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Characterization of wheat (*Triticum aestivum*) genotypes on the basis of metabolic changes associated with water stress*

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With the passage of time, increasing population will need further improvement in wheat (*Triticum aestivum* L. emend Fiori & Paol.) grain production, but major part of India and other countries across the world have been facing tremendous water scarcity due to falling of water-table (Wollenweber *et al.* 2003). Drought is probably the most deadly limiting factor that affects crop production and productivity. Therefore, along with the scientific and judicial exploitation of high-yielding varieties, crop damage/yield loss of wheat is to be checked by introducing drought-tolerant/resistant genotype (s). Recently, drought tolerance is being evaluated on the basis of grain yield and morpho-physiological characters of wheat grown under moisture stress (Kumar and Gupta 2009), but the biochemical aspects related to water retention capacity of plant still needs to be assessed. The inherent levels of these biochemicals and their variation with water stress would be interpreted for the measure of tolerance index. Therefore, a study of the biochemicals involved in metabolic mechanism (s) for water retention, viz proline, reducing sugar, free amino acids, total polyphenol, antioxidants, superoxide dismutase, catalase, peroxidase along with relative water content (RWC) in crossbred wheat genotypes was undertaken. These wheat genotypes were developed for the purpose of stress-resistant character along with high productivity and as evaluated by All India Coordinated Project for Wheat and Barley 2006–09, possessed leading positions nationally in terms of yield, nutritional quality and disease tolerance characters (DWR Progress Report 2009). They also carried recommended characteristics for rainfed plant types (tall plant types, waxy leaves and well organized root systems) and hence were taken to evaluate their drought tolerance potentialities biochemically.

A pot experiment was conducted to study the effect of water stress on biochemical parameters of eight wheat genotypes, *viz* RSP 511, RSP 529, RSP 560, RSP 561, RSP 564 RSP 566 and two national varieties PBW 175 and PBW 343. Seeds were surface sterilized with 0.1% $HgCl_2$ before sowing in 36 cm pots filled with soil and farmyard manure (FYM) mixture in 3:1 ratio. The leaf samples at mid flowering stage (before and after imposing of water stress) were taken for biochemical analysis of the parameters related to water stress. Proline, reducing sugar, free amino acid and relative water content (RWC) in fresh leaves were determined following standard methods. Total polyphenol content of fresh leaf sample was refluxed with 80% hot methanol for 1 hr, filtered and was assayed by measuring blue colour developed (due to reduction of phosphotungsto-molybdic acid by polyphenol) at 725nm spectrophotometrically (Schanderi 1970). For antioxidant assay, dried and powdered leaves samples (100 mg) were extracted with 20 ml of 50% methanol for 2 hr at room temperature and antioxidant activity (AOA) of the extract was estimated by auto oxidation of á-carotene and linoleic acid coupled reaction according to method given by Emmons and Peterson (1999). Enzymes of fresh leaf sample (0.5 g) were extracted with 10 ml ice cold 0.1 M Tris-HCl buffer (*p*H 7.5) containing 5×10^{-3} M mercaptoethanol at 4°C with centrifugation for 10 000 rpm × 15 min. Catalase, Superoxide dismutase (SOD) and peroxidase (POD) activities were assayed spectrophoto-metrically using standard methods.

Table 1 revealed that the accumulation of osmolytes in leaf increased remarkably with the corresponding decline of RWC in response of water stress in all the eight wheat genotypes/varieties under study. Proline, reducing sugar and free amino acid levels raised by 4.21– 4.87, 2.70–3.09 and

*Short note

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Table 1 Effect of water stress on proline, free amino acid, reducing sugar and relative water contents of different wheat genotypes

Wheat genotype	Proline µmole/g leaves control	Proline µmole/g leaves stress	RWC (%) control	RWC (%) stress	Reducing sugar (%) control	Reducing sugar (%) stress	Free amino acid (%) control	Free amino acid (%) stress
PBW 175	2.37	12.35 (+4.21x)	71.83	57.33 (–20.22%)	0.57	2.33 (+3.09x)	0.78	3.00 (+2.85x)
RSP 561	1.59	9.33 (+4.87x)	68.94	55.25 (–19.37%)	0.50	2.00 (+3.00x)	0.71	2.77 (+2.90)
RSP 566	1.09	6.07 (+4.57x)	58.33	47.06 (–19.82%)	0.56	2.15 (+2.84x)	0.68	2.58 (+2.79x)
RSP 529	2.68	14.96 (+4.58x)	76.25	60.48 (–19.67%)	0.96	3.92 (+3.08x)	0.85	3.58 (+3.21x)
PBW 343	1.60	8.87 (+4.54x)	61.68	50.20 (–18.65%)	0.48	1.95 (+3.06x)	0.52	2.02 (+2.88x)
RSP 564	2.70	14.37 (+4.32x)	80.68	61.71 (–23.47%)	0.67	2.55 (+2.81x)	1.13	4.41 (+2.90x)
RSP 511	1.85	9.83 (+4.31x)	75.29	60.28 (–20.94%)	0.56	2.13 (+2.80x)	0.85	3.44 (+3.06x)
RSP 560	1.67	8.74 (+4.32x)	65.50	51.65 (–21.15%)	0.54	1.99 (+2.70x)	0.76	2.85 (+2.75x)
CD (*P* = 0.5)	0.692	0.492	1.465	1.105	0.026	0.172	0.024	0.179

2.58–4.41 times, respectively with respect to control. Genotype RSP 564 raised proline, reducing sugar and free amino acid accumulation from control to water stress condition (2.70–14.37 µmole/g), (0.67–2.55%) and (1.13–4.41%) respectively whereas genotype RSP 529 showed rise of proline (2.68–14.96 µmole/g), reducing sugar (0.96–3.92%) and free amino acid (0.85–3.44%) respectively and were found statistically superior and followed by national cultivar PBW 175 which had third position in all three parameters. But other national cultivar PBW 343 and RSP 566 showed lowest values in these parameters. On the other hand, water stress led to fall in leaf RWC of the genotypes from the control values (58.33–80.68%) to the level (47.06–61.71%) in RSP 564 and RSP 529 genotypes which declined from 80.68 to 61.71 and 76.25 to 60.48 respectively, maintained highest water retention capacities even under water stress condition (at par to each other). RSP 566 which carried lowest values of RWC both in control (58.33%) and under water stress (47.06%) was assessed as poorest in water retention.

Proline is reported to accumulate in response to cold, high temperature, drought and salinity stress conditions in living cells and function as protectant for enzymes and cellular structure under these adverse conditions (Rana and Bansal 2006). Increase in proline accumulation is interpreted as cumulative effect comprising enhanced *de novo* proline biosynthesis, loss/decrease in feed back inhibition of proline synthesis and proline oxidation. Proline accumulation is also correlated to increase in total amino acids and sugar during moisture stress. Tolerant genotypes are seen to accumulate high concentration of osmolytes, *viz.* proline, sugars, free amino acid etc. which have been shown to be introduced by drought stress conditions in majority of cases (Mulla *et al.* 2006). Moisture stress causes loss of water content in leaves of plants. Crop that appears to be drought tolerant also maintains higher leaf water potential in leaves during direct or indirect moisture stress. The decrease in water availability for transport-associated processes lead to change in the concentration of many metabolites followed by distribution in amino acid, carbohydrate metabolism and increase in synthesis of compatible solutes such as special reducing sugars and amino acids as observed in wheat. The increase in reducing sugar level under water stress may also be ascribed to an increase in starch hydrolysis (Gang-ping *et al.* 2008).

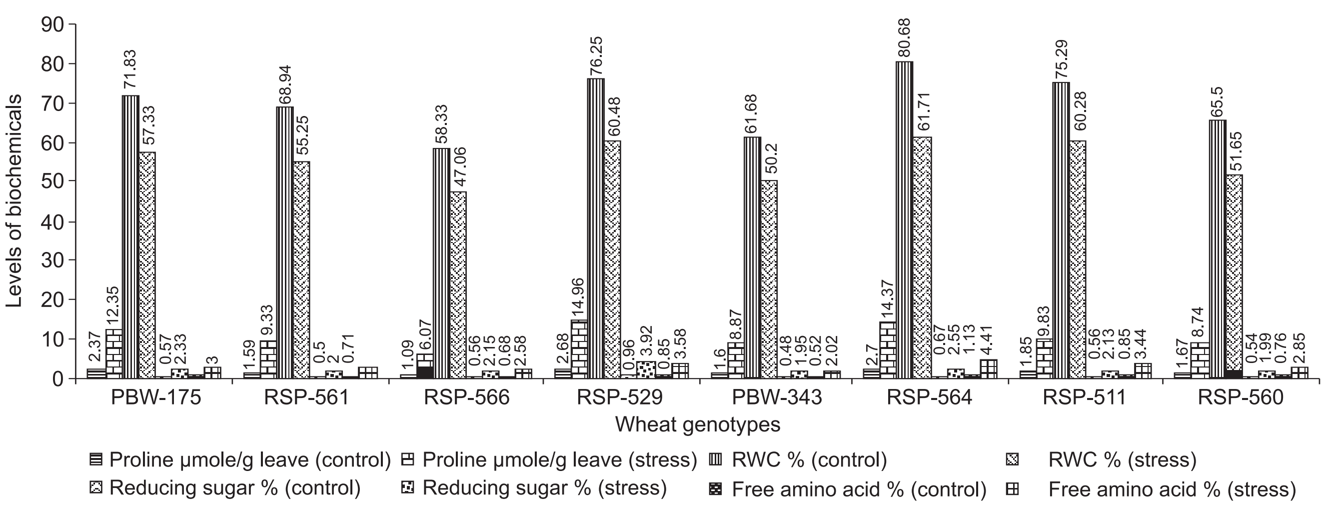

Fig 1 Proline, RWC, reducing sugar and free amino acids acculation in response to water stress in different wheat genotype

Free amino acids, reducing sugar, proline and other osmolytes largely accumulate in plant in response to water stress to maintain RWC and also to act as active oxygen scavengers (Mahatma *et al.* 2007). Thus, comparatively high leaf water retention is due to the cumulative effects of high amount of proline, reducing sugar and free amino acid accumulation under water deficit conditions. Recently, identification of drought-tolerant/resistant wheat genotypes is being executed on the basis of accumulated levels of osmolytes and also relative leaf water content (Mulla *et al.* 2006). Therefore, RWC along with proline, reducing sugar and free amino acid can be considered as an index for determining drought tolerance in crop species and hence could be used as marker to evaluate the genetic potential of a genotype for water stress tolerance in crop species. In the present experiment, genotype RSP 564 and RSP 529 which accumulated maximum osmolytes and maintained highest leaf relative water content were considered as best water stress-tolerant wheat genotypes and both of them were found to be better than national cultivars PBW 175 and PBW 343. RSP 566 was weak in this aspect, followed by PBW 343. In context to these biochemicals, the comparative potentials of the genotypes are depicted in Fig 1.

Data of secondary metabolites and detoxification enzymes (Table 2) elucidated that total polyphenol, antioxidant content and the activities of the detoxification enzymes, viz catalase, SOD and peroxidase in wheat genotypes under water stress increased remarkably from 13.67 to 33.10%, 21.9 to 92.45%, 15.72 to 37.72%, 8.6 to 25.5%, and 17.53 to 29.14% respectively as a response to water stress. RSP 564 showed maximum rise in total polyphenol (24.40–28.38 mg/g), catalase (167.90–210.72 mMH_2O_2/min/100 mg fr. wt.) and peroxidase (158.92–186.76 unit/min/100 mg fr. wt) and second highest in antioxidant (17.71–24.87%) and SOD (49.15–57.78 unit/min/g fr. wt.), whereas RSP 529 with (22.11–25.66 mg/g), (36.51–44.51%, (54.58–64.83 unit/min/g fr. wt), (170.55–200.09 mMH_2O_2/min/100 mg fr. wt) and (152.07–180.83 unit/min/100 mg fr. wt), respectively were best and second best followed by PBW 175. RSP 566 was poorer among the wheat genotypes. Like osmolytes, phenolics, antioxidant, catalase, SOD and peroxidase enzymes were reported in higher levels in resistant varieties under abiotic stresses including moisture stress for imparting tolerance. Soluble polyphenol were explained to act as antioxidants for plant cell protection from oxidative stress by trapping free radicals (Oncel *et al.* 2003). The scavenging of toxic superoxide radical starts with SOD catalyzed reaction. Catalase and peroxidase also play important role in cell protection by lignifications and are found in higher levels under stress. These detoxification enzymes offer protection to the living cell against reactive singlet oxygen species produced as a response to both abiotic and biotic stresses (Rampino *et al.* 2006). Hence, these parameters are also considered for assessing genetic potential of water stress tolerance of wheat genotypes. RSP 564 showed highest value in phenol content, superior in catalase, peroxidase and second highest in SOD and RSP 529 possessed maximum antioxidant, second in polyphenol with better SOD and considered to have highest potential in water stress tolerance. But national cultivars PBW 343 and PBW 175 were low in total phenol and antioxidant but found superior in SOD and catalase activity. It may be interpreted due to their tolerance to biotic stresses. The relative tolerance potentials of the genotypes in context to accumulated phenol, antioxidant and detoxification enzymes under control and water stress conditions are depicted in Fig 2.

On basis of overall performance of the wheat genotypes in context to osmolytes' accumulation and levels of detoxification enzymes, it is concluded that among the genotypes, RSP 564 and RSP 529 are most potential in water stress tolerance and also better than two national cultivars, viz PBW 175 and PBW 343, whereas RSP 566 is considered the most susceptible genotype against water stress. Among national varieties, PBW 175 possessed better drought tolerance compared to PBW 343.

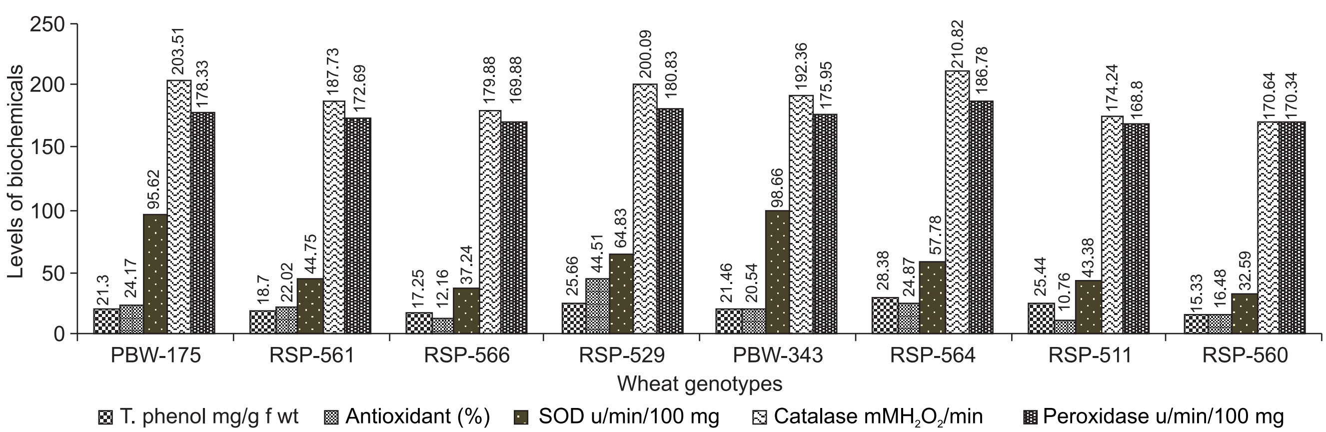

Fig 2 Comparison of wheat genotypes on the basis of total phenols, antioxidant, SOD, catalase and peroxidase levels under stress condition

Table 2 Changes in secondary metabolites, viz total phenols and antioxidant contents, and enzymes SOD, catalase and peroxidase activities in wheat genotypes under water stress

Wheat genotype	Total phenol mg/g fresh leaves wt control	Total phenol mg/g fresh leaves wt stress	Antioxidant in leave (%) control	Antioxidant in leaves (%) stress	SOD unit/min/ 100 mg fresh leaves wt. control	SOD unit/min/ 100 mg fresh leaves wt. stress	Catalasem M H_2O_2/ min/100 mg fresh leaves wt. control	Catalasem M H_2O_2/ min/100 mg fresh leaves wt. stress	Peroxidase unit/min/ 100 mg fresh leaves wt. control	Peroxidase unit/min/100 mg fresh leaves wt. stress
PBW 175	17.70	21.30 (+20.34%)	17.55	24.17 (+37.72%)	69.43	95.62 (+37.72%)	170.46	203.51 (+12.85%)	149.57	178.33 (+19.23%)
RSP 561	15.32	18.70 (+22.06%)	16.67	22.02 (+32.09%)	38.67	44.75 (+15.72%)	168.24	187.73 (+11.58%)	140.52	172.69 (+22.9%)
RSP 566	14.36	17.25 (+20.13%)	7.54	12.16 (+61.27%)	28.32	37.24 (+31.50%)	165.60	179.88 (+8.6%)	136.42	169.88 (+24.53%)
RSP 529	22.11	25.66 (+16.06%)	36.51	44.51 (+21.9%)	54.58	64.83 (+18.78%)	170.55	200.09 (+17.32%)	152.07	180.83 (+18.9%)
PBW 343	18.88	21.46 (+13.67%)	14.82	20.54 (+38.60%)	72.95	98.66 (+35.24%)	172.10	192.36 (+18.25%)	144.54	175.97 (+21.74%)
RSP 564	24.40	28.38 (+16.31%)	17.71	24.87 (+40.5%)	49.15	57.78 (+17.56%)	167.90	210.72 (+25.50%)	158.92	186.78 (+17.53%)
RSP 511	21.98	25.44 (+15.74%)	5.43	10.76 (+92.45%)	34.48	43.38 (+25.80%)	151.85	174.24 (14.74%)	130.71	168.8 (+29.14%)
RSP 560	11.51	15.33 (+33.19%)	10.99	16.48 (+49.95%)	27.35	32.59 (+19.16%)	154.47	170.64 (+10.47%)	133.85	170.34 (+27.3%)
CD (*P* =0.5)	0.651	1.466	1.225	1.751	2.627	4.796	1.812	3.631	2.520	2.793

Figures in parentheses indicate values increased (+) (in time/percentage) over control under water stress conditions

SUMMARY

Drought stress responses in cross bred wheat genotypes, viz RSP 561, RSP 566, RSP 529, RSP 564, RSP 511 and RSP 560 was studied by measuring the changes in osmolytes and cell protecting biochemicals, viz proline, reducing sugar, free amino acid, total polyphenol, antioxidant, superoxide dismutase (SOD), catalase and peroxidase (POD) along with relative water content (RWC) from the leaves samples after induction of water stress at flowering stages. National wheat cultivars, PBW 175 recognized as drought resistant and PBW 343 susceptible, were taken as references. It was recorded that all the parameters under water stress except relative water content increased significantly in all the wheat genotypes. The inter-genotypic variation of proline, reducing sugar, free amino acid, total polyphenol, antioxidant, SOD, catalase and peroxidase levels in controls ranged from 1.09–2.70 mmole/g, 0.48–0.96%, 0.68–1.13%, 11.51–24.40 mg/g, 5.43–36.51%, 27.35– 72.95 mM $H_2O_{2/}$min/100 mg, 151.85–172.10 unit/min/100 mg and 130.71–158.92 unit/min/100 mg and the raised levels ranged from 6.07–14.96 mmole/g, 1.93– 3.92%, 2.02–4.41%, 15.33– 28.38 mg/g, 10.76–44.51%, 32.59–98.66 mM $H_2O_{2/}$min/100 mg, 170.64–210.72 unit/min/100 mg, 168.8–186.78 unit/min/100 mg respectively, whereas the RWC decreased from 58.33–80.68% to 47.06–61.71%. The wheat genotypes RSP 564 and RSP 529 carried significantly high values of proline, free amino acid, reducing sugar, total polyphenols, antioxidant content and detoxicating enzymes SOD, catalase and POD activities both in control and water stress conditions along with maximum leaf water retention and hence can be considered highly tolerant to water stress, followed by national cultivar PBW 175 with the values in the third position in order. PBW 343 though possessed high values in total polyphenol, antioxidant and detoxicating enzymes but showed very low values in osmolytes and RWC and can be assessed low tolerant to water stress. RSP 566 which showed lowest values in most of these parameters can be elucidated as drought stress susceptible genotype.

REFERENCES

Directorate of Wheat Research (DWR) *Progress Report 2009*.CI, Vol 1, page 26.1.

Emmons C L and Peterson D M. 1999. Antioxidant activity and phenolics contents of oat groats and hulls. *Cereal Chemistry* **76**: 902–6.

Gang- Pong X, McIntyre C L, Colin L D, Jenkins, Donna G, van Herwaarden A F and Shorter R. 2008. Molecular dissection of variation in carbohydrate metabolism related to water- soluble carbohydrate accumulation in stems of wheat. *Plant Physiology* 146: 441–54.

Kumar B and Gupta B B.2009. Drought tolerance in wheat using half diallel analysis. *Indian Journal of Agricultural Sciences* **79** (5) : 394–8.

Mahatma M K, Bhatnagar R, Solanki R K and Mittal G K. 2007. Effect of seed soaking treatments on salt induced biochemical contents and polypeptide pattern of wheat (*Triticum aestivum* L.). *Indian Journal of Agricultural Biochemistry* **20**: 73–7.

Mulla M S, Munjal S V, Satbhai R D and Naik R M. 2006. Metabolic alterations associated with water stress at critical growth stages in rabi sorghum. *Indian Journal of Agricultural Biochemistry* **19**: 5–9.

Oncel I, Keles Y and Vstun A S. 2003. Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedling. *Journal of Environment pollution* **107**: 315–20.

Rampino P, Pataleo S, Gerardi C, Mita G and Perrota C. 2006. Detoxification activities of SOD, catalase and peroxidase in wheat under water stress. *Plant Cell and Environment* **29**: 2143–47.

Rana U and Bansal G L. 2006. Role of calcium and exogenous aminoacids in the promotion of proline and amino acid accumulation in *Phaseolus vulgaris* seedling under water stress. *Indian Journal of Agricultural Biochemistry* **19**: 11–4.

Schanderi S H. 1970. (*in*) *Methods in Food Analysis*, 709 pp. Academic press, N Y.

Wollenweber B, Porte J R and Schellberg J. 2003. Lack of interaction between extreme high temperature events at vegetative and reproductive growth stages in wheat. *Journal of Agronomy and Crop Sciences* **189**: 142–50.