Indian Journal of Agricultural Sciences **84** (7): 839–43, July 2014/Article
https://doi.org/10.56093/ijas.v84i7.41995

Soluble starch synthase activity in relation to thermal tolerance of developing wheat (*Triticum aestivum*, *Triticum durum)* and maize (*Zea mays*) grains

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Received: 18 September 2012; Revised accepted: 28 March 2014

ABSTRACT

Triticum aestivum (var HD 2987), *T. durum* (var HD 4719) and *Zea mays* (var HQPM 7) were exposed to control (C) and elevated (E) temperature (2.1-3.8°C higher) in open top chambers during post anthesis period. Soluble starch synthase (SSS) activity in the developing grains and grain yield components at maturity were determined in C and E grown plants. Excised developing grains at 20 DAA (days after anthesis) of ambient grown *T. aestivum* (var HD 2987), *T. durum* (var HD 4719) and *Zea mays* (var HQPM 7, HM 10 and DHM 117) were also incubated at 25, 35 and 45°C for 2 hours and then analysed for the activities of SSS. The kinetic parameters of SSS in the grains of ambient grown plants were also determined. The study revealed a higher catalytic efficiency and relatively thermostable SSS in maize grains compared to wheat. Among tested wheat varieties, aestivum wheat showed a better thermostability of SSS *in vitro* and *in vivo* than durum wheat. An association of thermostability of SSS and thermotolerance for grain growth was indicated. The above observation of a highly efficient and relatively thermostable SSS in maize grains may possibly be utilized for improving thermotolerance of SSS and grain growth in wheat. The improved thermotolerance for grain growth in wheat will go a long way in enhancing wheat productivity, which suffers heavy losses due to frequent hot winds during grain filling period.

Key words: Grain growth, Maize, Soluble starch synthase, Thermotolerance, Wheat

High temperature during the grain filling stage is an important yield limiting factor in wheat (Howard 1924). The situation may further aggravate with the increasing concentration of CO_2 and other greenhouse gases in the atmosphere, which are projected to increase the global temperature (Ghildiyal and Sharma-Natu 2000, Ravi *et al.* 2001, Solomon *et al.* 2007). The main effect of high temperature during grain development is a reduction in grain size. Physiologically, the rate of grain filling reflects the rate of biochemical reactions involved in the synthesis of reserves (Zeeman *et al.* 2010). It was observed that a decrease in grain growth and starch accumulation in wheat under high temperature is mainly through a decrease in SSS activity (Prakash *et al.* 2003, Prakash *et al.* 2009, Sumesh *et al.* 2008). There is a need, therefore, to identify thermostable form of SSS that would possibly pave the way for improving thermotolerance for grain growth in wheat. Considering that species grown in a warmer climate may have thermostable SSS and better thermotolerance for grain growth, an attempt was made to examine durum wheat and maize along with aestivum wheat varieties. An earlier study indicated a relatively thermostable SSS in maize grains (Pandey *et al.* 2012). The present study, therefore, aimed at evaluating thermostability of SSS in maize in comparison to wheat grains *in vitro* and *in vivo,* in relation to their thermotolerance for grain growth.

MATERIALS AND METHODS

Experiments were conducted during *rabi* season of 2009-10 and 2010-11 on wheat and maize varieties. Since, similar trends were observed; the data of only 2010-11 are presented. *T. aestivum* var HD 2987, *T. durum* var HD 4719 and *Zea mays* var HQPM 7 were grown in field inside open top chambers (OTCs). Standard cultural practices were followed (Singh *et al.* 2003). Sowing was done on 19 November. The construction of OTCs (300 cm × 200 cm) was based on the design of Leadley and Drake (1993). In one set of OTCs, warm air was supplied by hot air blower, blown by an axial fan, during day time, from anthesis to maturity. The warm air entered the chamber through double walled plenum around the base perforated toward inside. To eliminate the chamber environment effect, chambers in which only air was blown served as control. The maximum and minimum temperature of control (C) and hot air blown (E) OTCs on daily basis were recorded to assess the temperature difference. SSS activity in the grains from the

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middle portion of MS ear was determined at 20 and 30 days after anthesis (DAA) in wheat genotypes and 10, 20 and 30 days after silk appearance in maize in C and E grown plants. The yield components of C and E grown plants were determined at maturity. There were five replications for each observation taken. The heat susceptibility index (S) was calculated for grain yield and 1000 grain weight as described by Fischer and Maurer (1978):

S = (1-Y/Yp)/(1-X/Xp), where, Y = mean grain weight of a genotype grown at high temperature, Yp = mean grain weight of a genotype grown at controlled conditions, X = mean Y of all genotypes, Xp = mean Yp of all genotypes (S $\leq$ 1.0 means stress tolerant and S $\geq$ 1.0 means stress susceptible).

T. aestivum var HD 2987, *T. durum* var HD 4719 and *Zea mays* varieties (HQPM 7, HM 10 and DHM 117) were also grown in ambient field condition following normal cultural practices. The sowing was done on 19 November 2010. Minimum and maximum temperatures on daily basis during grain development were obtained from meteorological laboratory. At 20 DAA (a stage when soluble starch synthase in grain attains its maximum activity) (Prakash *et al.* 2003), grains from middle portion of MS ear were analysed for kinetic characterization of SSS. The excised grains were also incubated at different temperatures of 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 exposure to different temperatures were analysed for soluble starch synthase activity.

SSS was extracted following the method of George *et al.* (1994). SSS 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 in the grains was also determined at different ADPG concentrations. The kinetic constants, V_{max} and K_m (ADPG) of 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 each genotype were also determined to express enzyme activity on dry weight basis.

RESULTS AND DISCUSSION

In E-OTCs (open top chambers with elevated temperatures), genotypes were exposed to a mean maximum temperature of 2.1 to 3.8°C higher than C-OTCs (open top chambers with normal or control temperatures) during grain development period (Table 1). It may, however, be mentioned that mean of maximum temperature in both C and E-OTCs during grain development in maize was 7-9°C higher than wheat. In spite of such a high temperature regime during grain development in maize, SSS activity in maize grains was more than 2-3 times higher than that of wheat (Table 2).

Table 1 Mean of maximum and mean of minimum temperatures during grain development of wheat and maize genotypes grown under control (C) and elevated (E) temperatures in open top chambers (OTC)

Genotype	OTC	Mean of maximum temperature (°C)	Mean of minimum temperature (°C)
T. aestivum HD 2987	C	28.66	13.33
	E	31.30	13.33
T. durum HD 4719	C	27.48	13.31
	E	31.24	13.65
Zea mays HQPM 7	C	36.51	20.83
	E	38.62	20.83

Table 2 Soluble starch synthase (SSS) activity (nmol/g dry wt./min) in the grains of wheat and maize genotypes grown under control (C) and elevated (E) temperatures in open top chambers

Genotype	Days after anthesis	C	E	Per cent decrease
T. aestivum HD 2987	20	271.79±25.21	261.49±40.26	3.79NS
	30	280.96±14.37	201.04±12.54	28.44*
T. durum HD 4719	20	430.65±11.63	437.20±14.98	+1.52NS
	30	240.48±16.12	192.01±16.27	20.15*
Zea mays HQPM 7	10	796.48±12.88	808.00±45.14	+1.51NS
	20	1037.64±26.90	980.51±38.31	5.50NS
	30	642.72±12.17	608.27±14.34	5.36*

* Represents significant at 5% P, NS for non-significant and values are represented as mean ± SE

SSS activity was maximum at 20 DAA. In *T. aestivum* var HD 2987, SSS activity remained more or less same at 20 and 30 DAA in C grown plants, whereas, in E-grown plants 23.11% decrease was observed at 30 DAA as compared to that of 20 DAA. In *T. durum* var HD 4719, SSS activity decreased from 20 DAA to 30 DAA by 44.16% and 56.08% in C and E grown plants respectively. In maize, decrease in SSS activity from 20 to 30 DAA was 38.05% and 37.96% in C and E grown plants respectively. There was no significant difference in SSS activity between C and E grown plants at 20 DAA. SSS activity in grains was significantly less in E-grown plants compared to C-grown plants of wheat at 30 DAA, but no such effect was observed in maize. Since, *T. durum* var HD 4719 showed a sharp decrease in SSS activity from 20 to 30 DAA, the overall decrease in SSS activity under E was maximum in this variety, indicating its greater susceptibility to elevated temperature (Table 2). *T. durum* var HD 4719 also showed greater susceptibility to elevated temperature in terms of grain growth and yield as indicated by its higher S (Table 3). An association of thermostability of SSS and thermotolerance for grain growth was therefore, indicated. The decrease in grain yield of E-grown plants of all the genotypes was attributed mainly to a decrease in 1000 grain

Table 3 Yield components and heat susceptibility index (S) of wheat and maize genotypes grown under control (C) and elevated (E) temperatures in open top chambers

Genotype	Treatment	Grain yield (g/plant)	Grain number/ plant	Thousand grain wt. (g)	Total dry matter (g/plant)	Harvest index (%)	S for grain growth	S for grain yield
T.aestivum	C	2.83	68.28	41.45	7.77	36.42		
HD 2987	E	2.57	66.97	38.57	7.46	34.45	0.727	0.769
	% decrease	9.19*	1.92NS	6.95*	3.99NS	5.41*		
T. durum	C	4.03	92.11	43.75	9.39	42.92		
HD 4719	E	2.90	80.70	35.93	8.61	33.68	1.636	2.23
	% decrease	28.04*	12.39*	17.87*	8.31*	21.53*		
Zea mays	C	99.16	368.68	268.96	233.96	42.38		
HQPM 7	E	86.91	358.02	242.75	208.02	41.78	0.909	1.00
	% decrease	12.35*	2.89NS	9.74*	11.09*	1.41NS		

* Represents significant at 5% P, NS for non significant

weight. *T. durum* var HD 4719 showed a greater decrease in 1000 grain weight in E grown plants (Table 3).

In ambient grown plants, the mean of maximum temperatures during grain development period in maize was 8.2 to 11.1°C higher than wheat (Table 4). In spite of such a high temperature regime during grain development in maize, the SSS activity in the developing grains of all genotypes of maize was more than 2-3 times higher than that of wheat (Fig 1). *Zea mays* var HM 10 had the highest SSS activity. Among wheat genotypes, *T. durum* var HD 4719 had higher SSS activity than *T. aestivum* var HD 2987. The exposure of excised grains to high temperature for two hours *in vitro* did not significantly affect the activity of SSS in all genotypes of maize, except in var. HQPM 7, showing 15.46% decrease at 45ºC (Fig 1 and Table 5). On the other hand, both the wheat genotypes showed a significant decrease in SSS activity by *in vitro* high temperature treatment to excised grains. *T. durum* var HD 4719, however, showed a greater decrease than *T. aestivum* var HD 2987 at both the temperature treatments, indicating higher thermosensitivity of SSS in *T. durum* var HD 4719. SSS from maize genotypes had higher V_{max} and lower K_m (ADPG) compared to wheat genotypes (Table 6). *Zea mays* var HM 10 appeared to have highly efficient SSS with higher V_{max}, lower K_m (ADPG) and higher catalytic efficiency (V_{max}/K_m) (Table 6).

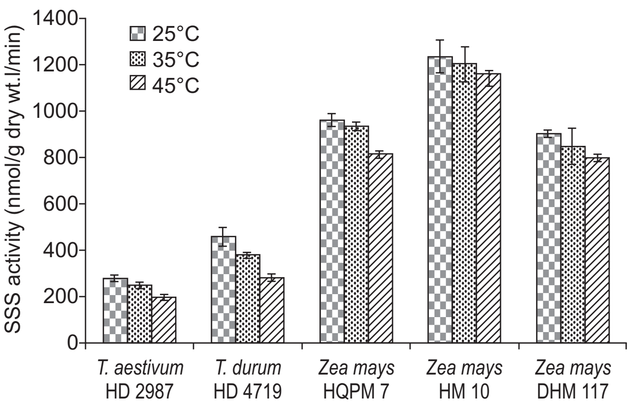

Fig 1 Soluble starch synthase (SSS) activity in the excised grains (20 DAA) of ambient grown wheat and maize genotypes following exposure to different temperatures. Bars represent mean ± SE

Table 4 Mean of maximum and mean of minimum temperatures (ºC) during grain development of ambient grown wheat and maize genotypes

Genotype	Mean of maximum temperature	Mean of minimum temperature
Triticum aestivum HD 2987	27.19	11.92
Triticum durum HD 4719	26.54	11.50
Zea mays HQPM 7	35.78	18.96
Zea mays HM 10	35.36	18.23
Zea mays DHM 117	37.62	20.66

Table 5 Per cent decrease in soluble starch synthase (SSS) activity in the excised grains (20 DAA) of ambient grown wheat and maize genotypes following exposure to different temperatures

Genotype	35°C	45°C
T. aestivum HD 2987	10.76*	29.84*
T. durum HD 4719	17.16*	38.50*
Zea mays HQPM 7	3.00NS	15.46*
Zea mays HM 10	2.65NS	6.26NS
Zea mays DHM 117	5.89NS	11.35NS

* Represents significant at 5% P, NS for non significant, per cent decrease was calculated with reference to 25°C exposure

Table 6 Vmax [nmol/mg (protein)/min], K_m ADPG (mM) and V_{max}/K_m of soluble starch synthase in the grains (20 DAA) of ambient grown wheat and maize genotypes

Genotype	V_{max}	K_m ADPG	V_{max}/K_m
T. aestivum HD 2987	17.20	0.56	30.71
T. durum HD 4719	22.60	0.54	41.85
Zea mays HQPM 7	28.00	0.40	70.00
Zea mays HM 10	38.00	0.30	126.66

High temperature during grain filling stage in wheat, as experienced in India and many other wheat growing regions of the world, is an important yield limiting factor (Howard 1924). It is considered that decrease in grain growth and starch accumulation in wheat under high temperature is mainly through a decrease in SSS activity (Prakash *et al.* 2003, 2004, 2009, Sumesh *et al.* 2008, Sharma-Natu *et al.* 2010). SSS in wheat has been reported to be extremely sensitive to high temperature (Rijven 1986). SSS is thus considered to be a key trait for improving high temperature tolerance for grain growth in wheat and efforts are being made to look for thermostable variant of SSS. Although, varietal variation in high temperature tolerance for grain growth and SSS activity in wheat are reported but the magnitude of variation is not remarkable (Sharma-Natu and Ghildiyal 2005). Considering that the species grown in a warmer climate may have thermostable SSS and better thermotolerance for grain growth, an attempt was made to examine maize and durum wheat along with aestivum wheat varieties. Maize grows in warmer climate and is also a cereal crop, accumulating mostly the starch in grains was considered an appropriate choice for comparative analysis. In commonly grown *kharif* maize, grain development takes place at a decreasing temperature. Hence, *rabi* maize was taken for study, whereby, grain development occurs in increasing temperature as in wheat. The study showed a highly efficient and relatively thermostable SSS in maize grains compared to wheat (Table 2, 5, Fig 1) and reaffirms earlier observation (Pandey *et al.* 2012). The SSS from maize grains has higher V_{max}, lower K_m (ADPG) and higher catalytic efficiency (V_{max}/K_m) (Table 6). Among wheat varieties, aestivum wheat showed a better thermostability of SSS *in vitro* and also *in vivo* and was associated with better thermotolerance for grain growth compared to durum wheat. Greater sensitivity of durum wheat to abiotic stress has also been reported by others (Munns and Tester 2008). The association of thermostability of SSS and thermotolerance for grain growth further strengthens the view that SSS is a key trait for improving thermotolerance for grain growth in wheat.

An important aspect of the above study is the identification of a highly efficient and relatively thermostable SSS in maize. This information can be utilized by plant biotechnologists and plant breeders for incorporation of the thermostable form of SSS in an otherwise high yielding cultivar of wheat. Genes encoding for enzymes of C_4 pathway have been transferred to C_3 plants particularly in rice (Bandyopadhyay *et al.* 2007). In wheat breeding, while the introduction of genes from outside of the *Triticeae* tribe is not a routine procedure, chromatin from C_4 species (*Zea mays* L. and *Tripsacum dactyloides*) has been introduced into wheat (Laurie and Bennett 1986, 1989, Li *et al.* 1996). Greater success has been achieved in oat (*Avena sativa* L.), with the production of a complete set of disomic additions of each of the maize chromosomes (Kynast *et al.* 2001). Expression of C_4 photosynthetic enzymes in some of these oat-maize chromosome addition lines has been reported (Kowles *et al.* 2008). It appears that with the availability of advanced molecular techniques and breeding, it may be possible to introduce SSS of maize into wheat. The study, thus, paved the way for improving thermotolerance for grain growth in wheat. The improved thermotolerance for grain growth in wheat will go a long way in enhancing wheat productivity which suffers heavy losses due to frequent hot winds during grain filling stage.

ACKNOWLEDGEMENTS

The financial assistance in the form of an Emeritus Professor scheme provided to fourth author by Council of Scientific and Industrial Research (CSIR), New Delhi, is gratefully acknowledged.

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