High Temperature Response Leads to Altered Membrane Permeability in Conjunction with Carbon Utilization in Wheat

Shashi Bala¹ • Bavita Asthir² • Navtej Singh Bains³

¹ Department of Biochemistry, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana - 141004, Punjab, India
² Department of Biochemistry, Punjab Agricultural University, Ludhiana - 141004, Punjab, India
³ Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, 141004 - Punjab, India

Corresponding author: b.asthir@rediffmail.com

ABSTRACT

Sensitivity of grain developmental stage to high temperature was investigated in four genotypes of wheat i.e. PBW 343, PBW 550, C 273, and C 518 using various morpho-physiological and biochemical indices under high temperature stress. A significant increase in membrane injury index and thiobarbituric acid reactive substances (TBARS) was found in PBW 343 and PBW 550 genotypes with considerable reduction in chlorophyll content at all stages. Delayed planting led to reduction in yield and yield component of all genotypes with significant effect on C 518 and PBW 343. Invertase activity increased under late planting in correspondence with total sugars. Conversely, activities of sucrose synthase and sucrose phosphate synthase decreased indicating high temperature mediated alteration in carbohydrate metabolism in all genotype with pronounced effect on PBW 550 and PBW 343. It was concluded that pre-era tall genotypes C 273 and C 518 possessed higher membrane thermostability and were responding better under high temperature while PBW 550 and PBW 343 possessed higher carbohydrate metabolizing efficiency and hence high yielding genotypes. Therefore, evolution of new genotypes by continuous genetic recombination can be utilized in developing tolerant wheat varieties by combining the genes for heat tolerance and high yield potential.

Keywords: carbohydrate, invertases, membrane injury, sucrose synthase, sugars, thermostability, yield, wheat

INTRODUCTION

High temperature (> 30°C) at the time of grain filling is one of the major constraints in increasing productivity of wheat in sub-tropical and tropical regions. For every 1°C rise in temperature above ambient temperature during the period between the end of tillering and the beginning of grain filling reduced the grain yield by 4% under heat stress conditions. The demand for wheat is expected to grow by approximately 1.6% per year worldwide and by 2% per year in developing countries up to the year 2020 (Rane and Nagarajan 2004). Consequently, the development of a heat-tolerant cultivar is of prime importance to alleviate future threats to food availability in a rapidly expanding human population.

Cellular membrane thermostability (CMS) as estimated by electrolyte leakage has long been seen as a potentially effective assay for thermotolerance in various crop plants. Wheat lines of high CMS tended to yield better than lines of low CMS when grain filling occurred under hot conditions (Shanahan et al. 1990). Exposure of plant cells to heat causes cellular membrane disruptions that are apparently related to temperature-specific phase changes in the membrane lipid bilayer (Suss and Yordanov 1986; Fokar et al. 1998). Heat stress was shown to cause impairments in mitochondrial functions and result in the induction of oxidative damage that manifested in lipid peroxidation (Larkindale and Knight 2002; Suzuki and Mittler 2006). One of the products of lipid peroxidation is malonaldehyde (Sairam et al. 1998). Photosynthetic apparatus in plant is highly thermostable and is damaged before visible symptoms of high temperature injury are manifested. High temperature affects thylakoid related reactions, changing the amount of absorbed light energy that is transduced from photosystem II (PSII) to photosystem I (PSI) and in turn, altering the pattern of chlorophyll fluorescence. Therefore determining mechanism associated with heat tolerance and identifying screening methods are vital for improvement of heat tolerance in plants.

Water soluble carbohydrates (WSC) mobilises from the stem during the later phase of grain filling and thus can become an important source of assimilate for grain yield in wheat under terminal drought stress conditions. Stem WSC accumulation is influenced by environmental factors (Blum et al. 1998; Ruuska et al. 2008). However, considerable genotypic variation in stem WSC concentration has been observed in wheat. Positive relationships between stem WSC concentration and yield have been observed in several studies (Asseng and van Herwaarden 2003; Ruuska et al. 2006; Xue et al. 2008). Therefore, high WSC concentration is considered to be a potentially useful trait for improving grain weight and yield in water limited wheat production environments (Asseng and van Herwaarden 2003; Ruuska et al. 2006; Foulkes et al. 2007).

Sucrose-metabolizing enzymes are important determinants of sink capacity by generating a sucrose gradient to support unloading of sucrose from the phloem. For these reasons, the enzymes responsible for the first metabolic reaction of sucrose and are probably critical links between photosynthate production in source leaves and growth capacity of sink organs (Farrar 1996; Balibrea et al. 2000; Roitsch et al. 2003).

Yield is the function of many components which when modified has direct influence on the productivity. Several physiological traits like plant height, tiller no, kernel no per
spike and 1000 grain weight have been shown to be associated with potential crop improvement under abiotic stress (Araus et al. 2001; Foulkes et al. 2007; Hamam and Khalad 2009). Therefore, information regarding inheritance pattern of some traits as plant height, grain yield would be of great importance in the selection of desirable parents for an effective breeding program to evolve new varieties of economic importance. Development of new genotypes with established heat tolerance and high yield potential by continuous genetic recombination is the need of today and these genotypes can be utilized in developing tolerant wheat varieties having high yield potential. Therefore, the present work was conducted to study the effect of terminal heat stress in terms of membrane thermostability and carbohydrate metabolizing capacity in four wheat varieties responding differentially to high temperature.

MATERIALS AND METHODS

Plant material

Four wheat (Triticum aestivum) genotypes viz. PBW 343, PBW 550, C 518 and C 273 used in this study represents cultivars belonging to different years (Goyal and Ashtir 2009). C 518 and C 273 are pre-dwarfing era tall wheat varieties which were grown in rainfed environment and are known to possess drought and heat tolerance traits (Table 1) whereas PBW 550 and PBW 343 were high yielding genotypes for cultivation under irrigated conditions. Plants were raised in the experimental area of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India. The crop was raised under normal (24th Oct 2007) and late planting (6th Dec 2007) conditions in plots. Each plot consisted of 4 rows of 1 m each. Row to row spacing was maintained at 23 cm and the material was sown in three replications. Irrigation and fertilizer application was as per the standard commercial practices of the region. All the genotypes were tested for various physiological and biochemical parameters at various stages of plant development. Plant tissue leaf and internodes were taken at different stages i.e. 30 days after sowing (DAS) and at anthesis. Fresh tissue was used for enzyme assays and various heat tolerance indices. There were three replications for each determination.

Yield and yield components

Yield was recorded on per plot basis. Three observations per plot were used for plant height. Five random ears were taken for recording no of grains per spike, numbers of tillers were counted from randomly chosen 1 m row length and a random sample of 1000 grains from each plot was weighed to obtain 1000-grain weight. There were three replications for each determination.

Membrane injury index

Membrane injury index was estimated by method of Blum et al. (2001). For each treatment 0.5 g tissue of grain was excised and washed with distilled water to remove adhering electrolytes. The tissue was then immersed in test tubes containing 20 ml of distilled water and stirred continuously at 28°C. After 5 h the electrolyte leakage was estimated by conductivity meter. The sample was then boiled for 30 min and conductivity was measured again. The percentage leakage was calculated as:

\[
\text{conductivity before boiling} \times 100
\]

\[
\text{conductivity after boiling}
\]

Lipid peroxidation

The lipid peroxidation products were determined from the thiobarbituric acid reactive substances (TBARS) contents resulting from the thiobarbituric acid (TBA) reaction as described by Asthir et al. (2009). Briefly, 0.5 g of grains were homogenized in 3 ml 20% (w/v) trichloroacetic acid (TCA) and 0.5% (v/v) TBA (2:1 ratio) and incubated at 95°C for 30 min. The reaction was stopped by placing the reaction tubes in an ice bucket. The lipid peroxidation as malonaldehyde (MDA) contents was determined by its extinction coefficient of 155 mM⁻¹cm⁻¹.

Chlorophyll content

Chlorophyll content was measured by Arnon (1949), 0.5 g fresh tissue was homogenized in 5 ml of ammonical acetone, prepared by mixing 81.8 ml acetone and 0.2 ml ammonium hydroxide and making final volume 100 ml with distilled water. The samples were then centrifuged at 3000 × g for 3 min. The absorbance of the supernatants of all samples was determined at the following wavelengths 645, 663 and 710 nm. Wavelength of 710 nm was used as an isobestic point which was deducted from all other absorbance readings.

Chlorophyll a (mg/g) = (12.7 x A663) - (2.69 x A645)

Chlorophyll b (mg/g) = (22.9 x A645) - (4.68 x A663)

Enzyme assays

Freshly collected samples were used for extraction of soluble acid invertase (EC 3.2.1.26; pH 4.8), soluble neutral invertase (EC 3.2.1.27; pH 7.5) and sucrose synthase (synthesis, EC 2.4.1.13), following the procedure employed by Asthir et al. (1998). Grain samples (4 g) homogenized at 0-4°C in 50 mol.m⁻³ Hepes-NaOH buffer (pH 7.5) containing 5 mol.m⁻³ MgCl₂, 1 mol.m⁻³ mM Na-EDTA, 2.5 mol.m⁻³ DTT, 0.5 mg ml⁻¹ BSA and 0.05% (v/V) Triton X-100. Homogenates were centrifuged at 10,000 × g for 15 min and the pellets resuspended in extraction buffer and centrifuged as before. The supernatants were pooled and passed through Sephadex G-25 column (17 cm) equilibrated with the above buffer without EDTA and Triton X-100.

Soluble acid and neutral invertases were assayed from the test extracts according to Asthir and Singh (1995). The reaction mixture (1 ml) consisted of 0.6 ml of 0.2 mol L⁻¹ Na-acetate, pH 4.8 (for soluble acid invertase) or 0.2 mol L⁻¹ Na-phosphate, pH 7.5 (for soluble-neutral invertase), 0.2 ml of 250 mol m⁻³ sucrose and 0.2 ml of enzyme extract. The contents were incubated at 37°C for 20 min and the reaction was terminated by addition of 1 ml of Nelson Reagent C. The amount of reducing sugars were measured (Nelson 1944) and the concentration of hydrolysed sucrose was calculated by multiplying the reducing sugar concentration by a factor of 0.95.

Sucrose synthase was assayed from the test extracts as described by Morell and Copeland (1985). The reaction mixture (0.5 ml) for sucrose synthase (synthesis) contained 3 mM UDPG 10 mM fructose, 5 mM MgSO₄, 40 mM Tris-HCl buffer (pH 8.2) and 0.2 ml dialysed enzyme preparation.

Extraction and estimation of sugars

Free sugars were extracted sequentially with 80 and 70% EtOH from ethanol-preserved samples. The test extracts were clarified with basic lead acetate and from these extracts concentrations of total sugars were determined (Asthir and Singh 1995). Sugars react with conc. sulphuric acid to form a dehydration product i.e., furfural or 5-hydroxyl methyl furfural. This dehydration product then react with phenol which act as a chromophore and gives orange yellow colour. The intensity of colour developed can be measured at 520 nm. Using glucose standard (10-60 μg) content of soluble sugars was calculated.

Table 1

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>30 (DAS)</th>
<th>60 (DAS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NS</td>
<td>LS</td>
</tr>
<tr>
<td>Maximum</td>
<td>26.0</td>
<td>17.2</td>
</tr>
<tr>
<td>Minimum</td>
<td>8.9</td>
<td>7.7</td>
</tr>
<tr>
<td>Mean</td>
<td>17.3</td>
<td>12.4</td>
</tr>
</tbody>
</table>

**Table 1** Maximum, minimum and mean temperature of wheat under normal sown (NS) and late sown (LS) crop during growth and development of wheat.

**DAS days after sowing**
Table 2 Morpho-physiological parameters of wheat genotypes under normal (NS) and late sown (LS) conditions. Values are determination of three replications ± SD.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Plant height</th>
<th>No of tillers</th>
<th>No of grains/spike</th>
<th>1000 grain weight</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NS</td>
<td>LS</td>
<td>NS</td>
<td>LS</td>
<td>NS</td>
</tr>
<tr>
<td>PBW 343</td>
<td>78.5 ± 3.3</td>
<td>74.5 ± 3.2</td>
<td>33.2 ± 1.8</td>
<td>45.3 ± 2.4</td>
<td>61.0 ± 2.3</td>
</tr>
<tr>
<td>PBW 550</td>
<td>79.0 ± 3.5</td>
<td>73.5 ± 3.6</td>
<td>22.7 ± 1.5</td>
<td>31.3 ± 1.6</td>
<td>56.0 ± 2.3</td>
</tr>
<tr>
<td>C 273</td>
<td>118 ± 5.2</td>
<td>105 ± 5.0</td>
<td>30.7 ± 1.6</td>
<td>31.0 ± 1.5</td>
<td>43.7 ± 1.8</td>
</tr>
<tr>
<td>C 518</td>
<td>113 ± 5.3</td>
<td>89.9 ± 4.8</td>
<td>32.2 ± 1.5</td>
<td>37.5 ± 1.6</td>
<td>28.8 ± 1.2</td>
</tr>
</tbody>
</table>

Statistical analysis

All the values reported in this paper are the means of three replicates. All data obtained was subjected to analysis of variance (factorial experiment in completely randomized design) by using CPCSSI software package. In all the tables ± values represent standard error of the means.

RESULTS AND DISCUSSION

The late planting reduced plant height in all the genotypes, the maximum to the extent of 24 cm in C 518. Genotype PBW 550 had highest 1000-grain weight under normal planting with lowest reduction of 29% under late planting (Table 2). In wheat, both grain weight and grain number appeared to be sensitive to heat stress, as the number of grains per ear at maturity declined with increasing temperature (Ferris et al. 1998). Under favourable temperatures, the genotypes which were relatively late in heading possessed better vegetative growth, reflected by increased plant height and more number of internodes but when growth resources are limited by heat stress, the size of plant organs such as leaves, tillers and spikes are reported to be reduced (Stone et al. 1991; Wahid et al. 2003). MII is influenced by all known and unknown factors (Araus et al. 2001; Sial et al. 2005; Mirbahi et al. 2009).

The phenomenon of yield depression due to late planting was also computed in each genotype. As high as 49.8 and 47% yield reduction were observed in genotypes PBW 343 and C 273, respectively. Genotype PBW 550 showed lowest reduction (38%) in yield, when planted late and seems to possess tolerance to high temperature to some extent. Yield of a genotype is the most integrative trait, because it is influenced by all known and unknown factors (Araus et al. 2001). In general, genotypes produced higher grain yield, when planted on 24th October as compared to the 7th December.

There was a significant increase in membrane injury index in all genotypes under late planting compared to normal planting (Fig. 1). MII increases from 30 days after sowing (DAS) to anthesis thus indicating a proportional increase in membrane injury with increase in temperature. However, at anthesis leakage was less in internodes than leaves both under normal and late planting conditions. Under late planting PBW 343 showed maximum increase in MII compared to other genotypes in vegetative tissue (leaf and internode). While minimum leakage was shown by C 273 in response to heat stress. The increased solute leakage, as an indicator of decreased cell membrane thermostability has long been used as an indirect measure of heat-stress tolerance in diverse plant species, including wheat (Blum et al. 2000). MII is influenced by plant tissue, sampling, organ, development age, growing season and degree of hardening of plant species (Deshmukh et al. 1991; Wahid et al. 2007). Hence, plants have evolved a variety of response to elevated temperature that minimize damage and ensure protection of cellular homeostasis.

TBARS is a measure of malonaldehyde content that increased under late planting in all cultivars (Fig. 1). Maximum TBARS was observed in PBW 343 and PBW 550 and minimum in C 273 and C 518. Environmental stresses differently affect plant processes that lead to loss of cellular homeostasis accompanied by formation of active oxygen species, which cause oxidative damage to membrane lipids (Srivalli et al. 2003).

Minimum reduction in chlorophyll content at vegetative and anthesis stages were recorded in leaves of PBW 550 and PBW 343 as compared to C 273 and C 518 at all stages under both planting (Fig. 1). Leaf and internode photosynthesis is one of the most heat sensitive processes which can be completely inhibited by high temperature before other symptoms of stress appear in the plant (Camejo et al. 2005). Adverse effects for photosynthesis result in structural and functional disruption of plastid membrane that leads in reduced accumulation of chlorophyll under high temperature stress (Xu et al. 1995; Dekov et al. 2000).

Sucrolytic enzymes were increased gradually at all stages of plant growth in all genotypes under late planting conditions (Fig. 2). Invertase activity remained at lower levels in internode as compared to leaves at anthesis. Highest acid invertase activity was shown by PBW 343 and PBW 550 in leaf and internode while minimum activity was shown by C 273 and C 518. Invertases (β-D fructofuranoside fructohydrolase, EC 3.2.1.26) have been isolated from several plant tissues, especially those engaged in active growth and development (Morris and Arthur 1985) or in which sucrose content was low or declined rapidly. It was concluded that at least in wheat temperature effect on source and sink activities, from such results increase mobilization efficiency of reserves from leaves, stem and other plant part has been
suggested as a potential strategy to improve grain filling and yield in wheat under heat stress (Xue et al. 2008).

Highest activity of neutral invertase was shown by PBW 550 and in leaves and internode (Fig. 2). It was found that under late sown conditions, genotype C 273 and C 518 had lower activity in both the tissues. It is generally accepted that the grain filling rate is closely related with sink strength (Huang et al. 2001). The sink strength can be described as a product of sink size and sink activity. Sink activity is a physiological restraint that includes multiple factor and key enzyme involved in carbohydrate utilization and storage (Wang et al. 1993).

Sucrose synthase (synthesis) and sucrose phosphate synthase was found to be more active in internode than in leaves under both normal and late planting condition (Fig. 2). Highest activity of sucrose synthase (synthesis) was reported in the leaves of PBW 343 and C 518 and in the internode of PBW 550 and C 273 under both plantings. Highest sucrose phosphate synthase activity was shown in the leaves and internodes of PBW 550 and C 518 under both plantings. Castrillo (1992) has also reported that sucrose synthase synthesis and sucrose phosphate synthase activity decrease when a plant is subjected to stress.

Total sugars were estimated at anthesis and post-anthesis stages under terminal heat stress (late planting) in flag leaf and internode (Fig. 2). Total sugars increased at post anthesis but there was more build-up of sugars in leaves compared to internode at anthesis. Conversely, at post anthesis, level of sugars in leaf decreased significantly in comparison to internode showing decrease in leaf photosynthesis at post anthesis. However, their accumulation in stem indicates their potential role as source for developing sink tissues. Metabolism of storage reserves in the endosperm of cereal seeds is highly regulated and has a primary pivotal role in the interaction among sugars (Mohammudkhani and Heidari 2008). Soluble sugar content proved to be a better marker for selecting improvement of heat tolerance in wheat.

CONCLUSION

Genotypes C 518 and C 273 are pre-dwarfing era tall wheat varieties which were grown in rainfed environment and are known to possess drought and heat tolerance. The mechanism for this tolerance was not well known however, C 273 and C 518 have stable membranes as indicated by membrane injury index and TBARS contents. Genotypes PBW 550 and PBW 343 were high yielding varieties for cultivation under irrigated conditions as they have better carbohydrate utilization. Water soluble carbohydrates (WSC) accumulate in the stem and leaf sheath of wheat during the early reproductive phase of wheat and other grasses (Fu and Dernoeden 2009). WSC can accumulate in wheat stems to more than 40% of total stem dry weight (Houses 2000). Osmotic adjustment is effected by accumulation of sugars and is involved in protection against stresses. Metabolism of storage reserves in the endosperm of cereal seeds is highly regulated and has a primary pivotal role in the interaction among sugars (Mohammudkhani and Heidari 2008). Soluble sugar content proved to be a better marker for selecting improvement of heat tolerance in wheat.

ACKNOWLEDGEMENTS

This work was financially supported by Department of Biotechnology, New Delhi, India. Sincere thanks are also to Dr S. S. Gos- sal, Additional Director of Research, for coordinating research projects running parallel in other Departments of Punjab Agricultural University, Ludhiana, Punjab, India.

REFERENCES


Azzed R, Singh R (1995) Invertase-mediated interconversion of sucrose and hexose during their translocation in growing pearl millet plant. Journal of...
High temperature effects on carbohydrate metabolism. Bala et al.

Plant Biochemistry and Biotechnology 4, 23-28


