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BRIEF COMMUNICATION

Starch synthase activity and heat shock protein in relation to thermal tolerance of developing wheat grains

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Abstract

Wheat (*Triticum aestivum* L.) cvs. HD 2285 (relatively tolerant) and WH 542 (susceptible) were exposed to ambient and elevated temperature (3 - 4 °C higher) in open top chambers during post anthesis period. The grain yield components were determined at the time of maturity. In order to elucidate the basis of differential tolerance of these cultivars, the excised developing grains (20 d after anthesis) of ambient grown plants were incubated at 15, 25, 35 and 45 °C for 2 h and then analysed for the activities of soluble starch synthase (SSS), granule bound starch synthase (GBSS), kinetic parameters of SSS and content of heat shock protein (HSP 100). The elevated temperature during grain development significantly decreased grain growth in WH 542 whereas no such decrease was observed in HD 2285. High temperature tolerance of HD 2285 was found to be associated with higher catalytic efficiency (V_{max}/K_m) of SSS at elevated temperature and higher content of HSP 100.

Additional key words: grain growth, granule bound starch synthase, soluble starch synthase, *Triticum aestivum*

High temperature during grain filling stage is an important yield limiting factor in wheat (Howard 1924, Chinoy 1947, Asana and Williams 1965, Wardlaw *et al.* 1989). It has been reported that single grain mass falls by 3 - 5 % for every 1 °C rise in temperature above 18 °C (Wiegand and Cuellar 1981, McDonald *et al.* 1983, Wardlaw *et al.* 1989). Physiologically, the rate of grain filling reflects the rate of biochemical reactions involved in the synthesis of reserves (Ghildiyal and Sirohi 1986). As starch constitutes around 70 % of dry matter in cereal grains, the synthesis and deposition of starch may be an important determinant of the size of the grain. It has been suggested that control of starch synthesis in wheat endosperm is vested largely in the activity of soluble starch synthase (SSS; Jenner *et al.* 1993) and SSS is extremely sensitive to high temperature (Rijven 1986, Keeling *et al.* 1993, 1994). Prakash *et al.* (2003) observed a parallelism in the effect of high temperature on grain growth, starch accumulation and SSS activity.

Plants exposed to high temperature are also induced to synthesize heat shock proteins (HSPs). HSPs are known to function as molecular chaperones that aid in refolding proteins denatured by heat and prevent them from aggregating (Vierling 1991, Boston *et al.* 1996, Iba 2002). Wahid and Close (2007) reported expression of dehydrins under heat stress in sugarcane. Accumulation of high molecular mass HSPs has been directly implicated in induction of thermotolerance in plants (Boston *et al.* 1996). Katiyar-Agarwal *et al.* (2003) introduced *Arabidopsis thaliana* HSP 101 (Athsp 101) cDNA into Pusa Basmati cultivar of rice. The transgenic rice lines showed significantly better growth performance in the recovery phase following the heat stress. The HSP 100 kDa protein has been reported to have a definite role in thermotolerance (Sanchez and Lindquist 1990, Lee *et al.* 1994, Schirmer *et al.* 1994). However, information regarding HSP 100 in developing grains and its role in providing thermotolerance to starch synthesis, consequently, grain growth in wheat is not clear. The present study analysed HSP 100 and starch synthase activity in grains exposed to different temperatures in wheat cultivars differing in heat tolerance, in an attempt to elucidate the basis of their differential heat tolerance for grain growth.

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Abbreviations: ADPG - adenosine diphosphate glucose; DAA - days after anthesis; GBSS - granule bound starch synthase; HSP - heat shock protein; OTC - open top chamber; S - heat susceptibility index; SSS - soluble starch synthase.

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Two wheat (*Triticum aestivum* L.) cultivars differing in their response to high temperature were used in this experiment: HD 2285 (relatively tolerant) and WH 542 (susceptible). Seeds of these cultivars were obtained from Genetics Division, Indian Agricultural Research Institute, New Delhi and grown in earthen pots (35 × 40 cm) containing sandy loam soil with added N, P and K. There were 100 pots for each variant and four healthy plants were kept in each pot. At anthesis these pots were shifted to open top chambers (OTC). The construction of OTC (300 × 200 cm) was based on the design of Leadly and Drake (1993) except that top of chamber was partially closed with poly vinyl chloride film to achieve warming. The warm air supplied by hot air blower, blown by an axial fan entered the chamber through double walled plenum around the base perforated towards inside. To eliminate chamber environment effect, chambers in which only air is blown served as control. The maximum and minimum temperature of control and hot air blown OTCs were recorded daily to assess the temperature difference. The mean maximum and mean minimum temperatures during grain development of control OTCs were 34.6 and 15.9 °C, respectively. For heated OTCs these values were 37.8 and 17.7 °C respectively. The control and high temperature grown plants were analysed for grain yield components at maturity. The heat susceptibility index (S) was calculated for grain yield per pot and thousand grain mass as described by Fischer and Maurer (1978): $S = (1 - Y/Y_P) / (1 - X/X_P)$, where, Y = mean grain mass of a genotype grown at high temperature, Y_P = mean grain mass of a genotype grown at control conditions, X = mean Y of all genotypes, X_P = mean Y_P of all genotypes ($S \leq 1.0$ means stress tolerant and $S > 1.0$ susceptible genotype).

The ears of main shoot (MS) of plants grown in control OTCs were sampled 20 d after anthesis (DAA). Basal grains from middle portion of ear were separated. The excised grains were incubated at different temperatures of 15, 25, 35 and 45 °C for 2 h in glass vials lined with moist filter paper and capped with non-absorbent cotton wool (Prakash *et al.* 2004). The grains after different temperature exposure were stored in liquid nitrogen for subsequent determination of starch synthase activity and HSP 100.

Soluble starch synthase (SSS) and granule bound starch synthase (GBSS) were extracted following the method of George *et al.* (1994). Starch synthase (SSS and GBSS) activity was estimated by the amount of adenosine diphosphate (ADP) formed from adenosine diphosphate glucose (ADPG). ADP estimation was carried out by using a preparation of pyruvate kinase which catalyses the transfer of phosphate from phosphoenol pyruvate to ADP. Pyruvate liberated was estimated (Leloir and Goldenberg 1960). SSS activity was also determined at different ADPG concentrations (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 mM) in the grains pre-exposed to 25 and 45 °C temperatures. The kinetic constants, V_{max} and K_m (ADPG), for SSS were determined from the double reciprocal plot of the ADPG saturation curves. Protein content in the enzyme extract was determined by Bradford method (Bradford 1976). Fresh and dry mass of comparable grains of both the cultivars were also determined to express the enzyme activity on dry mass basis.

Samples were analysed for HPS 100 by Western blot. Proteins from fresh grains (0.5 g) were extracted by rapid homogenization in 0.1 M Tris-HCl buffer (pH 7.8) containing 0.02 M sodium sulphite, 5 mM mercapto-ethanol, 5 mM benzamidine and polyvinylpoly-pyrrolidone (1 %). Protein was subjected to SDS-PAGE and electro blotted onto a nitrocellulose membrane according to Khyse-Anderson (1984). Blots were blocked using 4 % casein in phosphate buffer saline (PBS) for 12 - 16 h at 4 °C. Later they were probed with primary antibody (HSP 100) for 2 h at room temperature. Antibody raised in rabbit using rice HSP 100 were employed at a dilution of 1:25 000 (Pareek *et al.* 1995). The bands in Western blots were visualized after incubating with alkaline phosphatase conjugated anti-rabbit IgG for 1 h at room temperature. The blot was developed by using nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3 indolyl-phosphate (BCIP) as a substrate (Engvall and Perlmann 1972).

Wheat cultivars examined in the present study differed in grain yield components. HD 2285 had bolder grains but lesser grain number compared to WH 542. A significant decrease (17.10 %) in grain yield was observed in WH 542 under elevated temperature (ET) in

Table 1. Yield components and heat susceptibility index (S) of wheat cultivars under control (C) and elevated (ET) temperatures in open top chambers.

Cultivar	Treatment	Grain yield [g plant^{-1}]	Grain number [plant^{-1}]	Grain mass [mg]	Total dry mass [g plant^{-1}]	Harvest index	S for grain growth	S for grain yield
HD 2285	C	15.07	319.75	47.14	36.93	40.80		
	ET	14.92	321.34	46.43	36.70	40.65	0.146	0.102
	CD at P = 5 %	NS	NS	NS	NS	NS		
WH 542	C	17.74	467.00	37.98	36.38	48.76		
	ET	14.71	499.00	29.47	33.08	44.46	2.064	1.755
	CD at P = 5 %	1.73	NS	1.72	1.05	4.10		

Table 2. Soluble starch synthase (SSS) and granule bound starch synthase (GBSS) activity [nmol g^{-1}(d.m.) min^{-1}] in the excised grains (20 DAA) following exposure to different temperatures in wheat cultivars. Means ± SE, *n* = 3.

Cultivar		15 °C	25 °C	35 °C	45 °C
HD 2285	SSS	299.54 ± 23.96	352.03 ± 10.08	243.34 ± 13.89	183.44 ± 15.22
WH 542		228.29 ± 13.62	243.95 ± 9.60	189.19 ± 13.89	92.64 ± 13.21
HD 2285	GBSS	117.19 ± 4.51	109.26 ± 8.03	123.37 ± 7.10	95.62 ± 7.16
WH 542		121.29 ± 7.86	121.40 ± 4.62	104.35 ± 7.37	72.31 ± 9.73

OTCs compared to that of control (C) chambers. HD 2285, however, showed no significant decrease in grain yield by ET (Table 1). This decrease in grain yield under ET in WH 542 was mainly due to a decrease in grain mass. This cultivar also showed a significant decrease in dry matter production and harvest index (HI) under ET. The decrease in yield under ET associated with decrease in HI as observed in the present study, therefore, indicated that grain mass was more affected than biomass under ET. The heat susceptibility index calculated for grain growth and yield revealed that HD 2285 is a relatively stress tolerant cultivar, whereas, WH 542 is a susceptible cultivar (Table 1).

SSS activity was significantly higher than GBSS in both the cultivars at all the temperature treatments. HD 2285 showed significantly higher SSS activity as compared to WH 542 at all the temperature treatments but not so for GBSS activity (Table 2). SSS from the grains pre-exposed to 25 °C in HD 2285 showed lower values of V_{max} and K_m (ADPG) compared to WH 542. Pre-exposure of grains to 45 °C increased the K_m without affecting the V_{max} as compared to that of 25 °C treatment in HD 2285. In WH 542, however, high temperature treatment decreased V_{max} and increased the K_m. The ratio of V_{max}/K_m is the combined effect of temperature on V_{max} and K_m and is used as an indication of the efficiency of the enzyme for catalysing the reaction (Fersht 1985). High temperature treatment decreased V_{max}/K_m ratio in both the cultivars. HD 2285, however, maintained higher V_{max}/K_m compared to WH 542 at both the temperature treatments. HD 2285, therefore, had an efficient SSS at both the temperature treatments compared to WH 542 (Table 3).

Table 3. V_{max} [nmol mg^{-1}(protein) min^{-1}], K_m ADPG [mM] and V_{max}/K_m of soluble starch synthase in the grains exposed to two different temperatures in wheat cultivars.

Cultivar	Parameters	25 °C	45 °C
HD 2285	V_{max}	20.00	20.00
WH 542		25.00	16.67
HD 2285	K_m ADPG	0.45	0.71
WH 542		1.89	2.00
HD 2285	V_{max}/K_m	44.44	28.17
WH 542		13.22	8.34

HSP 100 in the developing grains pre-exposed to different temperatures was analysed by Western blot (Fig. 1). HD 2285 had higher content of this protein in the grains than WH 542 at all the temperature treatments. HSP 100 content, however, did not differ much with the different temperature treatments.

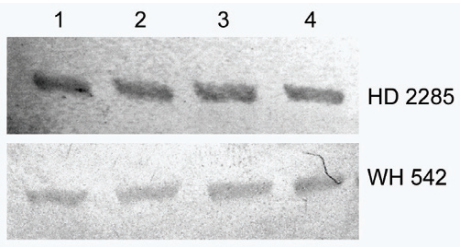

Fig. 1. HSP 100 in the excised grains (20 DAA) following exposure to different temperatures in wheat cultivars. *Lane 1* corresponds to 15, *lane 2* to 25, *lane 3* to 35 and *lane 4* to 45 °C.

The present observation, that tolerance to high temperature for grain growth is associated with catalytic efficiency of soluble starch synthase (SSS) suggests, that this enzyme could be an important target for improving the high temperature tolerance for grain growth in wheat. The higher sensitivity of this enzyme to high temperature has also been reported (Rijven 1986, Chaudhary-Mehra *et al.* 1994, Jenner 1994, Keeling *et al.* 1994, Prakash *et al.* 2003, 2004).

A thermostable form of SSS is to be identified in order to improve the thermotolerance for grain growth in wheat. In the present study HD 2285 had higher SSS activity and also showed lesser decrease in SSS activity under high temperature treatment compared to WH 542. SSS from HD 2285 had lower K_m (ADPG) and higher V_{max} than WH 542 under high temperature treatment. HD 2285 maintained higher V_{max}/K_m at 25 and 45 °C indicating greater efficiency of its SSS as compared to SSS from WH 542. Increase in K_m values of SSS for ADPG and amylopectin under high temperature treatment was also observed by Jenner *et al.* (1995). Zahedi *et al.* (2003) observed cultivar differences in the efficiency of SSS in wheat with regard to their thermostability. Activity of GBSS was considerably lower than SSS at different temperature treatments and was not much affected by high temperature probably because GBSS is bounded to the granule. The relative stability of GBSS at moderately

high temperature may explain the increase in the percentage of amylose in the grains (Shi *et al.* 1994, Tester *et al.* 1995). Over expressing this enzyme for greater temperature tolerance may possibly result in starch having more of amylose than amylopectin which is not desirable from the point of view of starch quality (Shewmaker and Stalker 1992, Morell *et al.* 2001). In rice leaves however, *GBSS I* gene expression was found to be higher at low temperature and decreased at temperatures higher than 30 °C (Wang *et al.* 2006).

In the present study, high temperature tolerant HD 2285 maintained higher content of HSP 100 than sensitive WH 542 at all the temperature treatments. However, there was no increase in HPS 100 content with increasing temperature. It could be that HSP 100 was induced earlier in the grain and is developmentally controlled. Singla *et al.* (1998) reported that developing rice grains (35 DAA) accumulate SAP 100 (a HSP 100 family protein) without any externally imposed stress. High content of SAP 104 was also observed in dry grains of wheat, sorghum and maize and under heat stress the content of SAP 104 remained unaltered (Singla *et al.* 1998). It is not known whether heat induced HSPs are functionally different from developmentally controlled HSPs that accumulate in the grain in absence of heat stress (Maestri *et al.* 2002). Since, HSP 100 family proteins reported to have a definite role in thermo-tolerance, their presence in developing grains would possibly provide thermotolerance for grain growth. The high SSS activity and its lesser depression under high temperature in HD 2285 than WH 542 could be due to presence of higher content of HSP 100 may therefore, not be ruled out. The study indicated the possibility of improving thermotolerance for grain growth through incorporation of thermostable form of SSS and over expression of HSP 100 in an otherwise high yielding cultivar of wheat.

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