efghi}	13.9 ± 1.3^{a}	0.74 ± 0.06^{bc}	14.3 ± 1.48^{pqrs}
PBW 527	50.0 ± 0.9^{ghijk}	206 ± 13^{efghijk}	17.4 ± 1.3^{bcd}	0.62 ± 0.04^{fg}	10.1 ± 0.71^{lmnop}
PBW 596	48.6 ± 2.8^{hijkl}	229 ± 1.4^{cdefg}	16.9 ± 0.8^{efghijk}	0.51 ± 0.06^{fg}	9.95 ± 1.06^{lmnop}
WH 1080	32.7 ± 0.9^{p}	212 ± 23^{efghi}	12.9 ± 0.1^{cdefg}	0.55 ± 0.01^{de}	12.1 ± 0.78^{qrs}
PBW 644	47.3 ± 1.1^{ijklm}	275 ± 19^{b}	12.8 ± 0.5^{cdef}	0.56 ± 0.06^{bc}	14.9 ± 0.92^{qrs}
Mean	46.3	227	14.7	0.59	12.2
Advanced breeding line					
BW 6866	54.1 ± 4.7^{efg}	210 ± 3.5^{efghij}	21.7 ± 1.6^{ab}	0.69 ± 0.02^{bc}	14.9 ± 0.92^{ghij}
BW 4101	53.3 ± 1.8^{fgh}	267 ± 8.5^{bc}	23.5 ± 0.8^{cde}	0.59 ± 0.06^{ghijk}	8.65 ± 1.91^{defg}
PBW 676	41.3 ± 1.8^{no}	282 ± 8.5^{ijklmn}	27.8 ± 0.7^{ijklm}	0.45 ± 0.02^{ghijk}	8.45 ± 0.49^{bc}
BWL 931	40.7 ± 0.9^{o}	177 ± 3.5^{qrst}	27.8 ± 1.8^{hijklm}	0.46 ± 0.06^{fgh}	9.35 ± 0.78^{bc}
BWL 932	69.3 ± 1.8^{b}	122 ± 9.9^{lmnopq}	22.1 ± 0.7^{hijklm}	0.46 ± 0.03^{fg}	9.90 ± 0.57^{fghi}
BWL 934	63.3 ± 1.0^{c}	158 ± 16^{hijklm}	17.1 ± 0.8^{efghij}	0.51 ± 0.02^{jkl}	7.05 ± 0.07^{lmnop}
BWL 83	59.3 ± 1.1^{cd}	185 ± 21^{bcdef}	19.6 ± 1.5^{cde}	0.57 ± 0.02^{ijkl}	7.55 ± 0.21^{hijklm}
BW 6280	46.1 ± 0.9^{klmn}	237 ± 13^{efghi}	21.2 ± 0.8^{ab}	0.70 ± 0.04^{fg}	9.55 ± 0.49^{ghijk}
BWL 936	43.3 ± 2.8^{mno}	212 ± 23^{bcd}	14.4 ± 1.8^{ab}	0.70 ± 0.04^{lm}	6.05 ± 0.78^{opqr}
RAJ 4134	59.3 ± 1.1^{cd}	253 ± 11^{defgh}	27.1 ± 0.4^{bcd}	0.61 ± 0.02^{m}	5.15 ± 0.49^{bcd}
BWL 927	61.3 ± 1.8^{c}	216 ± 12^{b}	26.8 ± 1.5^{a}	0.74 ± 0.02^{m}	4.75 ± 0.49^{bcd}
PBW 668	58.7 ± 1.9^{cde}	268 ± 12^{hijklm}	30.8 ± 1.8^{mnop}	0.38 ± 0.02^{fghij}	8.80 ± 1.56^{a}
PBW 687	56.4 ± 1.3^{def}	182 ± 12^{efghij}	18.1 ± 0.8^{lmno}	0.39 ± 0.04^{fg}	9.85 ± 0.07^{jklmno}
BW 7197	45.3 ± 1.8^{klmno}	210 ± 28^{efghij}	18.1 ± 0.8^{ijklmn}	0.45 ± 0.02^{fg}	9.95 ± 1.06^{jklmno}
BWL 924	50.7 ± 1.9^{ghij}	243 ± 26^{bcde}	23.1 ± 1.3^{ghijkl}	0.47 ± 0.03^{ijkl}	7.35 ± 1.06^{efgh}
BWL 73	44.0 ± 3.7^{lmno}	314 ± 9.9^{a}	25.5 ± 0.7^{fghijk}	0.48 ± 0.02^{jkl}	7.15 ± 0.78^{cdef}
BW 7296	40.6 ± 2.8^{o}	162 ± 9.2^{lmnop}	24.1 ± 1.5^{op}	0.36 ± 0.06^{m}	5.10 ± 0.57^{defg}
Mean	52.1	217	22.8	0.53	8.21
Australian cultivars					
Cook	19.3 ± 1.1^{r}	194 ± 23^{ghijkl}	19.1 ± 0.8^{p}	0.30 ± 0.05^{b}	15.4 ± 0.57^{ijklmn}
Sunco	51.3 ± 2.8^{ghi}	152 ± 23^{mnopqr}	19.6 ± 1.6^{op}	0.36 ± 0.06^{fg}	9.75 ± 0.78^{hijkl}
Sunmist	$27.3 \pm .1.1^{q}$	134 ± 18^{opqrst}	15.8 ± 0.7^{nop}	0.36 ± 0.02^{ef}	10.5 ± 0.28^{mnopq}
Carnamah	52.0 ± 3.7^{fghi}	96.5 ± 15^{t}	14.2 ± 1.5^{p}	0.30 ± 0.02^{lm}	6.25 ± 0.49^{pqrs}
Stretton	18.7 ± 1.9^{r}	163 ± 23^{lmnop}	14.7 ± 1.3^{klmno}	0.42 ± 0.04^{m}	4.75 ± 0.49^{opqr}
Binnu	20.6 ± 2.8^{r}	118 ± 16^{rst}	15.8 ± 1.3^{defghi}	0.53 ± 0.02^{kl}	7.10 ± 0.57^{nopq}
Ruby	18.7 ± 1.9^{r}	130 ± 12^{pqrst}	12.9 ± 0.2^{fghijk}	0.48 ± 0.02^{hijkl}	7.75 ± 0.49^{qrs}

Table 6 continued

	Non-enzymatic antioxidants				
	DPPH percentage activity	H_2O_2 content (µ moles g^{-1} FW)	MDA content (n moles g^{-1} FW)	Proline content (µ moles g^{-1} FW)	Ascorbic acid content (µ moles g^{-1} FW)
Datatine	22.0 ± 0.9^{r}	113 ± 14^{st}	10.4 ± 0.8^{ghijklm}	0.46 ± 0.02^{fg}	9.75 ± 0.21^{s}
Mean	28.7	137	15.3	0.40	8.89

Values are mean ± SD of three replicates

Mean values with different letters in the same column are significantly different ($p < 0.05$)

Table 7 Status in terms of highest/lowest ratio of enzymatic antioxidants in flag leaf after 5 days of anthesis

	Enzymatic antioxidants				
Parameter	SOD	POX	GR	CAT	APX
Highest/lowest ratio	2.25	3.97	10.9	3.93	2.94
Genotype					
Highest	PBW 644	PBW 502	PBW 502	BWL 932	PBW 550
Lowest	BWL 936	Stretton	Stretton	BWL 936	RAJ 4134

Table 8 Status in terms of highest/lowest ratio of non enzymatic antioxidants in flag leaf after 5 days of anthesis

	Non enzymatic antioxidants				
Parameter	DPPH	H_2O_2	MDA	Proline	Ascorbic acid
Highest/lowest ratio	4.02	3.25	2.16	2.46	3.38
Genotype					
Highest	DBW 17	BWL 73	PBW 668	PBW 175	WH 1021
Lowest	Ruby	Carnamah	Datatine	Cook	BWL 927

cultivars. Within Australian cultivars wheat cultivars Carnamah has maximum SOD activity. Minimum SOD activity was observed in BW 7197 and PBW 175.

Activity of glutathione reductase (GR) is depicted in Table 9. Minimum GR activity was observed in Australian cultivars. Within Australian cultivars Stretton and Binnu has minimum GR activity. DBW 17, WH 1021, PBW 175, PBW 527, BWL 73, BW 7296 and BWL 676 have higher activity of GR. Among four groups rain-fed cultivars have the highest GR activity.

Mean comparison of catalase (CAT) activity did not show significant difference among four groups. Maximum CAT activity was present in advanced breeding lines while the minimum CAT activity was present in rain-fed cultivars (Table 9). BWL 927 and BWL 934 have high CAT activity.

Tukey's comparison indicated significant difference in ascorbate peroxidase (APX) activity in the wheat cultivars (Table 9). High APX activity was observed in rain-fed cultivars. Within advanced breeding lines BW 4101 and BWL 924 have highest APX activity. Esfandiari et al. (2007) showed that in the leaves of wheat cultivar having high APX activity was related with drought tolerance but no reports are available in the literature showing correlation between activity of APX in wheat grains and abiotic stress tolerance.

Mean comparison of DPPH did not show significant difference (Table 10). Data of non enzymatic antioxidants such as Proline, MDA and H_2O_2 showed the variability in their content in the mature grains of wheat. Tukey's comparison indicated significant difference in Proline, MDA and H_2O_2 content (Table 10). Among the four groups rainfed cultivars have maximum Proline, MDA and H_2O_2 content.

Kumar (2007) reported that high activity of APX and high proline content along with activity of GR and APX might be related to drought stress tolerance. Devi (2008) proposed that high activity APX and GR in mature grains of wheat could be associated with drought tolerance. Grains of DBW 17 (commercial cultivar), PBW 175, PBW 527 and BW 7296 (rainfed cultivars) and PBW 676, BWL 934, BW 7296 (advanced breeding lines) have both high

Table 9 Variability of enzymatic antioxidants in mature grains of wheat

	Enzymatic antioxidants			
	SOD (units min^{-1} g^{-1} FW)	GR (n moles of $NADP^{+}$ formed min^{-1} g^{-1} FW)	CAT (μ moles of H_2O_2 decomposed min^{-1} g^{-1} FW)	APX (n moles of MDA formed min^{-1} g^{-1} FW)
Commercial cultivars				
PBW 343	285 ± 27[bcdef]	511 ± 23[c]	187 ± 19[bcd]	3407 ± 294[d]
PBW 502	262 ± 26[cdefghi]	287 ± 13[defgh]	150 ± 14[defg]	1846 ± 146[efg]
WH 542	256 ± 17[defghi]	631 ± 20[abc]	134 ± 14[defg]	4228 ± 251[abcd]
PBW 550	279 ± 21[bcdefg]	369 ± 26[de]	152 ± 21[defg]	2132 ± 249[ef]
PBW 621	265 ± 23[cdefgh]	377 ± 31[d]	145 ± 11[defg]	2571 ± 294[e]
DBW 17	256 ± 20[defghi]	703 ± 25[ab]	152 ± 17[defg]	4385 ± 243[abc]
PBW 373	269 ± 22[bcdefgh]	585 ± 20[bc]	151 ± 6.4[defg]	4074 ± 212[bcd]
RAJ 3765	267 ± 21[cdefgh]	305 ± 19[defg]	117 ± 20[efg]	1842 ± 113[efg]
DBW 16	307 ± 18[abcde]	305 ± 19[defg]	185 ± 27[bcd]	1892 ± 61[efg]
WH 1021	228 ± 27[efghij]	658 ± 28[ab]	237 ± 29[b]	4467 ± 246[abc]
Mean	267	473	161	3084
Rainfed cultivars				
PBW 175	169 ± 23[ij]	695 ± 17[ab]	138 ± 16[defg]	4646 ± 260[abc]
PBW 527	223 ± 28[efghij]	685 ± 30[ab]	109 ± 17[efg]	5046 ± 147[a]
PBW 596	287 ± 29[bcdef]	255 ± 30[defgh]	120 ± 8[efg]	1735 ± 193[fgh]
WH 1080	258 ± 20[defghi]	233 ± 27[fgh]	139 ± 6[defg]	1589 ± 160[fg]
PBW 644	262 ± 13[cdefghi]	618 ± 20[abc]	126 ± 9[efg]	4378 ± 248[abc]
Mean	240	497	126	3479
Advanced breeding line				
BW 6866	180 ± 12[hij]	244 ± 13[efgh]	142 ± 11[defg]	1628 ± 98[fg]
BW 4101	214 ± 26[efghij]	293 ± 13[defgh]	144 ± 11[defg]	1803 ± 111[efg]
PBW 676	216 ± 23[efghij]	717 ± 21[a]	162 ± 20[cde]	4796 ± 278[ab]
BWL 931	190 ± 23[ghij]	220 ± 19[fgh]	122 ± 23[efg]	1474 ± 176[fg]
BWL 932	261 ± 23[cdefghi]	282 ± 48[defgh]	139 ± 2.9[defg]	1699 ± 236[fg]
BWL 934	292 ± 13[bcdef]	641 ± 20[ab]	316 ± 20[a]	4271 ± 284[abc]
BWL 83	207 ± 25[fghij]	616 ± 34[abc]	116 ± 16[efg]	4110 ± 224[bcd]
BW 6280	188 ± 12[ghij]	305 ± 14[defgh]	133 ± 15[defg]	2121 ± 193[ef]
BWL 936	214 ± 21[efghij]	261 ± 4[defgh]	161 ± 23[cde]	1889 ± 138[efg]
RAJ 4134	270 ± 29[bcdefgh]	641 ± 23[ab]	163 ± 23[cde]	4275 ± 158[abc]
BWL 927	209 ± 30[fghij]	592 ± 41[abc]	328 ± 13[a]	3817 ± 237[cd]
PBW 668	294 ± 9.5[abcdef]	263 ± 8[defgh]	123 ± 6.7[efg]	1732 ± 91[fg]
PBW 687	249 ± 10[defghi]	256 ± 10[defgh]	140 ± 17[defg]	1739 ± 26[efg]
BW 7197	144 ± 30[j]	313 ± 24[def]	100 ± 7.8[g]	2089 ± 160[ef]
BWL 924	284 ± 25[bcdef]	254 ± 7.9[defgh]	120 ± 10[efg]	1732 ± 91[fg]
BWL 73	258 ± 20[defghi]	712 ± 15[a]	102 ± 7[fg]	4564 ± 230[abc]
BW 7296	229 ± 20[efghij]	657 ± 20[ab]	123 ± 10[efg]	4189 ± 288[bcd]
Mean	229	427	155	2819
Australian cultivars				
Cook	294 ± 22[abcdef]	216 ± 38[fgh]	122 ± 13[efg]	1418 ± 243[fg]
Sunco	341 ± 19[abcd]	197 ± 22[fgh]	127 ± 17[efg]	1324 ± 197[fg]
Sunmist	355 ± 12[abc]	202 ± 29[fgh]	158 ± 19[def]	1487 ± 186[fg]
Carnamah	387 ± 25[a]	189 ± 14[fgh]	159 ± 19[def]	1149 ± 53[g]
Stretton	333 ± 10[abcd]	184 ± 37[gh]	158 ± 21[def]	1193 ± 248[g]
Binnu	363 ± 23[ab]	178 ± 37[h]	148 ± 20[defg]	1514 ± 49[g]
Ruby	334 ± 26[abcd]	197 ± 26[fgh]	131 ± 7.4[defg]	1132 ± 157[g]

Table 9 continued

	Enzymatic antioxidants			
	SOD (units min^{-1} g^{-1} FW)	GR (n moles of $NADP^{+}$ formed min^{-1} g^{-1} FW)	CAT (μ moles of H_2O_2 decomposed min^{-1} g^{-1} FW)	APX (n moles of MDA formed min^{-1} g^{-1} FW)
Datatine	363 ± 23^{ab}	194 ± 22^{fgh}	219 ± 14^{bc}	1132 ± 156^{g}
Mean	346	195	153	1293

Values are mean ± SD of three replicates

Mean values with different letters in the same column are significantly different ($p < 0.05$)

Table 10 Variability of non-enzymatic antioxidants in mature grains of wheat

	Non-enzymatic antioxidants			
	DPPH percentage activity	H_2O_2 content (μmol g^{-1} FW)	MDA content (n moles g^{-1} FW)	Proline content (μmol g^{-1} FW)
Commercial cultivars				
PBW 343	49.9 ± 1.8^{hi}	311 ± 24^{abcde}	13.0 ± 0.78^{cdefghi}	0.294 ± 0.002^{c}
PBW 502	59.6 ± 2.8^{f}	268 ± 12^{efghi}	13.5 ± 0.33^{cdefghi}	0.356 ± 0.001^{a}
WH 542	51.3 ± 1.8^{h}	251 ± 13^{fghijk}	11.5 ± 0.85^{ghijklmno}	0.261 ± 0.001^{cde}
PBW 550	71.1 ± 2.8^{ab}	278 ± 26^{defgh}	10.6 ± 0.98^{ijklmnop}	0.171 ± 0.004^{ijklm}
PBW 621	50.6 ± 2.7^{h}	181 ± 26^{mn}	13.5 ± 0.49^{bcdefghi}	0.196 ± 0.004^{fghij}
DBW 17	59.6 ± 2.7^{f}	302 ± 11^{abcde}	12.8 ± 0.48^{cdefghij}	0.230 ± 0.001^{def}
PBW 373	48.1 ± 2.8^{hijk}	252 ± 9.9^{fghijk}	13.4 ± 0.49^{bcdefghi}	0.143 ± 0.004^{mn}
RAJ 3765	61.2 ± 2.8^{ef}	234 ± 14^{hijkl}	10.7 ± 0.49^{jklmnop}	0.206 ± 0.004^{fghi}
DBW 16	69.2 ± 1.8^{abcd}	209 ± 3.5^{jklmn}	11.2 ± 0.28^{ijklmnop}	0.183 ± 0.002^{hijkl}
WH 1021	42.9 ± 2.6^{ijklm}	300 ± 9.1^{abcde}	12.2 ± 0.64^{efghijkl}	0.187 ± 0.006^{ghijk}
Mean	56.3	259	12.2	0.223
Rainfed cultivars				
PBW 175	45.5 ± 2.7^{hijklm}	340 ± 3.5^{a}	11.9 ± 0.99^{ghijklmn}	0.334 ± 0.002^{ab}
PBW 527	47.4 ± 1.8^{hijkl}	252 ± 9.9^{fghijk}	14.0 ± 0.17^{abcdef}	0.223 ± 0.001^{efg}
PBW 596	64.7 ± 2.6^{bcdef}	277 ± 23^{defgh}	14.9 ± 0.7^{abc}	0.195 ± 0.003^{fghij}
WH 1080	63.4 ± 2.7^{cdef}	213 ± 24^{jklmn}	13.1 ± 0.71^{cdefghi}	0.193 ± 0.005^{fghij}
PBW 644	70.5 ± 1.7^{abc}	221 ± 9.1^{jklm}	9.7 ± 0.42^{nop}	0.288 ± 0.008^{c}
Mean	58.3	261	12.7	0.246
Advanced breeding line				
BW 6866	51.9 ± 2.9^{gh}	268 ± 10^{efghi}	13.6 ± 0.28^{bcdefg}	0.278 ± 0.001^{c}
BW 4101	65.9 ± 0.9^{bcdef}	217 ± 14^{jklm}	12.8 ± 0.48^{cdefghij}	0.163 ± 0.008^{jklm}
PBW 676	50.6 ± 2.7^{h}	169 ± 20^{n}	9.35 ± 0.35^{op}	0.214 ± 0.006^{fgh}
BWL 931	63.4 ± 2.6^{cdef}	244 ± 20^{ghijk}	13.9 ± 0.13^{abcdef}	0.143 ± 0.001^{mn}
BWL 932	75.6 ± 1.8^{a}	294 ± 22^{bcdef}	12.3 ± 0.71^{efghijkl}	0.195 ± 0.008^{fghij}
BWL 934	67.2 ± 2.7^{bcde}	253 ± 10^{fghij}	14.6 ± 0.49^{abcd}	0.204 ± 0.006^{fghi}
BWL 83	60.8 ± 2.6^{def}	250 ± 12^{fghijk}	10.0 ± 0.64^{lmnop}	0.121 ± 0.007^{h}
BW 6280	62.6 ± 2.3^{ef}	293 ± 24^{bcdef}	16.0 ± 0.92^{a}	0.187 ± 0.001^{ghijk}
BWL 936	71.1 ± 0.9^{ab}	334 ± 17^{ab}	15.6 ± 0.71^{ab}	0.220 ± 0.006^{fgh}
RAJ 4134	50.6 ± 2.6^{h}	309 ± 2.8^{abcde}	14.2 ± 0.35^{abcde}	0.164 ± 0.002^{jklm}
BWL 927	58.9 ± 1.9^{fg}	328 ± 25^{abc}	10.4 ± 0.78^{jklmnop}	0.266 ± 0.004^{cd}
PBW 668	41.0 ± 1.8^{klm}	250 ± 12^{fghijk}	13.1 ± 0.57^{cdefghi}	0.212 ± 0.006^{fgh}

Table 10 continued

	Non-enzymatic antioxidants			
	DPPH percentage activity	H_2O_2 content (µmol g^{-1} FW)	MDA content (n moles g^{-1} FW)	Proline content (µmol g^{-1} FW)
PBW 687	48.7 ± 1.8^{hij}	268 ± 10^{efghi}	14.6 ± 0.71^{abcd}	0.142 ± 0.003^{mn}
BW 7197	40.4 ± 2.8^{lm}	291 ± 21^{bcdef}	9.2 ± 0.42^{p}	0.152 ± 0.003^{klmn}
BWL 924	48.1 ± 2.7^{hijk}	270 ± 23^{efghi}	12.4 ± 0.49^{defghijk}	0.195 ± 0.008^{fghij}
BWL 73	50.6 ± 2.6^{h}	284 ± 10^{cdefg}	10.2 ± 0.64^{jklmnop}	0.159 ± 0.003^{jklmn}
BW 7296	60.8 ± 0.9^{ef}	207 ± 22^{klmn}	12.5 ± 0.85^{defghijk}	0.203 ± 0.007^{fghi}
Mean	57	267	12.7	0.189
Australian cultivars				
Cook	64.1 ± 1.2^{bcdef}	213 ± 24^{jklmn}	11.3 ± 0.85^{hijklmnop}	0.299 ± 0.008^{abc}
Sunco	48.1 ± 0.9^{hijk}	191 ± 10^{lmn}	13.5 ± 0.48^{bcdefgh}	0.283 ± 0.004^{c}
Sunmist	41.0 ± 1.8^{klm}	237 ± 10^{hijk}	10.3 ± 0.16^{jklmnop}	0.205 ± 0.003^{fghi}
Carnamah	49.4 ± 0.9^{hi}	229 ± 1.4^{ijkl}	10.4 ± 0.25^{jklmnop}	0.193 ± 0.005^{fghij}
Stretton	41.6 ± 0.9^{jklm}	322 ± 21^{abcd}	9.85 ± 0.49^{mnop}	0.204 ± 0.006^{fghi}
Binnu	38.4 ± 1.2^{m}	324 ± 2.8^{abc}	14.6 ± 0.49^{abcd}	0.145 ± 0.006^{lmn}
Ruby	47.4 ± 1.8^{hijkl}	212 ± 22^{jklmn}	9.3 ± 0.71^{op}	0.169 ± 0.008^{ijklm}
Datatine	44.8 ± 1.6^{hijklm}	276 ± 21^{efgh}	12.0 ± 0.92^{efghijklm}	0.221 ± 0.008^{fgh}
Mean	46.9	251	11.4	0.215

Values are mean ± SD of three replicates

Mean values with different letters in the same column are significantly different ($p < 0.05$)

Table 11 Proposed genotypes for studying tolerance to different abiotic stresses on the basis of status of enzymatic and non enzymatic antioxidants

Proposed genotypes at different stages of development		
Vegetative stage	Flag leaf stage	Mature grains
PBW 550	PBW 343	PBW 542
PBW 621	PBW 550	DBW 17
BW 4101	PBW 16	PBW 16
BWL 932	PBW 644	WH 1021
BWL 83	BWL 932	PBW 175
	Binnu	PBW 527
	Ruby	PBW 644
	Datatine	PBW 676
		BWL 73

APX and GR activities. These advanced breeding lines can be tested in the field for drought tolerance.

In the Table 11, an attempt has been made to identify genotypes which could show tolerance to different abiotic stresses. On the basis of available information in the literature, high activity of antioxidant enzymes with low H_2O_2 and MDA content in tissues of normally growing wheat genotypes could help in tolerating different abiotic stresses (Kahrizi et al. 2012, Sairam et al. 1998, Hasheminasab et al. 2012, Valifard et al. 2012, Ashraf et al. 2010).

A genotype if having three characters (high antioxidant enzymes like APX, GR, SOD, CAT, POX and low H_2O_2 and MDA content) out of proposed seven, it might be worth studying for tolerance towards different abiotic stresses. However, it is difficult to pin point the abiotic stress to which genotypes could be tolerant as of non specificity in the role of antioxidant enzymes to different kinds of stresses. Though PBW 550 has been recommended for cultivation under irrigated conditions, but because of high antioxidant activity in leaves during vegetative and flag leaf stage, it could be worthwhile to study the performance of this high yielding cultivar under different abiotic stresses like water deficit conditions (rainfed) and high temperature stress (late sowing). In study conducted over fifty genotypes, Kumar (2007) proposed high activity of APX and proline content of wheat grains could be related with drought tolerance. Devi (2008) proposed that high activity APX and GR in mature grains of wheat could be associated with drought tolerance. On this basis, nine genotypes have at least two of three characters in mature grains. Two advanced breeding lines namely PBW

676 and BWL 73 could be drought tolerant. However, a field study is necessary for validating this proposal.

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