4. Kumar, P., Kumar, S. and Manchanda, M. L., Satellite stereo data for DEM surfaces and derivatives. *J. Ind. Soc. Remote Sens*., 2004, **32**, 81–90.
5. Jenson, S. K. and Domingue, J. O., Extracting topographic structure from digital elevation data for geographic information system analysis. *Photogram. Eng. Remote Sensing*, 1988, **54**, 1593–1600.
6. Martz, L. W. and Garbrecht, J., Numerical definition of drainage network and the sub-catchment areas from digital elevation models. *Comp. Geosci.*, 1992, **18**, 747–761.
7. Vogt, J. V., Colombo, R. and Bertolo, F., Deriving drainage networks and catchment boundaries: a new methodology combining digital elevation data and environmental characteristics. *Geomorphology*, 2003, **53**, 281–298.
8. Saha, S. K., Prioritization of sub-watersheds based on erosion loss estimation – a case study of part of Song river watershed, Doon valley using satellite data. Proceedings of the National Symposium on Remote Sensing for Sustainable Development, 1992, pp. 181–186.
9. Rao, D. P., Gautam, N. C., Nagaraja, R. and Ram Mohan, P., IRS-1C application in land use mapping and planning. *Curr. Sci.*, 1996, **70**, 575–578.
10. Moore, I. D. and Wilson, J. P., Length-slope factors for the revised Universal soil loss equation simplified method of estimation. *J. Soil Water Conserv.*, 1992, **45**, 423–428.
11. Jasrotia, A. S., Rainfall–runoff and soil erosion modeling using remote sensing and GIS technique – a case study of tons watershed; paper in of photonirvachak. *J. Indian Soc. Remote Sensing*, 2002, **30**, 167–180.
12. Kothyari, U. C. and Jain, S. K., Sediment yield estimation using GIS. *Hydrol. Sci. J., Proc. IAHS*, 1997, **42**, 833–843.
13. Hydrology. In *SCS National Engineering Handbook*, USDA Soil Conservation Service, US Department of Agriculture, DC, 1972, section 4.
14. Singh, G., Venkataramanan, C., Sastry, G. and Joshi, B. P., *Textbook on Manual of Soil and Water Conservation Practices*, Oxford and IBH, New Delhi, 1990.

ACKNOWLEDGEMENTS. We thank the Dept of Environmental Science, Pune University for the technical and other support extended for the study; Prof. Ghole, Former Head of Environmental Science, University of Pune for giving his technical inputs and encouragement during the various phases of the work.

Received 6 January 2009; revised accepted 7 December 2009

ADP-glucose pyrophosphorylase activity in relation to starch accumulation and grain growth in wheat cultivars

Vaibhav D. Lohot, Poonam Sharma-Natu, Rakesh Pandey and M. C. Ghildiyal*

Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi 110 012, India

ADP-glucose pyrophosphorylase (AGPase) activity in the developing grains of four wheat (*Triticum aestivum* L.) cultivars DL153-2, C306, HD2329 and WH542 grown under normal (27 November) and late (28 December) sown conditions was determined in relation to their grain growth and starch content. In order to analyse the temperature sensitivity of AGPase, excised developing grains (20 days after anthesis) of normal sowing were exposed for 1 h at 25°C, 35°C and 45°C and subsequently analysed for AGPase activity. AGPase activity in the developing grains was also determined in presence of PGA and Pi to evaluate the sensitivity of the enzyme to allosteric effectors. The study showed a highly significant correlation of AGPase activity with starch accumulation and grain growth in wheat under normal sowing but not so under late sowing. However, AGPase was not found to be that sensitive to moderate heat so as to be responsible for decreased starch accumulation and grain growth under late sowing. PGA helped in overcoming inhibition by Pi but did not activate the AGPase further. However, genotypic differences in the sensitivity of AGPase to allosteric effectors were observed. An efficient AGPase insensitive to regulation by PGA and Pi in wheat grain would lead to faster starch accumulation and early filling of grains and may thus avoid extreme terminal high temperature experienced during grain development.

Keywords: ADP-glucose pyrophosphorylase, grain growth, heat tolerance, starch, wheat.

STARCH constitutes around 70% of dry matter in wheat grain. Synthesis and deposition of starch may, therefore, be an important determinant of the size of the grain and thus directly have an impact on yield[1,2]. Starch in grains is deposited in amyloplasts involving ADP-glucose pyrophosphorylase (AGPase), starch synthases and branching enzymes[3,4]. A number of genetic and biochemical studies have established that AGPase is a rate limiting and regulatory enzyme in the pathway of starch synthesis[2,3,5]. AGPase is allosterically activated by 3-phosphoglycerate (3PGA) and inhibited by Pi. In fact, the ratio of 3PGA/Pi governs the catalytic activity of AGPase in leaves[2,6]. The extent to which this enzyme in non-photosynthetic tissues,

*For correspondence. (e-mail: mc_ghildiyal@rediffmail.com)

particularly in wheat endosperm is subjected to allosteric regulation is not clear[2,4]. A number of studies provided evidence that change in allosteric properties of AGPase led to increased starch production and yield[5,7–10].

In India as well as in many other wheat growing regions, grain growth takes place under a rapidly ascending temperature when hot winds are frequent. With the prevalence of rice–wheat cropping system, late planting of wheat is generally practised which pushes grain development further to high temperature regime[11]. The situation may further aggravate with the increasing concentration of CO_2 and other greenhouse gases in the atmosphere which are expected to increase global temperature[12–14]. It has been reported that single grain weight falls by 3–5% for every 1°C rise in temperature above 18°C[15–17]. This is attributed largely to a decrease in the activity of soluble starch synthase which is extremely sensitive to high temperature[18–23]. However, high temperature sensitivity of AGPase has not been adequately investigated[24]. The present study therefore analysed AGPase activity, its sensitivity to allosteric effectors and high temperature in wheat cultivars in order to elucidate the relationship of AGPase with starch accumulation and grain growth.

Wheat (*Triticum aestivum* L.) cultivars DL153-2, C306, HD2329 and WH542 were grown in earthern pots (35×40 cm) containing sandy loam soil under normal (27 November) and late (28 December) sown conditions. Four healthy plants were kept in each pot. Standard cultural practices were followed[25]. The anthesis dates of main shoot (MS) ear were recorded on tags placed on each plant. Minimum, maximum and mean temperatures on daily basis during grain development were obtained from Meteorological Lab of Indian Agricultural Research Institute, New Delhi. The late sowing pushed grain growth period to a higher temperature regime compared to normal sowing. The mean minimum temperature during grain growth duration of different wheat cultivars at normal sowing was between 14.20°C and 15.71°C and mean maximum temperature between 30.48°C and 33.11°C. Under late sowing, mean minimum temperature was between 17.0°C and 17.8°C and mean maximum temperature between 34.10°C and 34.83°C. The differences among cultivars are due to differences in date of anthesis and maturity. Under late sowing, wheat cultivars were, therefore, exposed to mean maximum temperatures of up to 3.65°C and mean minimum temperatures of up to 2.83°C higher during grain growth duration compared to normal sowing. The normal and late sown plants were analysed for yield components at maturity. Starch content was determined in the basal grains of the middle spikelet of MS ear following the method described elsewhere[26].

AGPase activity was determined in the basal grains from the middle spikelets of the MS ear of normal and late sown plants 10, 20, 30 and 40 days after anthesis. Samples were taken between 10 and 11 a.m. and stored in liquid nitrogen. AGPase was extracted following the method described by Singletary *et al.*[27]. AGPase activity was estimated by the procedure of Espada[28]. The glucose-1-P formed by incubation of ADP glucose and PPi with the enzyme was measured by adding phosphoglucomutase, glucose-6-P dehydrogenase and NAD^+. The reduced NAD was estimated by its absorbancy at 340 nm using UV-spectrophotometer (Model CE588 Cecil Instruments, Cambridge). Enzyme assay was carried out in two steps. In the first step, AGPase activity was assayed in a small test tube by adding 0.1 ml of enzyme extract to 0.5 ml of reaction mixture containing 0.2 ml of 0.5 M Tris HCl buffer (pH 7.9), 0.1 ml of 0.1 M $MgCl_2$, 0.03 ml of 0.01 M ADPG, 0.03 ml of 0.02 M sodium pyrophosphate and 0.14 ml of water in a total volume of 0.6 ml. The reaction was started by adding the enzyme and incubated at 25°C for 10 min in water bath. The reaction was stopped by immersing the tube in boiling water bath for 1 min and were then cooled immediately in a water bath to room temperature. Blank was run with the boiled enzyme without PPi. The mixture was centrifuged and the supernatant was taken in small test tube for further assay.

In the second step, the supernatant fluid from first step was added to another tube containing 1 ml of 0.3 M Tris HCl buffer (pH 7.4) (containing 0.03 M $MgCl_2$ and 0.03 M mercaptoethanol), 0.01 ml of phosphoglucomutase and glucose-6-P dehydrogenase (50 units each of phosphoglucomutase and G-6-P dehydrogenase) and water was added to a final volume of 3 ml. The reaction was started by adding 0.03 ml of 0.01 M NAD. The OD was measured (after it became constant) 1 h after initiation of the reaction at 340 nm. The results were calculated from a standard curve drawn by using different concentrations (100–1000 nmol) of NADH. The enzyme activity was expressed on fresh weight basis.

Basal grains from the middle spikelets of the MS ear of normal sown wheat cvs. DL153-2 and C306, 20 days after anthesis, were incubated at different temperatures (25°C, 35°C and 45°C) for 1 h in glass vials lined with moist filter paper and capped with non-absorbent cotton wool[23]. The treated grains were then analysed for the activity of AGPase assayed at 25°C.

AGPase activity in the grains from the middle spikelets of the MS ear of normal sown wheat cvs. DL153-2 and C306, 20 days after anthesis was determined at different concentrations of PGA (0, 1, 2, 4, 6 and 8 mM) in presence of 2 mM Pi in the reaction mixture. Samples without PGA and Pi served as control.

Wheat cultivars examined in the present study differed in grain yield components. DL153-2 had bolder grains and greater starch content but lesser grain number per ear, whereas, WH542 had smaller grain size, lower starch content but higher grain number per ear, although less than HD2329. C306 and HD2329 were intermediate in starch content and grain size. Late sowing decreased these yield components differentially but varietal pattern remained more or less the same (Table 1).

Table 1. Yield components and grain starch content in normal and late sown wheat cultivars

Parameter	Cultivar	Normal sowing	Late sowing	Per cent reduction
Grain yield (g/plant)	DL153-2	15.75 ± 0.80	10.98 ± 0.61	30.28
	C306	16.73 ± 0.95	11.07 ± 0.72	33.83
	HD2329	21.80 ± 0.77	12.46 ± 0.61	42.81
	WH542	13.84 ± 0.90	8.47 ± 0.55	38.82
Grain number/plant	DL153-2	310.89 ± 3.10	222.26 ± 4.28	28.50
	C306	394.85 ± 4.49	290.62 ± 4.65	26.39
	HD2329	511.49 ± 5.47	356.20 ± 4.24	30.36
	WH542	400.23 ± 5.06	284.03 ± 3.67	29.03
100 grain weight (g)	DL153-2	50.66 ± 2.01	49.40 ± 1.45	2.48 NS
	C306	42.37 ± 1.90	38.09 ± 1.80	10.10
	HD2329	42.62 ± 1.80	34.98 ± 1.60	17.92
	WH542	34.58 ± 2.10	29.82 ± 1.20	13.76
Starch content mg/grain	DL153-2	34.23 ± 0.98	32.00 ± 1.28	6.51 NS
	C306	26.91 ± 0.89	26.10 ± 0.73	3.01 NS
	HD2329	30.52 ± 0.98	27.00 ± 1.60	11.53
	WH542	24.12 ± 0.73	21.54 ± 0.65	10.69

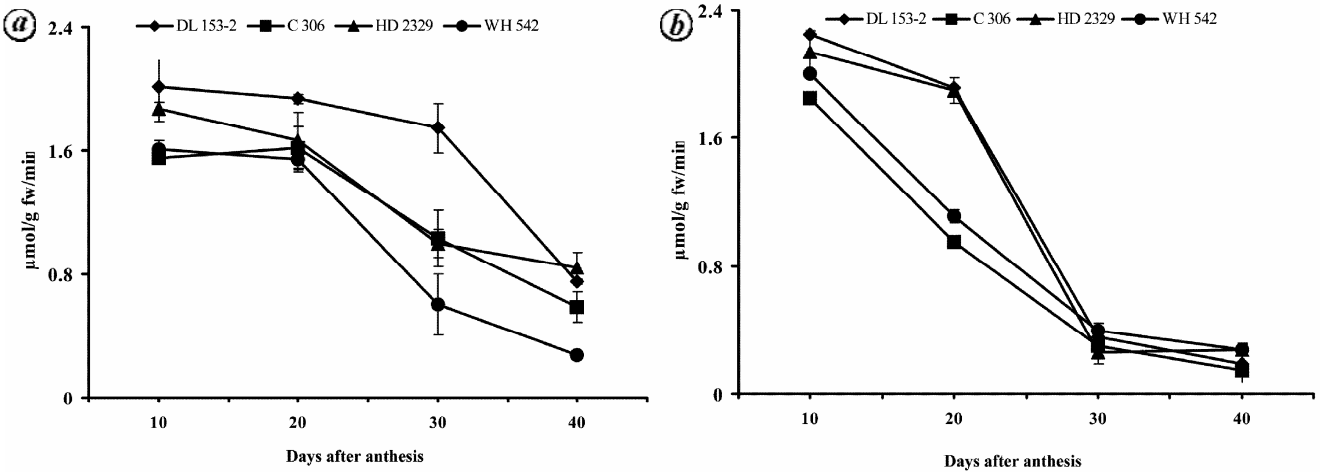

Figure 1. ADP-glucose pyrophosphorylase (AGPase) activity (µmol g^{-1} fw min^{-1}) in the developing grains of MS ear in wheat cultivars under normal (***a***) and late (***b***) sown conditions.

AGPase activity expressed on fresh weight basis was maximum initially and decreased beyond 20 days after anthesis (DAA) in all the cultivars under normal sowing (Figure 1 *a*). DL153-2, however, showed a lesser decrease in activity until 30 DAA. DL153-2 having bolder grains had the higher AGPase activity at most of the stages, whereas, WH542, a small grain type was found to be poorest in this regard. Under late sowing, AGPase activity g^{-1} fw was higher at 10 and 20 DAA and showed a sharp decrease thereafter sinking to lowest level at 30 DAA in DL153-2 and HD2329. In C306 and WH542, a steady decrease in AGPase activity was observed between 10 and 30 DAA (Figure 1 *b*). A highly significant (significant at 1% P) correlation of mean AGPase activity over grain growth period with grain growth and starch accumulation was observed under normal sowing. Such correlations, however, were not significant (at 1% P) in late sown condition (Figures 2 and 3).

AGPase activity in the excised grains (20 DAA) of DL153-2 and C306 pre-exposed to different temperature was between 1.8 and 2.0 µmol g^{-1} fw min^{-1}. There was no significant effect of high temperature pre-exposure of grains on AGPase activity in both the cultivars. AGPase activity in both the cultivars was inhibited by 2 mM Pi. However, this inhibition was greater in C306 than in DL153-2 (Figure 4). PGA (1 mM) was found to overcome this inhibition in C306, whereas, 2 mM PGA was required to overcome Pi inhibition in DL153-2. These observations suggested varietal differences in sensitivity of AGPase from wheat grains to allosteric effectors.

In the present study, a highly significant correlation (significant at 1% P) of AGPase activity with starch

accumulation ($r = 0.888$) and grain growth ($r = 0.970$) was observed in wheat cultivars under normal sowing but not so under late sowing. Under late sowing, grain development occurred under moderately high temperature conditions, hence, temperature might be responsible for such an effect. However, grains pre-exposed to high temperature had no significant effect on the activity of AGPase. Soluble starch synthase, which utilizes the ADP glucose formed by the action of AGPase for the synthesis of starch, has been reported to be extremely sensitive to high temperature[19–21]. The decrease in starch synthesis and grain growth under elevated temperature has been attributed to a decrease in activity of soluble starch synthase[21–23].

A significant correlation of AGPase activity with starch accumulation and grain growth under normal sowing would indicate that enhancing the efficiency of this enzyme would lead to increase in starch accumulation and grain growth. This would lead to a faster grain filling and may thus help in avoiding the terminal high temperature stress for grain growth in wheat. AGPase is allosterically activated by 3PGA and inhibited by Pi. In fact the ratio of 3PGA/Pi governs the catalytic activity of AGPase in leaves[2,6]. A number of studies provided evidence that change in allosteric properties of AGPase led to increased starch production and yield[5,7–10]. In these studies, mutant *E. coli* AGPase was expressed which is insensitive to regulation by PGA and Pi. AGPase activity from developing grains was found to be inhibited by 2 mM Pi in the study. This inhibition was overcome by PGA. PGA, however, was not found to activate the AGPase enzyme in wheat grain. PGA helped in overcoming inhibition by Pi. More or less, similar results were obtained by Gomez-Casati and Iglesias[29] for AGPase from wheat endosperm. In the present study, the differential sensitivity of AGPase from grains of wheat cultivars to allosteric effectors is of particular significance in this context in finding allosterically insensitive AGPase.

A significant correlation of AGPase activity with starch accumulation and grain growth was observed in wheat under normal (November) sowing. This indicates that enhancing the efficiency of this enzyme would lead to a faster grain filling. A change in allosteric properties of AGPase has been shown to increase starch production and yield in potato and maize. In the present study, the differential sensitivity of AGPase from grains of wheat cultivars to allosteric effectors is of particular significance in this context, in finding allosterically insensitive AGPase. Expressing an efficient AGPase insensitive to regulation by PGA and Pi in wheat grain would possibly lead to faster starch accumulation and early filling of

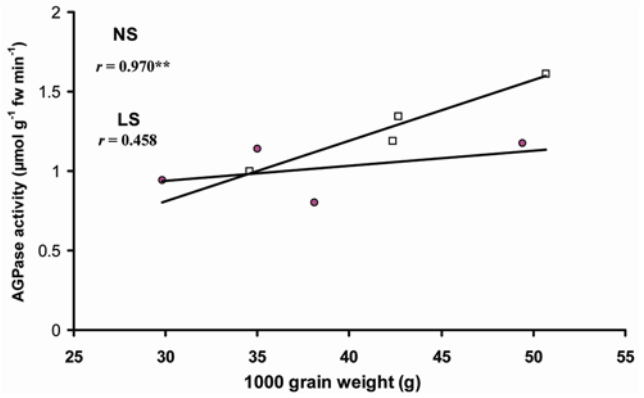

Figure 2. Relationship of mean AGPase activity (µmol g^{-1} fw min^{-1}) with 1000 grain weight in wheat cultivars under normal (NS, □) and late sowing (LS, ●). **Significant at 1% P.

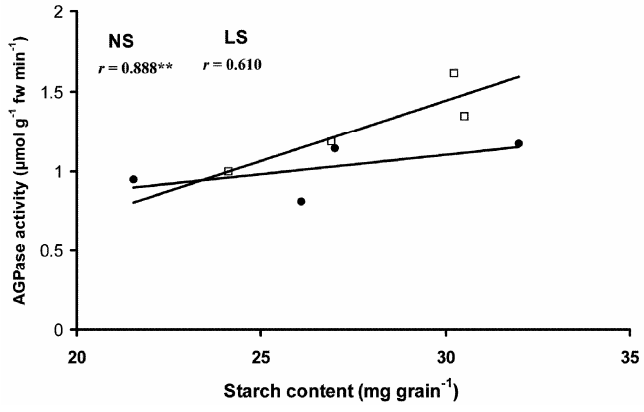

Figure 3. Relationship of mean AGPase activity (µmol g^{-1} fw min^{-1}) with starch content (mg $grain^{-1}$) in wheat cultivars under normal (NS, □) and late sowing (LS, ●). **Significant at 1% P.

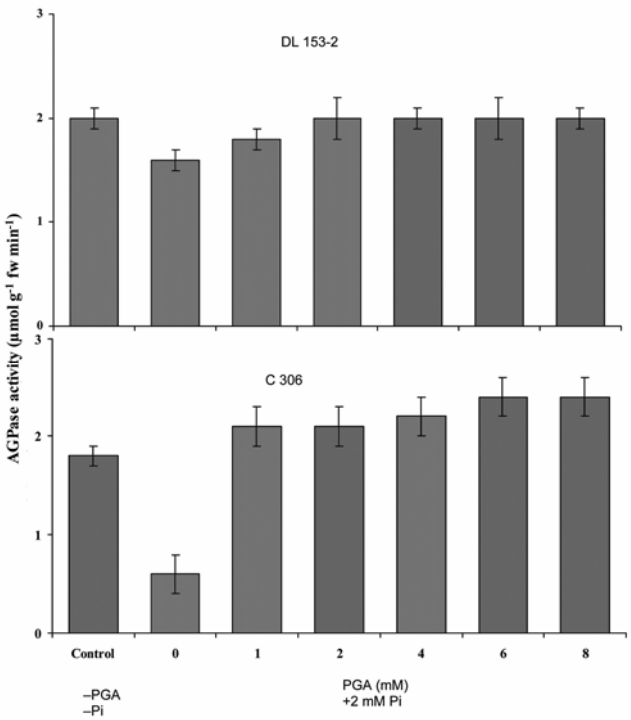

Figure 4. Effect of different concentrations of PGA + Pi (2 mM) on AGPase activity in the grains of wheat cultivars.

grains and may thus avoid extreme terminal high temperature experienced during later part of grain development.

1. Preiss, J., Biosynthesis of starch and its degradation. In *The Biochemistry of Plants*, Academic Press, Orlanto, FL, 1988, vol. 14, pp. 181–254.
2. Preiss, J. and Sivak, M. N., Starch synthesis in sinks and sources. In *Photoassimilate Distribution in Plants and Crops* (eds Zamski, E. and Schaffer, A. A.), Marcel Dekker Inc, New York, 1996, pp. 63–96.
3. Ball, S. G. and Morrel, M. K., From bacterial glycogen to starch: understanding the biogenesis of the plant starch granule. *Annu. Rev. Plant Biol*., 2003, **54**, 207–233.
4. Emes, M. J., Bowsher, C. G., Hedley, C., Burell, M. M., Scrase-Field, E. S. F. and Tetlow, I. J., Starch synthesis and carbon partitioning in developing endosperm. *J. Exp. Bot*., 2003, **54**, 569–575.
5. Stark, D. M., Timmerman, K. P., Barry, G. F., Preiss, J. and Kishore, G. M., Regulation of the amount of starch in plant tissues by ADP-glucose pyrophosphorylase. *Science*, 1992, **258**, 287–292.
6. Sanwal, G. G., Greenberg, E., Hardie, J., Cameron, E. and Preiss, J., Regulation of starch biosynthesis in plant leaves: activation and inhibition of ADP-glucose pyrophosphorylase. *Plant Physiol*., 1968, **43**, 417–427.
7. Giroux, M. J., Shaw, J., Barry, G., Cobb, B. G., Greene, T., Okita, T. and Hannah, L. C., A single gene mutation that increases maize seed weight. *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 5824–5829.
8. Smidansky, E. D., Clancy, M., Meyer, F. D., Lanning, S. P., Blake, N. K., Talbert, L. E. and Giroux, M. J., Enhanced ADP-glucose pyrophosphorylase activity in wheat endosperm increases seed yield. *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 1724–1729.
9. Smidansky, E. D., Martin, J. M., Hannah, L. C., Fischer, A. M. and Giroux, M. J., Seed yield and plant biomass increases in rice are conferred by deregulation of endosperm ADP-glucose pyrophosphorylase. *Planta*, 2003, **216**, 656–664.
10. Sakulsingharoj, C. *et al.*, Engineering starch biosynthesis for increasing rice seed weight: the role of the cytoplasmic ADP-glucose pyrophosphorylase. *Plant Sci.*, 2004, **167**, 1323–1333.
11. Zhong-hu, He, and Rajaram, S., Differential responses of bread wheat characters to high temperature. *Euphytica*, 1994, **72**, 197–203.
12. Bowes, G., Photosynthetic responses to changing atmospheric carbon dioxide concentration. In *Photosynthesis and the Environment* (ed. Baker, N. R.), Kluwer Academic Publ., Dordrecht, 1996, pp. 387–407.
13. Ghildiyal, M. C. and Sharma-Natu, P., Photosynthetic acclimation to rising atmospheric carbon dioxide concentration. *Indian J. Exp. Biol*., 2000, **38**, 961–966.
14. Ravi, I., Khan, F. A., Sharma-Natu, P. and Ghildiyal, M. C., Yield response of durum (*Triticum durum*) and bread wheat (*T. aestivum*) varieties to carbon dioxide enrichment. *Indian J. Agric. Sci*., 2001, **71**, 444–449.
15. Wiegand, C. L. and Cuellar, J. A., Duration of grain filling and kernel weight of wheat as affected by temperature. *Crop Sci.*, 1981, **21**, 95–101.
16. McDonald, G. K., Sutton, B. G. and Ellison, F. W., The effect of time of sowing on the grain yield of irrigated wheat in the Naomi Valley, New South Wales. *Aust. J. Agric. Res.*, 1983, **34**, 229–240.
17. Wardlaw, I. F., Dawson, I. A. and Munibi, P., The tolerance of wheat to high temperatures during reproductive growth II. Grain development. *Aust. J. Agric. Res*., 1989, **40**, 15–24.
18. Rijven, A. H. G. C., Heat inactivation of starch synthase in wheat endosperm tissue. *Plant Physiol*., 1986, **81**, 448–453.
19. Keeling, P. L., Bacon, P. J. and Holt, D. C., Elevated temperature reduces starch deposition in wheat endosperm by reducing the activity of soluble starch synthase. *Planta*, 1993, **191**, 342–348.
20. Jenner, C. F., Denyer, K. and Guerin, J., Thermal characteristics of soluble starch synthase from wheat endosperm. *Aust. J. Plant Physiol.*, 1995, **22**, 703–709.
21. Prakash, P., Sharma-Natu, P. and Ghildiyal, M. C., High temperature effect on starch synthase activity in relation to grain growth in wheat cultivars. *Indian J. Plant Physiol*. (*Spl. Issue*), 2003, **8**, 390–398.
22. Prakash, P., Sharma-Natu, P. and Ghildiyal, M. C., Effect of different temperature on starch synthase activity in excised grains of wheat cultivars. *Indian J. Exp. Biol.*, 2004, **42**, 227–230.
23. Sumesh, K. V., Sharma-Natu, P. and Ghildiyal, M. C., Starch synthase activity and heat shock protein in relation to thermal tolerance of developing wheat grains. *Biol. Plant*, 2008, **52**, 749–753.
24. Keeling, P. L., Banisadr, R., Barone, L., Wasserman, B. P. and Singletary, G. W., Effect of temperature on enzymes in the pathway of starch biosynthesis in developing wheat and maize grain. *Aust. J. Plant Physiol.*, 1994, **21**, 807–827.
25. Singh, C., *Modern Techniques of Raising Field Crops*, Oxford & IBH, New Delhi, 1983.
26. Ghildiyal, M. C. and Sinha, S. K., Physiological analysis of heterosis in *Zea mays* L. I. Changes in sugars and starch contents in relation to grain development. *Indian J. Exp. Biol.*, 1977, **15**, 899–904.
27. Singletary, G. W., Banisadr, R. and Keeling, P. L., Heat stress during grain filling in maize: effects on carbohydrate storage and metabolism. *Aust. J. Plant Physiol*., 1994, **21**, 829–841.
28. Espada, J., ADP-glucose pyrophosphorylase from corn grain. In *Methods of Enzymology: Complex Carbohydrates* (eds Neufeld, F. F. and Ginburg, V.), Academic Press, New York, 1966, vol. VIII, pp. 259–260.
29. Gomez-Casati, D. F. and Iglesias, A. A., ADP-glucose pyrophosphorylase from wheat endosperm. Purification and characterization of an enzyme with novel regulatory properties. *Planta*, 2002, **214**, 428–434.

ACKNOWLEDGEMENT. Financial assistance provided by Council of Scientific and Industrial Research, New Delhi is gratefully acknowledged.

Received 20 April 2009; revised accepted 11 January 2010