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An efficient and thermostable soluble starch synthase in developing maize (*Zea mays*) grains*

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High temperature during grain-filling stage, as experienced in India and many other wheat (*Triticum acstivum* L. emend. Fiori. & Paol.) growing regions of world, is an important yield-limiting factor (Howard 1924). With the prevalence of rice–wheat cropping system, late sowing of wheat is generally practised which pushes grain development further to high temperature regime. Despite the long standing evidence for the harmful effect of high temperature on grain development in wheat, much progress has not been achieved in improving the high temperature tolerance in wheat. Efforts in this direction have become all the more important in the light of increasing CO_2 concentration and other greenhouse gases in the atmosphere which are projected to increase global temperature (Ghildiyal and Sharma-Natu 2000, Ravi *et al.* 2001, Long *et al.* 2004).

Grain growth depends on availability of assimilates and the ability of grains to utilize assimilates for the synthesis of reserves. In wheat, assimilate availability is generally considered not to be limiting grain growth even under elevated temperature. Limitation in the grain growth under elevated temperature, therefore, lies mainly in the ability of grains to synthesize reserves (Richards 1996). Physiologically, the rate of grain-filling reflects the rate of biochemical reactions involved in the synthesis of reserves. Since, 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 grains (Preiss and Sivak 1996). Starch is deposited in amyloplasts involving ADP glucose pyrophosphorylase (AGPase), starch synthase and branching enzyme (Ball and Morell 2003, Lohot *et al.* 2010, Zeeman *et al.* 2010). Of these, soluble starch synthase (SSS) has been shown to be extremely sensitive to high temperature.

* Short note

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It was reported earlier that a 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, 2004, 2009, Sumesh *et al.* 2008). SSS, thus appeared to be a key trait for improving high temperature tolerance for grain growth in wheat. There is a need to identify thermostable form of SSS. The present study, therefore, aimed at identifying thermostable form of SSS in diverse genotypes. Maize (*Zea mays*), in which grain development takes place under relatively warmer conditions was, therefore, compared with *durum* and *aestivum* wheat varieties for soluble starch synthase (SSS) activity. The maize was grown in (*Rabi*) season so that grain development takes place in an increasing temperature as in wheat.

Six genotypes, including one *Zea mays* L. var. HQPM 7, one *T. durum* var. PDW 291 and four *T. aestivum* varieties (WH 542, Lok Bold, Pusa Gold and Halna), were grown in field following normal cultural practices (Singh 1983). The sowing was done on 23 November 2009. The date of anthesis in the main shoot (MS) of wheat and date of silk appearance in maize were recorded on the tags placed on each plant. Minimum and maximum temperatures on daily basis during grain development were obtained from meteorological laboratory of Indian Agricultural Research Institute (IARI), New Delhi. From the date of anthesis/silk appearance till maturity of grains, mean of maximum and mean of minimum temperatures were calculated for each genotype.

At 20 days after anthesis (a stage when soluble starch synthase in grain attains its maximum activity), basal grains from middle portion of MS ear were separated and stored in liquid nitrogen for subsequent determination of soluble starch synthase (SSS) and granule bound starch synthase (GBSS) activity. Fresh and dry weights of comparable grains of each genotype were also determined to express enzyme activity on dry weight basis. In an another experiment, basal grains from middle portion of MS ear of *Zea mays* var. HQPM 7, *T. aestivum* var. Lok Bold and *T. durum* var. PDW 291 were separated 20 days after anthesis. The excised grains were incubated at different temperatures of 25, 35 and 45°C for 2 hr 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 soluble starch synthase activity.

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). The ADP estimation was carried out by using a preparation of pyruvate kinase which catalyzes the transfer of phosphate from phosphoenol pyruvate to ADP. Pyruvate liberated was estimated (Leloir and Goldenberg 1960).

The wheat and maize varieties taken for investigation differed in date of anthesis. In *T. aestivum* var. Lok Bold, Pusa Gold and Halna, anthesis in MS took place on 14 February 2010, whereas in WH 542 and in *T. durum* var. PDW 291, date of anthesis was 27 February 2010. In *Zea mays* var. HQPM 7, grown in *rabi* season, the silk appearance occurred on 2 April 2010. The grains in these genotypes, therefore, developed under different temperature regimes. The mean of maximum and mean of minimum temperatures during grain development period of different genotypes were determined from daily temperature data, obtained from meteorological laboratory of IARI, New Delhi (Fig 1). The mean of maximum temperatures during grain development period for Lok Bold, Pusa Gold and Halna was 29.40–29.67 °C, whereas, for WH 542 and PDW 291, it was between 33.85 and 34.37°C. In maize, mean of maximum temperature during grain development period was as high as 39.82 °C (Table 1). In spite of such a high temperature regime during grain development in maize, the SSS activity in grains was 3–4 times higher than wheat. This indicated that SSS in maize is not only highly efficient in comparison to wheat but

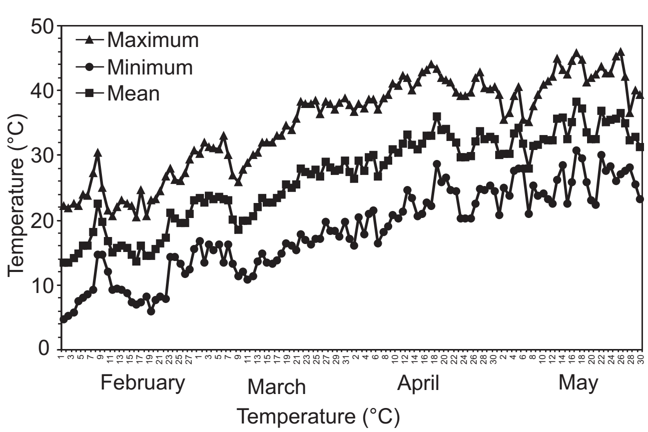

Fig 1 Changes in maximum, minimum and mean temperature during grain development period of wheat and *rabi* maize (*rabi* season 2009–2010)

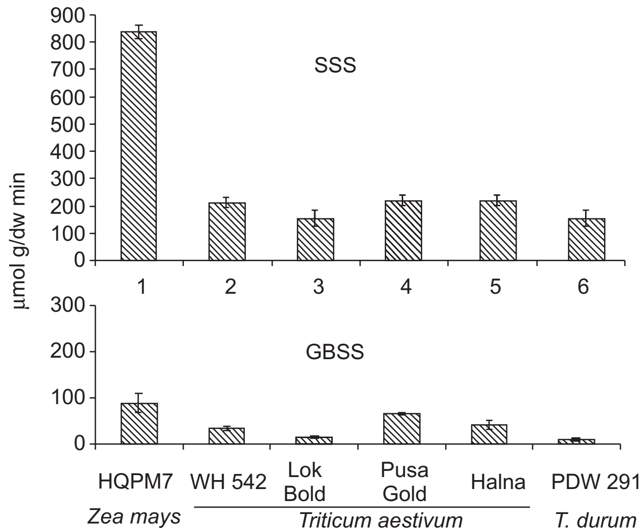

Fig 2 Soluble starch synthase (SSS) and granule bound starch synthase (GBSS) activity in the grains (20 DAA) of maize and wheat varieties. Bar represents mean ± SE.

also thermostable (Fig 2). Among wheat genotypes, Lok Bold and PDW 291 had significantly lower activity of SSS. It may however be mentioned that grain growth in PDW 291 took place at a relatively higher mean maximum temperature. On the other hand, in Lok Bold, lower SSS activity was observed, in spite of a lower mean maximum temperature during grain development period indicating its susceptible nature. The activity of GBSS was considerably lower than SSS activity indicating SSS is the major enzyme for starch synthesis (Fig 2). To evaluate the susceptibility of SSS to high temperature, the excised grains (20 DAA) of maize var. HQPM 7, *aestivum* wheat var. Lok Bold and *durum* wheat var. PDW 291 were exposed to elevated temperature *in vitro*. In maize, no significant effect of high temperature exposure of grains on SSS activity was observed. Lok Bold was found to be most susceptible showing a decrease of 66.84 % at 45 °C treatment compared to that of 25°C. *T. durum* var. PDW 291, showed a relatively lesser decrease in SSS activity at

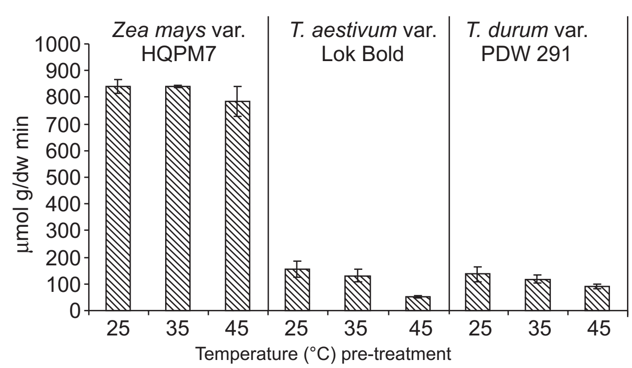

Fig 3 Soluble starch synthase activity in the excised grains (20 DAA) following exposure to different temperature in maize and wheat varieties. Bar represents mean ± SE

Table 1 Mean of maximum and mean of minimum temperatures (°C) during grain development of maize and wheat varieties

Genotype	Mean of maximum temperature	Mean of minimum temperature
Zea mays var. HQPM 7	39.82	22.68
T. aestivum var. WH542	34.37	17.10
T. aestivum var. Lok Bold	29.67	13.51
T. aestivum var. Pusa Gold	29.40	13.38
T. aestivum var. Halna	29.67	13.51
T. aestivum var. PDW291	34.37	17.10

high temperature exposure of grains (Fig 3).

The present study brings out that soluble starch synthase from maize var. HQPM 7 is not only highly efficient in comparison to wheat but also thermostable. The extent of difference between maize and wheat is 3-4 fold in comparison to lesser variation observed among genotypes of wheat. It was reported earlier that decrease in grain growth and starch accumulation in wheat under high temperature is through a decrease in (SSS) activity which is extremely sensitive to high temperature (Prakash *et al.* 2003, 2004, 2009, Sumesh *et al.* 2008, Sharma-Natu *et al.* 2010). SSS is thus considered to be a key trait for improving high temperature tolerance for grain growth in wheat. Efforts are being made to look for thermostable variant of the SSS. Although, varietal variations 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). With the advent of genetic engineering and biotechnology, there is now, no barrier for gene transfer. Hence, attempt was made to examine maize along with wheat genotypes. Since, maize grows in warmer climate and is also a cereal crop, accumulating mostly the starch in grains, it was considered an appropriate choice. 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 present observation of a highly efficient and relatively thermostable SSS in maize var. HQPM 7 could possibly pave the way for improving thermotolerance for grain growth in wheat.

SUMMARY

High temperature during grain-filling stage is an important yield-limiting factor in wheat. It was reported earlier that a decrease in grain growth under high temperature is associated with a decrease in soluble starch synthase (SSS) activity, as this enzyme is extremely sensitive to high temperature. In attempt to identify thermostable SSS, a maize variety, in which grain development takes place under relatively warmer conditions, was compared with *durum* and *aestivum* wheat varieties in the present study. Maize was grown in *rabi* season so that grain development takes place in an increasing temperature as in wheat. The mean of maximum temperature during grain development period was highest for maize. In spite of such a high temperature exposure during grain development, the maize grains had a remarkably higher (3-4 times high) SSS activity as compared to wheat. Granule bound starch synthase (GBSS) activity was considerably lower than SSS activity, indicating SSS is the major enzyme for starch synthesis. High temperature exposure of excised developing grains showed no significant decrease in SSS activity in maize, whereas, *T. aestivum* var. Lok Bold showed a significant decrease. The present study reveals an efficient and relatively thermostable SSS in maize var. HQPM7 as compared to wheat, which could possibly be utilized in improving thermotolerance for grain growth in wheat.

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