Determination of biochemical responses to drought stress in cultivated and exotic wheat genotypes

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ABSTRACT

Ten wheat genotypes of locally cultivated and exotic origins were grown under drought condition to determine the biochemical responses. Plants were grown in hydroponics in modified Hoagland solution in box container in the Growth Room with temperature, light and humidity control at the Plant Stress Breeding Laboratory (PSBL), at the dept. of Genetics and Plant Breeding, BAU, Mymensingh. Nutrient solution was refreshed after every ten days. Wheat plants were subjected to drought stress, induced by 3% and 6% polyethylene glycol 6000 (PEG 6000) 6000 in hydroponic solution after 47 days of transferring seedling in full strength nutrient solution. Plants were kept in drought treatment for 42 days and on harvesting, leaves were collected for biochemical assays. Six biochemical traits were determined from leaf tissues following standard protocol. Concentration of leaf biochemical *viz.* Catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), hydrogen peroxide (H_2O_2), malondialdehyde (MDH) and proline were found in both increasing and decreasing trend of fluctuation. Not a single genotype was found with the entire biochemical traits with favorable actions, but several genotypes demonstrated relatively better performance compared to control. The relationship among the biochemical traits were also determined and found a positive significant correlation between CAT and POD. A ranking table was also constructed considering mean performance of the genotypes under different PEG 6000 treatment. Considering mean performance, % change of bio-chemicals under drought condition and ranking table, genotypes Sonalika, BARI Gom 21, BARI Gom 29, BARI Gom 30, Summa and Burr were found with better biochemical mechanisms under both 3% and 6% PEG 6000 induced drought and recommend to consider for future wheat improvement program for drought tolerance.

Introduction

Wheat (*Triticum aestivum* L.) is the 2nd of the three major cereals (wheat, rice, and maize) with a global annual production of 756.40 million metric tons from 222.68 million hectares of land, in 2016-2017 (USDA, 2019). Wheat is grown under a wide range of climatic conditions covering temperate, Mediterranean to tropical areas. Wheat in tropical areas mostly grown in winter season which is predominantly dry with lower precipitation. This led wheat plants to suffer with drought stress. In developing countries, about 45% of the 120 million hectares are prone to drought (Macharia and Ngina, 2017). The situation will go further adverse for wheat production as drought affected area will be increased twofold by mid 21st C, threefold by end of 21st C for many regions (Sheffield and Wood, 2008). Seasonally about 250 – 350 mm water is necessary for wheat production with 1.5 – 4.0 mm as daily evapo-transpiration (Sattar, 2004). Drought stress reduces the efficiency of photochemicals, reduces the Rubisco efficiency, gathers of stress metabolites, antioxidative enzymes, such as: peroxidase, catalase, ascorbate peroxidase, glutathione reductase, malondoaldyhide, and reactive oxygen species

(ROS) accumulation *etc*. (Nezhadahmadi *et al*., 2013). These ingredients may initiate disturbing lipid peroxidation, chlorophyll, protein oxidation, and nucleic acids. Plants demonstrate various responses from drought stress, of which some response confers tolerance to drought in different extent (Raffi and Asaduzzaman, 2015). Drought tolerance is a complex mechanism which has a cumulative relation with morphological, physiological and biochemical activities. Among the biochemical mechanisms, antioxidant systems scavenge or detoxify of excess ROS which eventually reduces the damages of cellular activities due to oxidation (Noctor and Foyer, 1998). Antioxidative enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), glutathione-S-transferase (GST), glutathione peroxidase (GP), mono dehydro ascorbate reductase (MDHAR) are produced which eventually contribute to the scavenging of accumulated ROS in plants under water stress (Sairam *et al*., 1998; Nezhadahmadi *et al*., 2013). Biochemical traits show

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high variability and heritability as well as association with grain yield that can be exploited as selection indicators to enhance selection efficiency of breeding programs (Saed-Moucheshi *et al*., 2019). This experiment was aimed at understanding of biochemical mechanisms for drought tolerance in local and exotic wheat genotypes in response of induced drought stress.

Materials and Methods

Ten wheat genotypes (seven local: BARI Gom 20, BARI Gom 21, BARI Gom 28, BARI Gom 29, BARI Gom 30, BARI Gom 31, Sonalika and three exotic: Macya, Burr and Summa) with three replications in Randomized Completely Block Design (RCBD) design were used to study their biochemical mechanisms against drought condition. Seven local genotypes were primarily introduced or developed from CIMMYT lines by Wheat Research Centre (Now, Bangladesh Wheat and Maize Research Institute) for their better agronomic features. These genotypes are well adapted to Bangladesh and can be used to improve with new characteristics from exotic materials. The exotic materials are varieties brought from Saudi Arabia considering dry agro-climatic condition. Catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), hydrogen peroxide (H_20_2), malondialdehyde (MDA) and proline are the biochemical traits which were considered in this study. For seed germination and seedling establishment, standard procedures were followed as described by Malik *et al*. (2009). Plants were grown in 20 litre plastic containers popularly known as Milk Crate by RFL Plastic Industries Bangladesh, filled with hydroponic solution (Hoagland and Arnon, 1950) in controlled environment facilities at the Plant Stress Breeding Laboratory (PSBL) at the Department of Genetics and Plant Breeding, BAU. Optimum growth condition was maintained throughout the growing period of wheat genotypes (14h/10h light/dark period, $22^oC/18^oC$ day/night temperature, 60%/65% day/night RH and 4500-5500 lux light intensity) for their normal growth and development. The pH of the nutrient solution was maintained at 6.5. Nutrient solution was refreshed after every 10 days. Sometimes, humidifier was used to maintain the relative humidity of the growth room. Air bubbler was used to ensure availability of oxygen to the root zone inside the container. Drought was induced by applying PEG 6000 (Mark, India) at 0%, 3% and 6% in the nutrient solution on 47th day of transferring seedlings (Zadoks Scale 22; Zadoks *et al*., 1974) to full strength solution and kept for 6 weeks (Zadok's Scale 41). A schematic presentation of the activities done for the experiment was presented in Fig 1.

Biochemical analysis

Flag leaf was selected for biochemical data collection. This leaf sample was collected after 42 days of PEG 6000 treatment. Fresh leaf sample were used for biochemical analysis.

Sample extraction for CAT, POD and APX

Fifty milligrams of fresh plant sample was collected and homogenized with 3 ml of 50mM potassium phosphate buffer (p^H 8.0) in a mortar and pestle. The homogenate was centrifuged at 12,000 rpm for 10 min. In all stage $4^\circ C$ temperature was maintained. The clear supernatant was used for assaying CAT, POD and APX activity.
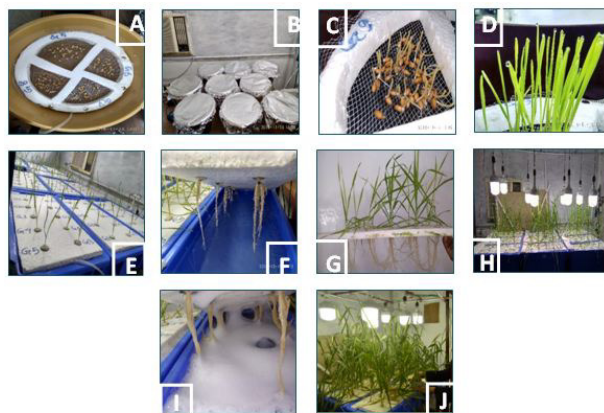

Fig. 1. Hydroponic Culture with drought treatment; (A) Placement of wheat seeds; (B) Germination of seedlings; (C) Four days old seedling; (D) Seedlings before transferring to the main container; (E) Seedlings transplantation; (F) Nutrient medium; (G) & (H) vegetative period; (I) Drought induction with 3% and 6% PEG; (J) Plants at reproductive stage grown under drought treatment

Determination of CAT activity

CAT activity was determined by following the method of Aebi (1984). 0.7 ml of 50 mM potassium phosphate buffer (p^H 8.0), 0.1 ml of EDTA and 0.1 ml H_2O_2, were added in an eppendorf and mixed well. In all stage $4^\circ C$ temperature maintained. Reaction was started by adding 0.1 ml of enzyme extract and changes in absorbance were recorded immediately at 240 nm at 30 seconds interval for two minutes. The activity of catalase was calculated from the decrease in absorbance in per minute according to following formula:

CAT (mM $min^{-1}g^{-1}$ F. W.) =

$$\frac{(Absorbance\ difference\ per\ min)\times Dillution factor\times 1000}{40\times 1000}$$

Here, extinction Coefficient = 40 $M^{-1}cm^{-1}$ of CAT

Determination of POD activity

POD activity was determined by following the method of Nakano and Asada (1981). In an eppendorf tube, 0.6 ml of 50 mM potassium phosphate buffer (p^H 8.0), 0.1 ml of EDTA, 0.1 ml of H_2O_2 and 0.1 ml of Guaiacol were added and mixed well. Reaction was started by adding 0.1 ml of enzyme extract and changes in absorbance were recorded immediately at 470 nm at 30 seconds interval for two minutes.

The activity of peroxidase was calculated from the increase in absorbance in per minute according to following formula.

POD (µmole $min^{-1}g^{-1}$ F. W.) =

$$\frac{(Absorbance\ difference\ per\ min \times 4) \times Dillution\ factor \times 1000}{26.6 \times 1000}$$

Here, Extinction Coefficient = 26.6 $M^{-1}cm^{-1}$ of POD

Determination of APX activity: APX activity was determined by following the method of Nakano and Asada (1981). In an eppendorf tube, 0.6 ml of 50 mM potassium phosphate buffer (p^H 8.0), 0.1 ml of EDTA, 0.1 ml of H_2O_2 and 0.1 ml of Guaiacol were added and mixed well. Reaction was started by adding 0.1 ml of enzyme extract and changes in absorbance were recorded immediately at 290 nm at 30 seconds interval for two minutes. The activity of ascorbate peroxidase was calculated from the decrease in absorbance in per minute according to following formula.

APX (µmole $min^{-1}g^{-1}$ F. W.) =

$$\frac{(Absorbance\ difference\ per\ min) \times Dillution\ factor \times 1000}{2.8 \times 1000}$$

Here, Extinction Coefficient = 2.8 $M^{-1}cm^{-1}$ of APX

Determination of H_2O_2 activity: H_2O_2 content was determined using the method given by Velikova *et al.,* (2000). About 0.1 g of fresh leaf sample was homogenized in a pre-chilled mortar with pestle using 1ml of 0.1% tri-chloro-acetic acid (TCA) and homogenize it at 4°C. The homogenate was centrifuged at 12,000 rpm for 15mins. 0.5m supernatant of sample was then mixed with 0.5mL of 10mM $PO4^{3-}$ buffer (pH 7) and 1.0mL of 1M potassium iodide. The absorbance was recorded at 390nm and calculation was done according to following formula.

H_2O_2 (µmole g^{-1} F. W.) =

$$\frac{(Absorbance}{Extincion\ Coefficient} \times Dilution\ factor$$

Here, Extinction Coefficient of H_2O_2 = 0.28 µ $M^{-1}cm^{-1}$

Determination of MDA activity

MDA activity was determined by following the method of Heath and Packer (1968). About 0.1 gm of leaf tissue was homogenized by adding 0.5 ml 0.1 % (w/v) TCA. The homogenate was then centrifuged for 10 min (10000 rpm) and supernatant was collected. Then 0.5 ml of supernatant was mixed with 1.5 ml 0.5% TBA diluted in 20 % TCA and incubate in water bath at 95°C for 25 min and then quickly cooled on ice. Absorbance was measured at 532 and following equation was used to calculate the amount of MDA in nmole g^{-1} F. W. All the steps were performed at 4°C except absorbance.

Melandialdehyde (MDA) (nmole g^{-1} F. W.) =

$$\frac{(Absorbance}{Extincion\ Coefficient} \times Dilution\ factor$$

Here, Extinction Coefficient of MDA = 155 m $M^{-1}cm^{-1}$

Determination of proline activity

Proline was determined according to the method described by Bates *et al.* (1973). Approximately 0.5g of fresh leaf was homogenized in 10 ml of 3% aqueous sulfosalicyclic acid and filtered through Whatman's No. 2 filter paper. Two ml of filtrate was mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 hr at 100°C. The reaction mixture was extracted with 4 ml toluene and the chromophore containing toluene was aspirated, cooled to room temperature, and the absorbance was measured at 520 nm. A standard curve was prepared with analytical grade proline and proline contents in sample were calculated by using the standard curve. The percentage of proline present in the leaves was expressed as mg/100g fresh leaves. Data were managed with MS Excel spreadsheet and analysed using STAR 2.0.1 (IRRI) software.

Results and Discussion

The results of the experiment conducted on ten wheat genotypes at PEG 6000 induced drought condition were analysed, presented, and discussed in the following section under respective sub-headings. Here, mean performances of ten genotypes under control and drought stress for six biochemical traits were presented in Table 1 and per cent changes of biochemical traits due to drought stress in Fig. 2.1 to 2.6.

Catalase content

Catalase detoxify plant cell by converting H_2O_2 to H_2O or O_2 (Abid *et al.*, 2018). In a study, Islam *et al.* (2015) found that leaf CAT concentration was produced more in drought tolerant variety than drought susceptible variety in drought condition. Here, synthesis of catalase in leaf was found with both increasing and decreasing trends under PEG treatments (Table 1 and Fig 2.1). Genotypes showed differences in CAT synthesis even in control condition indicating differences in inherent capability for CAT synthesis. Based on mean performance, under PEG treatments, Summa showed highest synthesis (0.39 mM/min/gm FW) followed by Sonalika (0.255 mM/min/gm FW) at 3% PEG, and BARI Gom 21 with highest CAT conc. (0.81 mM/min/gm FW) followed by Burr (0.37 mM/min/gm FW) and Sonalika (0.33 mM/min/gm FW) at 6% PEG. When considered % change of CAT conc. due to PEG treatment at 3% and 6% PEG over control and in-between (Fig. 2.1), Sonalika demonstrated highest increase at both 3% PEG (25400%) and 6% PEG (32900%) over control, as Sonalika demonstrated lowest synthesis of CAT in control condition. Thus, Sonalika was the most responsive genotype compared to others. By contrast, lowest synthesis was observed in BARI Gom 28 (-90.91%) and BARI Gom 31 (-96.18%) at 3% and 6% PEG, respectively.

Furthermore, highest per cent increase of catalase in 6% PEG over 3% PEG was observed in BARI Gom 21 (260%) and lowest in Summa (-96.16%). In summary, genotypes with higher CAT activity under drought stress were Sonalika, BARI Gom 21, Summa and Burr and therefore, recommended for future drought tolerance improvement program.

Table 1. Mean performance of wheat genotypes for biochemical traits under different concentrations of PEG 6000

Genotypes	CAT (mM/min/gm FW)			APX (microM/min/gm FW)			POD (microM/min/gm FW)		
				PEG concentrations					
	0	3%	6%	0	3%	6%	0	3%	6%
BARI Gom 20	0.6 b	0.075 f	0.1950 e	0.8568 g	1.928 e	0.6426 h	0.5175 e	0.1125 f	0.4275 c
BARI Gom 21	0.228 e	0.2250 c	0.8100 a	2.142 b	2.142 d	3.427 c	0.4275 f	0.3375 c	0.4050 c
BARI Gom 28	0.33 d	0.03 g	0.06 f	1.928 c	1.071 g	0.4284 i	0.4500 f	0.045 g	0.54 b
BARI Gom 29	0.528 c	0.1350 de	0.3150 c	1.288 f	1.499 f	4.927 a	0.3150 g	0.2025 de	0.63 a
BARI Gom 30	0.24 e	0.075 f	0.24 d	1.320 f	6.640 a	0.8568 g	0.7633 b	0.1125 f	0.54 b
BARI Gom 31	1.178 a	0.15 d	0.045 f	2.142 b	0.1 i	1.071 f	0.9675 a	0.2250 d	0.045 h
Sonalika	0.001 h	0.255 b	0.33 c	1.714 d	3.709 b	3.213 d	0.5625 d	0.3825 b	0.1350 g
Macya	0.135 g	0.1350 de	0.04503 f	1.928 c	2.785 c	1.928 e	0.6750 c	0.2025 de	0.2407 e
Burr	0.15 fg	0.1226 e	0.37 b	1.499 e	0.4284 h	4.070 b	0.2700 h	0.1800 e	0.2025 f
Summa	0.18 f	0.39 a	0.015 g	4.498 a	0.4284 h	3.354 c	0.7425 b	0.5850 a	0.3373 d
LSD	0.4202	0.02426	0.02426	0.1057	0.08046	0.08747	0.02426	0.02426	0.02426
CV (%)	22.34	23.66	5.38	10.12	5.04	4.71	6.58	6.11	8.7
Minimum	0.001	0.03	0.015	0.8568	0.1	0.4284	0.27	0.045	0.045
Maximum	1.178	0.39	0.81	4.498	6.64	4.927	0.9675	0.585	0.63
Mean	0.357	0.159	0.263	1.932	2.063	2.392	0.569	0.238	0.35

Table 1. (Continued)

| Genotypes	H$_2$O$_2$ (nmol/gm FW)			MDA (micro M/gm FW)			Proline (mg/ml.)		
				PEG concentrations					
	0	3%	6% PEG	Control	3% PEG	6% PEG	Control	3% PEG	6% PEG
BARI Gom 20	5.114 f	5.429 a	6.645 a	0.0157 ab	0.0128 bc	0.012 bc	0.0079 a	0.0101 a	0.004833 b
BARI Gom 21	3.657 g	3.629 f	4.429 d	0.007 b	0.0592 a	0.0263 a	0.0016 f	0.0051 bc	0.002067 c
BARI Gom 28	5.114 f	4.686 b	5.629 b	0.0041 b	0.0153 bc	0.007 d	0.0065 bc	0.0066 b	0.0078 a
BARI Gom 29	7.971 bc	4.571 c	3.114 g	0.0325 a	0.0354 ab	0.0074 d	0.003867 de	0.005867 b	0.0017 c
BARI Gom 30	7.914 c	4.4 e	2.629 h	0.0094ab	0.0058 c	0.0093 cd	0.0068 bc	0.0065 b	0.0017 c
BARI Gom 31	7.943 bc	4.486 d	4.114 e	0.00523 b	0.00753 c	0.0052 d	0.004233 d	0.0011 d	0.0014 c
Sonalika	7.714 d	3.457 g	3.486 f	0.004467 b	0.0172 bc	0.02320 a	0.0048 d	0.0021 d	0.0014 c
Macya	8.086 b	4.543 c	4.2 de	0.0035 b	0.0203 bc	0.0074 d	0.007433 ab	0.001367 d	0.00183 c
Burr	10.57 a	1.203 i	5.2 c	0.0037 b	0.006 c	0.0079 cd	0.0031 e	0.005567 b	0.0022 c
Summa	7.086 e	2.578 h	3.6 f	0.0056 b	0.0143 bc	0.01470 b	0.0063 c	0.0025 cd	0.0023 c
LSD	0.1495	0.04202	0.2533	0.02426	0.02426	0.004539	0.001085	0.002657	0.00203
CV (%)	1.45	1.33	7.65	49.16	44.78	48.24	30.92	45.57	40.88
Minimum	3.657	1.203	2.629	0.0035	0.0058	0.0052	0.0016	0.0011	0.0014
Maximum	10.57	5.429	6.645	0.0325	0.0592	0.0263	0.0079	0.0101	.0078
Mean	7.117	3.898	4.304	0.009	0.019	0.012	0.005	0.005	0.003
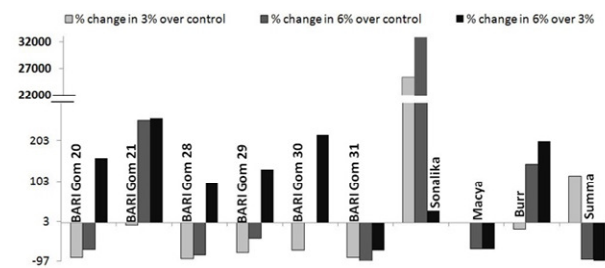
Fig. 2.1 Per cent changes in leaf Catalase (CAT) content in ten wheat genotypes under 3% and 6% PEG 6000 treatment

Ascorbate peroxidase (APX) content

According to Mittler (2002), APX content found in increased amount in drought tolerant plant against drought condition compared to control. APX uses ascorbate as the electron donor for the reduction of H$_2$O$_2$ to scavenge, especially when CAT is absent (De Gara *et al*., 2003, Huseynova, 2012). Similar to leaf catalase content, synthesis of APX in leaf was found with both increasing and decreasing trends under PEG treatments (Table 1, Fig. 2.2). Genotypes showed differences in APX synthesis even in control condition indicating differences in inherent genotypic capability for APX synthesis. Based on mean performance, under PEG treatments, BARI Gom 30 demonstrated highest synthesis of APX at 3% PEG (6.64 microM/min/gm FW), followed by Sonalika (3.709 microM/min/gm FW); but at 6% PEG, the highest by BARI Gom 29 (4.97 microM/min/gm FW), followed by Burr (4.04 microM/min/gm FW) and Summa (3.354 microM/min/gm FW). Considering % changes of APX due to PEG treatments, BARI Gom 30 demonstrated highest APX synthesis (403.03%) at 3% PEG over control but decrease (-87.09%) at 6% PEG over 3% PEG. Genotypes BARI Gom 29 (282.53%), Burr (171.51%), Sonalika 87.46%) and BARI Gom 28 (59.99%) demonstrated increase of APX synthesis in 6% PEG compared to control, suggesting inherent capability

Fig. 2.2 Per cent changes in leaf Ascorbate peroxidase (APX) content in ten wheat genotypes under 3% and 6% PEG 6000 treatment
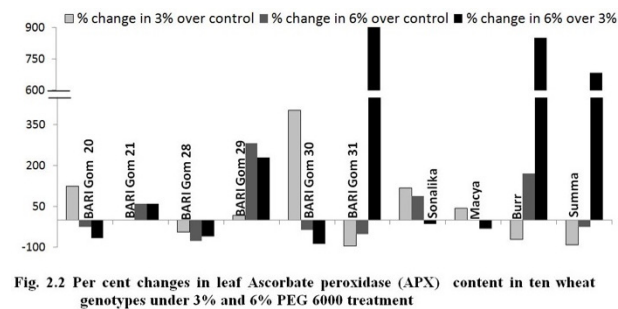

Fig 2.3 Per cent changes in leaf Peroxidase (POD) content in ten wheat genotypes under 3% and 6% PEG 6000 treatment

of synthesizing more APX under drought stress. Compared to 3% PEG, five of the ten genotypes demonstrated decrease in synthesis of APX at 6% PEG, indicating inability of continuing APX synthesis in higher drought stress. Therefore, other five genotypes *viz.* BARI Gom 21, BARI Gom 29, BARI Gom 31, Burr and Summa, deemed as better performers for profound capacity of APX synthesis in higher drought condition. Considering output from Table 1 and Fig. 2.2, genotypes BARI Gom 29, BARI Gom 30, Burr and Summa can be recommended for future exploitation.

Peroxidase (POD) content

Drought stress in the plant tissue is alleviated by a concerted action of both enzymatic and non-enzymatic antioxidant systems which includes POD enzymes (Prochazkova *et al.*, 2001). Islam *et al.* (2015) found that POD content was produced more in drought tolerant genotype than drought susceptible in drought condition. Synthesis of POD in leaf was found with both increasing and decreasing trends under PEG treatments (Table 1, Fig. 2.3). Genotypes showed differences in POD synthesis even in control condition indicating differences in inherent genotypic capability for POD synthesis. Based on mean performances, under PEG treatments, Summa performed better (0.58 microM/min/gm FW), followed by Sonalika (0.38 microM/min/gm FW) at 3% PEG. By contrast under 6% PEG treatment, BARI Gom 29 (0.63 microM/min/gm FW) enhanced POD synthesis ability followed by BARI Gom 28 and BARI Gom 30 (0.54 microM/min/gm FW). Considering % change of POD synthesis upon PEG treatments (Fig. 2.3), POD synthesis decreased in all genotypes at 3% PEG over control. However, in higher PEG treatmen, *i.e.* at 6% PEG compared to control, genotypes BARI Gom 29 (100%) and BARI Gom 28 (20%) demonstrated increase in POD synthesis. By contrast, at 6% PEG compared to 3% PEG, along with BARI Gom 28 (1100%) and BARI Gom 29 (211.11%), BARI Gom 30 (380%) demonstrated increased POD synthesis. Considering output from Table 1 and Fig. 2.2, genotypes BARI Gom 29 and BARI Gom 28 can be recommended for future utilization for improving drought tolerance.
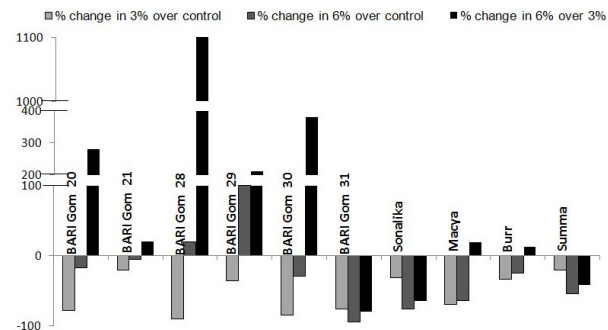
Hydrogen peroxide (H_2O_2) content

Drought stress reduces the carbon assimilation results in an imbalance between electron excitation and utilization through photosynthesis, which results in the production H_2O_2 (Abid *et al.*, 2018). At higher concentration, H_2O_2 is deleterious to cell, but at lower concentration, it acts as a signaling molecule to indicate drought stress (Caverzan *et al.*, 2016). Accumulation of H_2O_2 in leaf was found with both increasing and decreasing trends under PEG treatments (Table 1, Fig. 2.4). Genotypes showed differences in H_2O_2 synthesis even in control condition indicating differences in inherent genotypic capability for H_2O_2 synthesis. Based on mean performances, under PEG treatments, Burr (1.203 nmol/gm FW) followed by Summa (2.578 nmol/gm FW) produced lower amount H_2O_2 at 3% PEG among all. In higher amount of PEG (6%), BARI Gom 30 (2.629 nmol/gm FW) and BARI Gom 29 (3.114 nmol/gm FW) produced lower amount of H_2O_2 among all genotypes. BARI Gom 20 produced higher amount of H_2O_2 both at 3% (5.429 nmol/gm FW) and 6% PEG (6.645 nmol/gm FW), indicating its inability to scavenge H_2O_2 by anti-oxidants produced due to PEG treatments.

Considering % change of H_2O_2 production upon PEG treatments (Fig. 2.4), all the genotypes except BARI Gom 20 and BARI Gom 21 demonstrated increase in production of H_2O_2 at 3% PEG over control, where Burr demonstrated lowest production (-88.61%) followed by Summa (-63.61%). At 6% PEG over control, production of H_2O_2 increased in BARI Gom 20, BARI Gom 21 and BARI Gom 28, and decreased in other genotypes. Here, genotype BARI Gom 30 (-66.78%) demonstrated decrease in production of H_2O_2 followed by BARI Gom 29 (-60.93%). By contrast, only four genotypes demonstrated decrease in concentration of H_2O_2 at 6% PEG compared to 3%, *viz.* BARI Gom 29 (-31.87%), BARI Gom 30 -40.25%), BARI Gom 31 (-8.29%) and Macya (-7.55%) compared to other genotypes.
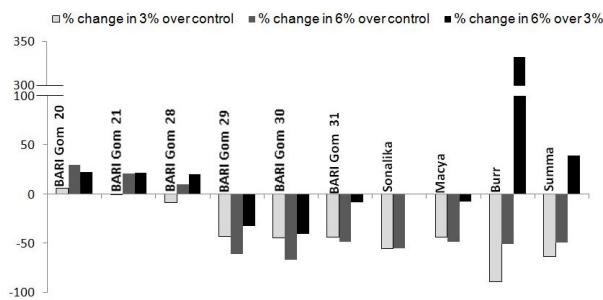
Fig 2.4 Per cent changes in H_2O_2 content in ten wheat genotypes under 3% and 6% PEG 6000 treatment

Fig 2.5 Per cent changes in Malondialdehyde (MDA) content in ten wheat genotypes under 3% and 6% PEG 6000 treatment

The decreasing rate of H_2O_2 accumulation in genotypes might be due to scavenging of H_2O_2 by the anti-oxidants molecules produced from PEG treatments. In summary form Table 1 and Fig. 2.4, genotypes BARI Gom 29, BARI Gom 30, Burr and Summa could be recommended for exploitation to improve H_2O_2 related drought response mechanism in wheat.

Malondialdehyde (MDA) content

The inter-cellular accumulation of MDA, a product of fatty acid peroxidation, in plants due to cellular membrane lipid peroxidation is a measure of oxidative stress induced membrane damage during water stress (Farooq *et al.* 2010; Abid *et al.*, 2018). Therefore, increased MDA content in drought stressed cell indicates more oxidative damage, which eventually refrains plants to tolerate drought stress. Accumulation of MDA in leaf was found with both increasing and decreasing trends under PEG treatments (Table 1, Fig. 2.5). Genotypes showed little difference in MDA accumulation in control condition indicating differences in inherent genotypic capability for oxidative damage to membrane. Based on mean performances, under PEG treatments, BARI Gom 30 (0.0058 micro M/gm FW), BARI Gom 31 (0.00753 micro M/gm FW) and, Burr (0.006 micro M/gm FW) accumulated lowest amount of MDA in leaf due to 3% PEG treatment. By contrast, BARI Gom 28, BARI Gom 29, BARI Gom 31 and Macya demonstrated lowest accumulation of MDA in leaf tissues ranging from 0.0052 to 0.007 micro M/gm FW at 6% PEG. Considering % change of MDA accumulation due to PEG treatments compared to control, and among PEG treatments, two genotypes, namely, BARI Gom 30 (-38.73%) and BARI Gom 20 (-18.47%) accumulated lower amount of MDA at 3% PEG compared to control. At 6% PEG compared to control, rate of accumulation of MDA in leaf tissues were found in BARI Gom 29 (-77.23%), BARI Gom 20 (-23.57%), BARI Gom 30 (-1.76%) and BARI Gom 31 (-0.63%). Among PEG treatments, accumulation rate of MDA found lower in six genotypes, namely, BARI Gom 20, BARI Gom 21, BARI Gom 28, BARI Gom 29, BARI Gom 30, BARI Gom 31 and Macya (range: -6.25% to -79.09%).
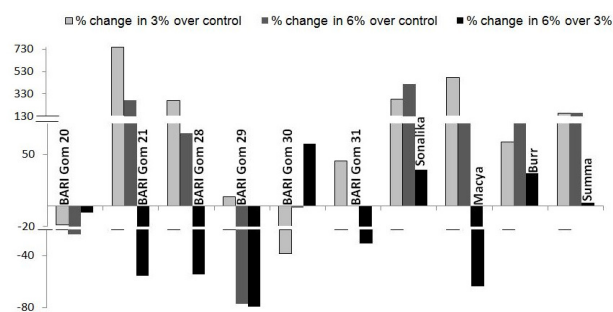
Considering Table 1 and Fig. 2.5, BARIGom20, BARI Gom 29, BARI Gom 30 could be recommended for improving MDA related traits in wheat.

Proline content

Plant brings osmotic adjustment by producing more proline which a compatible solute and helps plant to maintain turgor pressure and cell volume at lower water potential for facilitating metabolic functions (Hammad and Ali, 2014; Abid *et al.*, 2018). Therefore, increase of proline in drought suffered cells offers plants an ameliorating option to keep cellular activities by increasing the osmotic potential lowered due to drought (Errabii *et al.*, 2006). Synthesis of proline in leaf was found with both increasing and decreasing trends under PEG treatments (Table 1 and Fig. 2.6). Genotypes showed differences in proline synthesis even in control condition indicating differences in inherent capability for proline synthesis. Based on mean performance, under both PEG treatments, genotypes demonstrated little differences for proline synthesis, as BARI Gom 20 (0.0101 mg/ml) demonstrated the highest amount synthesis at 3% PEG and BARI Gom 28 (0.0078 mg/ml) at 6% PEG. Considering % change of proline due to PEG treatments, the highest increase rate of proline was found in BARI Gom 21 (218.75%) followed by BARI Gom 20, BARI Gom 28, BARI Gom 29 and Burr with a range of 1.53% to 79.58% at 3% PEG over control. Furthermore, only two genotypes demonstrated increasing rate of proline at 6% PEG over control, *viz.* BARI Gom 21 (29.18%) and BARI Gom 28 (20%), indicating enhanced potential of the genotypes to keep cellular activities undamaged under drought condition. When comparing increasing rate of proline at 6% PEG over 3% PEG, genotypes Macya (34.09%), BARI Gom 21 (27.27%) and BARI Gom 28 (18.18%) demonstrated the increase of proline under drought stress. In summary, considering Table 1 and Fig. 2.6, genotypes BARI Gom 20, BARI Gom 21, BARI Gom 28, Macya and Burr could be recommended for improving proline related tolerance mechanism in wheat genotypes.

Fig 2.6 Per cent changes in Proline content in ten wheat genotypes under 3% and 6% PEG 6000 treatment
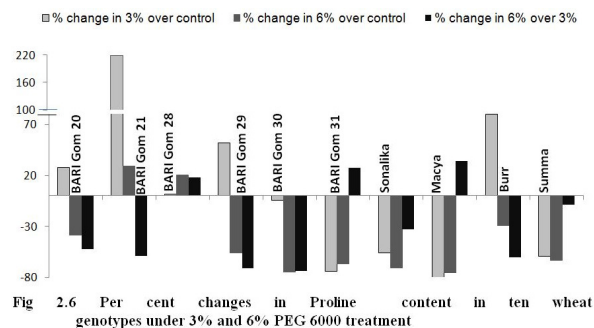
It is evident from the above results that not all the mechanisms conferring drought tolerance are present in a single genotype, which eventually depicts the gradient for drought tolerance in the genotypes studied. Drought tolerance is a complex mechanism and can be achieved with the interplay of different traits with different degree of contributions (Chaves *et al*., 2003). Different genotypes showed tolerances due to different

mechanisms (Bansal *et al*., 2016). For instances, some genotypes might have better osmoprotectant abilities, whereas some might have better ROS scavenging abilities (Abid *et al*., 2018), or some might have both (Laxa *et al*., 2019). Therefore, it is really important to find out inter-relationships between the biosynthesis of different bio-chemicals towards bringing drought tolerance. In the present study, positive and significant linear relationship was observed between POD and CAT in 3% drought condition but not in control condition, indicating that, these traits have synergistic relationship only when drought is induced. However, they did not show significant linear relationship in higher drought level, which indicated their possible independent role to drought tolerance in severe drought condition (Gong *et al*., 2005).

Table 2. Correlation coefficient among biochemical traits under different concentrations of PEG 6000

Traits →	CAT			APX			POD			H2O2			MDA		
	PEG concentrations														
	0	3%	6%	0	3%	6%	0	3%	6%	0	3%	6%	0	3%	6%
APX	-0.149	-0.19	0.529												
POD	0.394	0.973**	0.069	0.387	-0.202	-0.003									
H2O2	-0.06	-0.466	0.033	-0.06	0.278	-0.353	0.042	-0.481	-0.040						
MDA	0.254	0.23	0.513	-0.269	-0.017	0.289	-0.281	0.215	-0.045	-0.014	0.075	0.011			
Proline	-0.068	-0.16	-0.149	0.056	-0.11	-0.321	0.366	-0.218	0.267	-0.027	0.12	0.493	-0.026	0.216	-0.021

** indicates significant at 1% level.

Table 3. Ranking of wheat genotypes based on biochemical responses under different concentrations of PEG 6000

Genotypes	CAT			APX			POD			H2O2			MDA			Proline			Total			**Rank**		
	PEG concentrations																							
	0	3%	6%	0	3%	6%	0	3%	6%	0	3%	6%	0	3%	6%	0	3%	6%	0	3%	6%	0	3%	6%
BARI Gom 20	7	2	3	1	5	2	4	2	6	6	1	1	1.5	2.5	2.5	6	4	2	25.5	16.5	16.5	**3**	**9**	**10**
BARI Gom 21	4	5	7	6	6	7	3	5	6	7	6	4	2	1	1	1	2.5	1	23	25.5	26	**4**	**3**	**3**
BARI Gom 28	5	1	2	5	3	1	3	1	7	6	2	2	2.5	4	4.5	3	3	25.5	12.5	19	**3**	**10**	**8**		
BARI Gom 29	6	3.5	5	2	4	9	2	3.5	8	2.5	3	7	1	1.5	4	2.5	3	1	16	18.5	34	**8**	**7**	**1**
BARI Gom 30	4	2	4	2	9	3	7	2	7	3	5	8	1.5	3	3.5	4.5	3	1	22	24	26.5	**6**	**4**	**2**
BARI Gom 31	8	4	2	6	1	4	8	4	1	2.5	4	5	2	3	4	3	1	1	29.5	17	17	**1**	**8**	**9**
Sonalika	1	6	5	4	8	6	5	6	2	4	7	6	2	2.5	1	3	1	1	19	30.5	21	**7**	**1**	**6**
Macya	2	3.5	2	5	7	5	6	3.5	4	2	3	4.5	2	2.5	4	5.5	1	1	22.5	20.5	20.5	**5**	**6**	**7**
Burr	2.5	3	6	3	2	8	1	3	3	1	9	3	2	3	3.5	2	3	1	11.5	23	24.5	**9**	**5**	**4**
Summa	3	7	1	7	2	7	7	7	5	5	8	6	2	2.5	2	4	1.5	1	28	28	22	**2**	**2**	**5**

However, traits with no significant linear relationship not necessarily indicate no relationship but could be due to lower exposure time of drought, or lower number of sample taken during experiment. In the present study, biochemical traits varied among the ten wheat genotypes under drought stress. There was not a single genotype which performed consistently better for all of the traits against drought stress. Different genotypes showed better performance for different traits such as Sonalika, BARI Gom 21, Summa and Burr for leaf CAT content; BARI Gom 29, BARI Gom 30, Burr and Summa for leaf APX content; BARI Gom 29 and BARI Gom 28 for leaf POD content; BARI Gom 29, BARI Gom 30, Burr and Summa for leaf H_2O_2 accumulation; BARI Gom 20, BARI Gom 29 and BARI Gom 30 for MDA content; BARI Gom 20, BARI Gom 21, BARI Gom 28, Macya and Burr for proline content. Plants developed defense mechanisms against drought by increasing CAT, APX,

proline and POD content for osmotic adjustment as well as scavenging ROS and by decreasing MDA and H_2O_2 in leaf tissues. Considering the individual performances of the wheat genotype to different biochemical characters under drought stress, a ranking table was produced (Table 3) and presented. Genotypes with better performance were scored with higher grade based on the number of letters found while grading. Trait based scores of a genotype were then summed to obtain total score, and based on that, genotypes were ranked. Considering the above approach, cumulatively better biochemical mechanisms under drought stress were found in Sonalika, followed by Summa and BARI Gom 21, at 3% PEG.

At 6% PEG, BARI Gom 29, followed by BARI Gom 30 and BARI Gom 21 were found to have better biochemical responses under drought condition. In general, BARI Gom 21, exotic genotypes Summa and Burr demonstrated consistently better biochemical responses under drought compared to other genotypes. The above-mentioned genotypes, therefore, should be evaluate in more detail with yield data at different level of drought in different stages of growth before considering for future hybridization program to improve drought tolerance in wheat considering biochemical mechanism.

Conclusion

Biochemical parameter has effective responses against drought stress in wheat plants and demonstrated higher variability among the genotypes at different level of drought stress. So, these are very important indicators to recognize the drought stress tolerant variety. This performance profile of genotypes for different biochemical traits could be helpful to select genotypes for hybridization program for drought tolerance in future drought stress breeding program for wheat.

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