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STARCH SYNTHASE ACTIVITY AND GRAIN GROWTH IN WHEAT CULTIVARS UNDER ELEVATED TEMPERATURE : A COMPARISON OF RESPONSES

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SUMMARY

Wheat (*Triticum aestivum* L.) cvs. HD 2285 and C 306 (relatively tolerant) and HD 2329 and HD 2428 (susceptible type) were exposed during grain development period to 5-7°C higher temperature in a normal glasshouse and 8°C higher than control in temperature control glasshouse of phytotron facility. There was more severe depression in grain growth under elevated temperature in phytotron experiment and was associated with greater decrease in the activity of soluble starch synthase (SSS) in the grains. Furthermore, relatively tolerant cultivars showed less decrease compared to susceptible cultivars in grain growth and also showed less decrease in SSS activity. Such parallelism in the effect of elevated temperature on grain growth and SSS activity and relatively tolerant cultivars showing less depression, further suggests that soluble starch synthase is the key component imparting sensitivity to high temperature for grain growth in wheat. The study suggested that thermotolerance for grain growth in wheat could possibly be improved through incorporation of thermostable form of this enzyme.

***Key words*: Grain growth, starch synthase, thermotolerance, wheat**

INTRODUCTION

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). The main effect of high temperature after anthesis is a reduction in grain size (Wiegand and Cuellar 1981, McDonald *et al.* 1983, Sharma-Natu *et al.* 2006). With the prevalence of rice-wheat cropping system, late sowing of wheat is generally practiced which pushes grain development further to high temperature regime (Zhang-hu and Rajaram 1994). The situation may further aggravate with the increasing concentration of CO_2 and other greenhouse gases in the atmosphere, which are expected to increase the global temperature (Bowes 1993, Ghildiyal and Sharma-Natu 2000, Ravi *et al.* 2001). There is a need therefore, to develop wheat varieties having tolerance to high temperature particularly during grain development.

In was reported earlier that decrease in grain growth and starch accumulation in wheat under high temperature is through a decrease in soluble starch synthase (SSS) activity (Prakash *et al.* 2003). This was shown in the experiments whereby plants were exposed to high temperature during grain development in glasshouse or in control vs heated open top chambers (Prakash *et al.* 2003, Sharma-Natu *et al.* 2006, Sumesh *et al.* 2008). This was further verified in excised grains exposed to different temperature in the lab condition (Prakash *et al.* 2004, Sumesh *et al.* 2008). In the present study effect of high temperature on grain growth and soluble starch

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synthase activity was compared when high temperature exposure was given in a normal glasshouse with that of control environment exposure in phytotron.

MATERIALS AND METHODS

Wheat (*Triticum aestivum*) cvs. HD 2285 and C 306 (relatively tolerant) and HD 2329 and HD 2428 (susceptible types) were grown in glazed china clay pots (50 x 25 cm) containing sandy loam soil. All recommended cultural practices were followed (Singh 1983). At anthesis, fifty per cent of total number of pots of each cultivar were transferred to a normal glasshouse to provide elevated temperature (ET) condition. Plants remained outside in the pot culture served as control (C). The maximum and minimum temperature of glasshouse and pot culture were recorded daily to assess the temperature difference. The temperature in the glasshouse was around 5-7°C higher than outside control throughout the grain development period. These wheat cultivars were also grown in temperature controlled glasshouses of phytotron in the National Phytotron Facility at Indian Agricultural research Institute, New Delhi. All the plants were grown at 21°/18°C, day and night temperature respectively upto anthesis stage. After anthesis, half of the total number of pots continued to remain at 21°/18° (control) whereas, remaining half were shifted to another glasshouse of the phytotron facility for high temperature (30°/25°C) treatment during grain development. The day temperature was maintained from 8 AM to 6 PM whereas, the night temperature was maintained from 6 PM to 8 AM. The temperature control was regulated by an automatic time sensor and thermostat and change of temperature was attained within half hour period.

Dates of ear emergence and anthesis in the main shoot (MS) were recorded on tags placed on each plant. The ears of MS of four wheat varieties were harvested at anthesis and 10, 20, 30, 40 and 50 days after anthesis (DAA). The grains were separated from ears, dried in an over till constant weight and dry weights were recorded. Soluble starch synthase was determined in the basal grains of middle spikelets of MS ear at 20 and 30 days after anthesis representing peak starch synthesis period. Grain samples were taken around 11.00 h and stored in liquid nitrogen. SSS was extracted following the method described elsewhere (George *et al.* 1994). Soluble starch synthase activity was estimated by the amount of ADP formed from 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). There were three replications for each determination. The data were analyzed statistically to determine CD value (Panse and Sukhatme 1967).

RESULTS AND DISCUSSION

In the present study growth pattern of individual grains (expressed as 1000 grain weight) as influenced by elevated temperature exposure under two sets of conditions was examined in relation to SSS activity in the grains. Elevated temperature of glasshouse did not affect significantly the individual grain weight upto 20 days after anthesis. Thereafter, growth of grains slowed down considerably in high temperature grown plants. In control plants of HD 2285 and C 306, the grain growth continued even upto 50 days whereas, in HD 2329 and HD 2428 the maximum grain growth was attained 30 days after anthesis (Fig. 1). HD 2285 and C 306, which are considered to be relatively more tolerant showed 16.88 and 17.30 per cent depression in final grain weight, respectively by high temperature condition of glasshouse (Table 1). In phytotron grown plants, there was only marginal effect of high temperature on thousand grain weight, 10 days after anthesis. Thereafter, grain growth of high temperature grown plants was significantly less than control grown plants (Fig. 2). The depression in grain growth by high temperature in phytotron experiment was much more severe compared to that of glasshouse. This can be clearly seen from the fact that the depression in final grain weight by high temperature in glasshouse was between 16.88 to 22.42 %, whereas, under phytotron condition, high temperature decreased grain weight by 35.41 to 45.35 %. Temperature susceptible cv. HD 2329 and HD 2428 showed 45.54 and 44.14 per cent decrease in final grain weight by high temperature. On the other hand HD 2285 and C 306 considered to be relatively temperature tolerant showed a depression of 35.41 and 41.23 % respectively (Table 1).

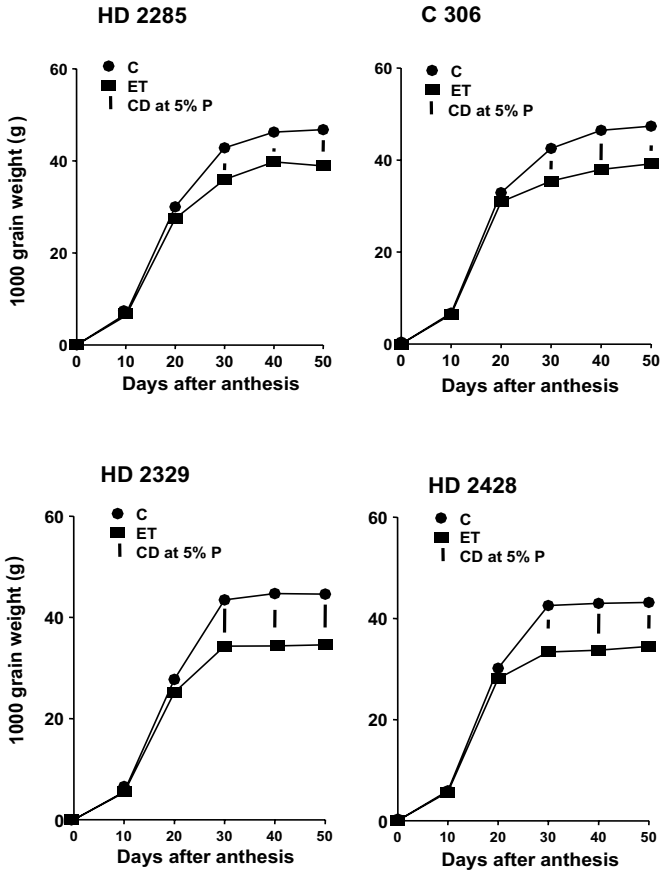

Fig. 1. Course of development of grains (1000 grain weight) of MS of wheat cultivars under control (C) and elevated temperature (ET) of glasshouse

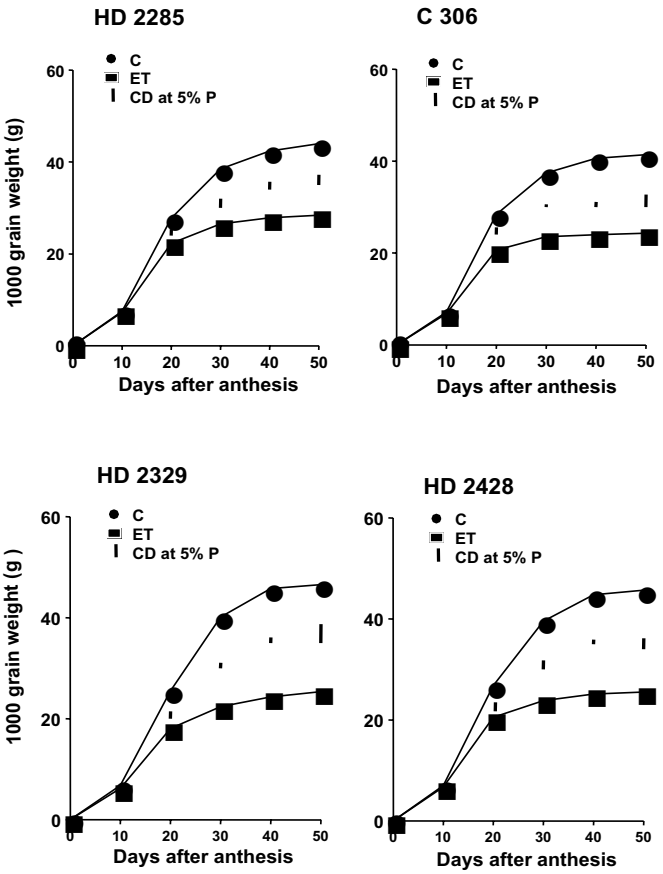

Fig. 2. Course of development of grains (1000 grain weight) of MS of wheat cultivars under control (C) and elevated temperature (ET) in phytotron

Table 1. Per cent decrease in thousand grain weight of MS in wheat cultivars by elevated temperature

Cultivar	Days after anthesis				
	10	20	30	40	50
			Glasshouse		
HD 2285	NS	NS	16.12	31.85	16.88
C 306	NS	NS	16.70	18.28	17.30
HD 2329	NS	NS	21.15	23.27	22.42
HD 2428	NS	NS	21.61	21.57	20.14
			Phytotron		
HD 2285	NS	19.64	31.08	34.27	35.41
C 306	NS	27.29	37.06	40.90	41.23
HD 2329	NS	29.17	44.25	46.88	45.53
HD 2428	NS	23.51	39.82	43.91	44.14

SSS activity in the grains was also found to be significantly lower under elevated temperature in glasshouse grown plants compared to control, 20 and 30 DAA in all the cultivars (Fig. 3). The decrease in SSS activity compared to control was 10.53 and 17.97 % in HD 2285, 20 and 30 DAA (Table 2). C 306 showed a depression of 10.76 and 25.97 % in SSS activity by elevated temperature 20 and 30 DAA. The decrease in SSS activity under elevated temperature was 37.48 and 33.29 % in HD 2329 and 20.19 and 22.89 % in HD 2428 at 20 and 30 DAA. It may be mentioned that relatively tolerant cultivars (HD 2285 and C 306) showed least decrease in SSS activity by elevated temperature compared to susceptible types (HD 2329 and HD 428), 20 DAA, the peak stage of starch synthesis in wheat grain. The activity of SSS was greatly reduced under

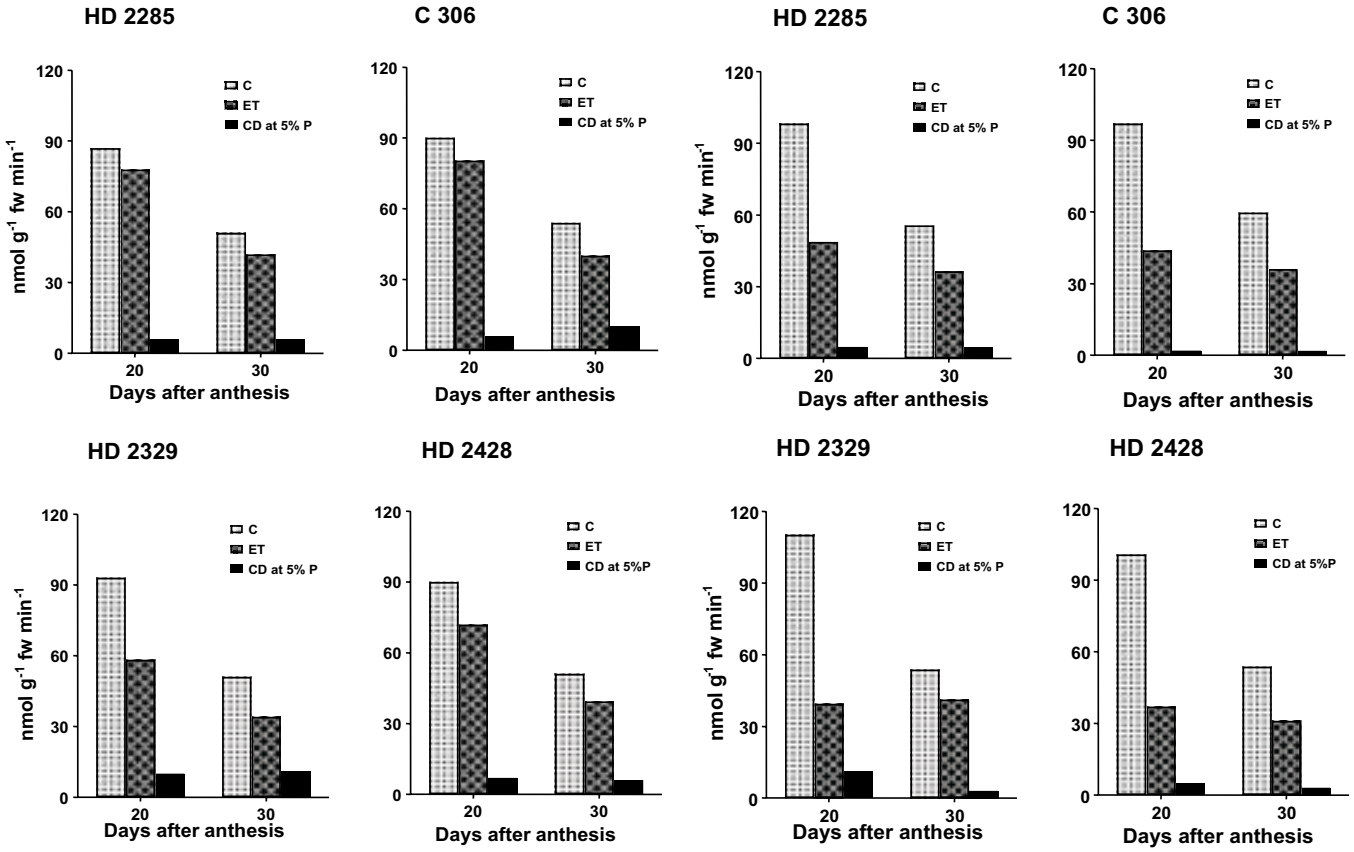

Fig. 3. Soluble starch synthase activity (nmol g^{-1} fw min^{-1}) in the developing grains of MS ear in wheat cultivars under control (C) and elevated temperature (ET) of glasshouse

Fig. 4. Soluble starch synthase activity (nmol g^{-1} fw min^{-1}) in the developing grains of MS ear in wheat cultivars under control (C) and elevated temperature (ET) in phytotron

Table 2. Per cent decrease in soluble starch synthase activity in the grains of MS in wheat cultivars by elevated temperature

Cultivar	Days after anthesis	
	20	30
	Glasshouse	
HD 2285	10.53	17.97
C 306	10.76	25.97
HD 2329	37.48	33.29
HD 2428	20.19	22.89
	Phytotron	
HD 2285	50.48	34.53
C 306	54.78	39.48
HD 2329	64.28	23.33
HD 2428	63.23	42.36

elevated temperature in phytotron (Fig. 4). The decrease in SSS activity 20 DAA in phytotron experiment was between 50.48 to 64.28 % (Table 2). Under phytotron condition also, HD 2285 and C 306 considered to be relatively tolerant to high temperature, showed lesser decrease in SSS activity under elevated temperature over control compared to susceptible types HD 2329 and HD 2428, 20 DAA, the peak stage of starch synthesis in wheat grain.

In the present study, plants were exposed to 5-7°C higher temperature in glasshouse than outside grown plants, whereas, in phytotron experiment the mean temperature was 8°C higher than control during grain development period. Furthermore, in phytotron experiment elevated temperature was much harsher in the sense that given temperature was attained within half

hour and maintained at that level, as a result, the overall energy received was greater under such conditions. On the other hand mean temperatures computed in other experiment conceal large diurnal fluctuations and the maximum is not retained for a longer period. Plants were therefore, exposed to much higher temperature stress in phytotron experiment than glasshouse. The more severe depression in grain growth and SSS activity under ET in phytotron experiment is therefore understandable. The cultivar difference in susceptibility and tolerance, however, remained the same.

The more severe depression in grain growth under elevated temperature in phytotron experiment was associated with greater decrease in SSS activity in the grain. Furthermore, relatively tolerant cultivars showed lesser decrease compared to susceptible cultivars in grain growth and also showed less decrease in SSS activity. Such a parallelism in the effect of elevated temperature on grain growth and SSS activity further suggests that SSS is the key component imparting sensitivity to high temperature for grain growth in wheat. These observations are in line with the report that synthesis of starch in wheat endosperm at high temperature is predominantly controlled by the activity of SSS (Jenner *et al.* 1993, Hawker and Jenner 1993, Keeling *et al.* 1994). The higher sensitivity of this enzyme to high temperature has also been reported (Rijven 1986, Chaudhary-Mehra *et al*. 1994, Prakash *et al*. 2003, 2004). Therefore, a thermostable form of SSS has to be identified in order to improve the thermotolerance for grain growth in wheat.

Cultivar differences in thermotolerance for grain growth were observed in the present study and were also reported earlier (Prakash *et al.* 2003, 2004, Zahedi *et al.* 2003). Sumesh *et al.* (2008) and Sharma-Natu *et al.* (2009) observed that high temperature tolerance of a cultivar for grain growth was associated with higher efficiency of SSS at elevated temperature and higher content of heat shock proteins (HSP 100 and HSP 18) in the grains. 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). It appeared that the association of SSS activity at higher temperature with level of HSP in wheat grain could be through the protection of SSS from heat denaturation. HSP 18 was found to be induced even at moderately high temperature and its association with thermotolerance for grain growth in wheat has been reported (Sharma-Natu *et al.* 2009). It seems that thermotolerance for grain growth in wheat could be improved through incorporation of thermostable form of SSS and by overexpressing HSP 18, which would possible protect SSS from heat denaturation.

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