Bioenergetic cost of heat tolerance in wheat crop

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Decline in national wheat productivity, and stagnation of yield in the national Advanced Varietal Trials in the past decade are partly attributed to high temperature stress during the period of grain-filling. In view of the predicted global warming, terminal heat stress is likely to increase. Heat shock proteins (HSPs) synthesized to cope with the heat stress in different organisms are known to provide protection and repair the cellular damage caused by heat. The production cost of HSPs from different plant species – wheat, *Arabidopsis*, *Phaseolus* and maize ubiquitin was calculated based on their reported amino acid composition, and the production value of individual amino acids published earlier. Glucose required for the production of wheat HSP 101 was 20% higher compared to the storage proteins in wheat grains. Thus, incorporation of terminal heat tolerance into high-yielding cultivars will have an energetic cost, and would require additional carbon assimilates and N inputs.

Keywords: Bioenergetic cost, heat shock proteins, production value, terminal heat stress, wheat.

THE food security of our country is dependent on wheat crop to a large extent. It accounted for 55.8% of the total foodgrain buffer stock in 2002, when the reserve had accumulated close to 60 million tonnes (mt). Stagnation of wheat yield and declining production in the past few years, after touching the peak of 76.37 mt in the crop season of 1999–2000, and recent imports have shaken the confidence of the nation, raising apprehensions for the country's ability to produce adequate food to meet the demand of the growing, economically ascendant population. Wheat yield in Punjab has declined[1] from 4.7 to 4.2 t/ha between 2000 and 2005. At the present economic growth rate[2] occasional imports are not a matter of concern. However, for maintaining the overall economic growth rate above 8%, a minimal agricultural growth rate of 4% is crucial[3]. Stagnation in wheat production and productivity is a matter of great concern[1,3,4]. This is all the more important for the near future in view of the predicted global warming by the United Nations Inter-Governmental Panel on Climate Change[5]. The negative impact of temperature increase of 0.4°C observed between 1980 and 2000 on global wheat production has been demonstrated[6]. Climate-driven yield change between 1981 and 2002 is estimated at –88.2 kg/ha. The authors infer that the observed negative impact is after taking into consideration the expected increase due to higher levels of atmospheric CO_2 since 1981.

In India, wheat is mainly grown during the winter season, planted during October–November and harvested in March–April. The Northwestern Plains Zone (NWPZ) comprising Punjab, Haryana, Delhi, parts of Rajasthan, and western Uttar Pradesh (UP) and the Northeastern Plains Zone (NEPZ) including eastern UP, Bihar, Jharkhand, Orissa, West Bengal and Assam respectively, account for 40 and 33% of the total wheat area grown (26 m ha). Nagarajan[4] in an in-depth analysis arrived at the following causes for declining wheat productivity in the country: (i) decrease in the use of fertilizers (N, P and K) in the NWPZ and NEPZ; (ii) micronutrient imbalance in soils; (iii) fatigue in genetic gain in varietal development, and terminal heat stress in the NWPZ; and (iv) low minimum support price fixed by the Government for the procurement of wheat. The requirement of wheat for 2020 is estimated at 109 mt. In this communication we examine the effect of heat stress considering the bioenergetic cost and resulting trade-offs involved in crop plant improvement[7–9]. The macronutrients (NPK) to be harvested in the crop providing 109 mt of grain by 2020 are also estimated.

Though India is the second largest producer of wheat in the world, the average productivity is 2770 kg/ha against 3885 kg/ha in China and 8043 kg/ha in UK, The Netherlands and other NW European countries. Productivity in India is only 35% of that in UK. The wheat crop in UK and NW European countries grows at much lower temperatures all through the crop duration with no water stress, and the grain-filling takes place over an extended period of 60 days at temperatures below 20°C. The semi-dwarf Indian cultivars when grown as spring wheat in experimental plots in Europe, also give much higher productivity. In India, the lower productivity is due to shorter crop duration and period of grain-filling, and higher temperatures during crop growth, particularly during grain-filling. Crop duration varies from 150 days in the north and goes down to 100 days in Maharashtra and further south, with corresponding decline in yield. A loss of 100 kg/ha for each 100 km distance is reported in the Indo-Gangetic Plain[4]. Besides heat stress, wheat crop also faces water stress during the period of grain-filling. Based on the photosynthetically active radiation and assuming 150-day crop duration, theoretically maximum biomass and grain yield of 28,000 and 11,200 kg/ha at Delhi was estimated[10]. Grain yield recorded[4] for the best cultivars in the Advanced Varietal Trials of the All-India Coordinated Wheat Improvement Program (AICWIP) between 1984–85 and 2002–03 was 5400 kg/ha. It is not likely that the mean yield levels of the NW European countries or the predicted theoretical maximum under Delhi environment can be realized in the country.

Wheat crop physiology has been extensively studied globally[11] and to some extent in India[12,13]. The pre- and

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*For correspondence. (e-mail: neil@bom7.vsnl.net.in) post-anthesis periods are the two distinct phases of crop growth. The pre-anthesis phase that determines the yield potential of the crop includes: (i) germination to seedling emergence; (ii) canopy development and conversion of the vegetative meristem to reproductive meristem and spike initiation; (iii) spikelet development – first spikelet initiation to the formation of the terminal spikelet; and (iv) ear emergence and anthesis. Post-anthesis phase includes the period of grain development to maturity. Higher temperatures affect all phases of crop growth, accelerate floral initiation, reduce the period of spike development, resulting in shorter spike with lower number of spikelets, and adversely affecting pollen development. The duration of grain growth in the post-anthesis period is considered the most significant determinant of yield in wheat[11]. Effect of temperature on the development of wheat grain under Indian conditions was investigated[12,13]. Both the day and night temperatures have a pronounced effect on the duration of grain-filling. It could extend to over 80 days at 15/10°C day/night temperature and is reduced to less than 40 days at 30/25°C. Higher temperatures further associated with limitation of water cause rapid shrinkage of grain volume.

Most of the carbohydrates in the wheat grain are derived from the photosynthates produced in the flag-leaf after anthesis[11]. A positive correlation ($r = 0.8$) between rate of photosynthesis and grain yield was observed in diverse germplasm[14]. Thus, the processes that determine yield are net canopy photosynthesis, translocation of the assimilates, and sink capacity in the developing grains. In the modern, well-adapted cultivars, the three are well balanced. Higher temperatures at earlier stages reduce the spike length and the number of spikelets (sink capacity). Heat stress after anthesis reduces the net availability of assimilates, reducing grain number and weight. Genetic variability for heat stress tolerance to these components is known. The challenge, however, is to incorporate heat tolerance into high-yielding genotypes.

High temperatures are known to have deleterious effects on photosynthesis, respiration and reproduction[11]. At molecular level, these effects are brought about by altered gene expression and manifested at the biochemical and metabolic level, membrane stability, and production of heat shock proteins (HSPs)[15,16]. All organisms are known to respond to higher temperature by altered gene expression. Activities of the various enzymes involved in cellular processes are affected by increased temperature. The concept of thermal kinetic window (TKW) of an enzyme is defined as the temperature range in which the Michaelis–Menten constant (K_m) of the enzyme remains within 200% of the optimum[17]. Whole plant response to temperature stress reflects the thermal dependence of K_m of different enzymes. A 5°C increase results in selective expression of HSPs, with continued synthesis of normal cellular proteins. With further increase in temperature, synthesis of HSPs predominate, while the others are inhibited. At still higher temperatures, even the synthesis of HSP is inhibited. HSPs were first reported in *Drosophila* and subsequently have been found in several plant species, including rice, maize, wheat and *Arabidopsis*[15–18]. Several families of HSPs on the basis of high (>70 kDa) and low (14–30 kDa) molecular weight have been identified[19,20]. Development of heat tolerance is correlated with HSP synthesis. Mutants defective in HSP synthesis are not able to acquire thermotolerance[20]. Over expression of *Arabidopsis* HSP101 in transgenic rice improved growth after heat stress[21]. HSPs are assigned the role of ‘housekeeping’ in the cell, and are involved in repair of the heat-induced damage in cellular proteins, especially in correct protein folding to ensure biological activities[18]. Gradual increase in temperature, as experienced by the plants in field, also elicits similar response.

The concept of production value (PV) was originally developed by Penning de Vries *et al.*[22] who after extensive examination of the biochemical pathways and energy requirements of the component reactions concluded that in plants, under aerobic conditions 1 g of glucose can be used to produce 0.83 g of carbohydrates, or alternatively 0.40 g of proteins (assuming nitrate to be the nitrogen source) or 0.33 g of lipids. The derivation of these values and the assumptions were discussed. PV is defined as the weight of the end-product divided by the weight of the substrate required for C-skeletons and energy production based on biochemical pathways expressed in units of g/g. Inverse of PV (1/PV) gives gram of glucose required to make 1 g of the end-product. Construction cost of the individual amino acids in terms of glucose equivalent (1/PV) has a significant correlation of 0.79**, with the heat of combustion (ΔH^0C) that represents the intrinsic energy content of a molecule[8]. We had earlier estimated bioenergetic cost of different plant-breeding objectives, such as improving grain protein content[7], grain amino acid[8] and fatty acid composition[23], yield[24] and for plant-produced chemicals that are known to provide protection from insect pests[25]. Here we have estimated the PVs of the known HSPs, reported to have a significant role in heat tolerance from different species – heat-induced maize ubiquitin, *Arabidopsis* HSP 70, wheat HSP 101 and *Phaseolus* HSP 100. Calculated PVs of different HSPs, based on their amino acid composition and the PVs of the amino acids are given in Table 1. The estimated PVs of the Rubisco large and small subunits and wheat grain proteins are included as they are the contenders competing for the assimilate diversion from the source at the time of grain development. Competing demand of assimilates for the synthesis of these proteins will be reflected in seed yield. The PVs of the proteins are the result of their constituent amino acid composition and proportion of those amino acids with low PVs and the number of amino acid residues of the protein molecule (cost of polymerization increases with the number of residues). The amino acid composition of the HSP and Rubisco is given in Table 2.

Table 1. Production value (PV) of the putative heat shock proteins (HSP) of plants

Protein	PV	Amount (g) of glucose required to produce 1 g protein	Per cent increase over wheat grain protein
Maize ubiquitin	0.401	2.49	19.1
Arabidopsis HSP70	0.408	2.45	17.2
Wheat HSP 101	0.398	2.51	20.1
Phaseolus HSP 100	0.397	2.52	20.6
Wheat grain protein	0.478	2.09	–
Rubisco LSU	0.394	2.54	21.5
Rubisco SSU	0.390	2.56	22.5
Wheat grain	0.710	1.41	

PV is defined as the weight of the end-product divided by the weight of the substrate required for carbon skeletons and energy production. Its unit is g/g. The number of amino acid residues and the predicted molecular weight of each protein were computed from the protein sequence data using Expasy Prot Param tool. Methods for the calculation of PVs of the above proteins are from Mitra *et al.*[8].

Sources of protein sequence data: Maize ubiquitin NCBI accession P69319; *Arabidopsis thaliana* HSP 70 NCBI accession AAN 71949; Wheat (*Triticum aestivum*) 101 kDa HSP NCBI accession AAF01280; *Phaseolus lunatus* HSP 100/ClpB NCBI accession AAF91178; maize Rubisco (ribulose 1,5-diphosphate carboxylase/oxygenase) large subunit NCBI accession CAA 60294; maize rubisco small subunit NCBI accession BAA00120; wheat grain protein PV from Mitra *et al.*[8].

Table 2. Amino acid composition of the putative HSPs and Rubisco large and small subunits (%)

Amino acid	PV of individual amino acids	Production cost of amino acid glucose (g) for 1 g amino acid	Maize ubiquitin	*Arabidopsis* HSP 70	Wheat HSP 101	*Phaseolus* HSP 100	Rubisco large subunit	Rubisco small subunit
Asp	0.8024	1.25	7.9	7.5	6.7	6.4	5.9	4.7
Glu	0.7054	1.42	6.6	6.8	9.0	7.7	6.5	2.9
Ser	0.6928	1.44	3.9	7.5	4.7	7.0	3.8	9.4
Gly	0.6091	1.64	7.9	9.6	7.3	5.9	9.9	6.5
Thr	0.5711	1.75	9.2	7.1	3.6	5.5	6.5	5.3
Ala	0.5527	1.81	3.9	8.8	10.7	8.0	9.5	7.1
Asn	0.5259	1.90	2.6	3.9	2.4	3.6	2.9	4.1
Gln	0.5054	1.98	7.9	4.0	4.4	4.3	2.3	4.1
Cys	0.4913	2.04	0.0	0.3	0.2	0.4	2.3	2.9
Val	0.4750	2.11	5.3	9.3	9.1	7.2	6.1	6.5
Pro	0.4469	2.14	2.6	4.0	3.5	3.1	4.6	7.1
Leu	0.4534	2.21	11.8	7.2	11.1	11.0	7.8	7.6
Tyr	0.4443	2.25	1.3	1.0	1.8	1.9	3.6	5.9
Met	0.4309	2.32	1.3	1.4	2.3	2.4	2.3	3.5
Phe	0.4248	2.35	2.6	3.8	2.0	3.3	4.6	4.7
Ile	0.4190	2.39	9.2	5.2	4.6	5.8	5.3	3.5
Lys	0.3952	2.53	9.2	7.1	5.4	6.9	5.5	5.9
Trp	0.3880	2.58	0.0	0.1	0.2	0.3	5.7	2.4
Arg	0..3601	2.78	5.3	4.9	8.8	7.7	5.9	5.3
His	0.3499	2.86	1.3	0.4	2.3	1.7	3.2	0.6

Amino acids are arranged in the order of decreasing PVs. 1/PV is the construction cost in terms of glucose equivalent. PVs of amino acids are from Mitra *et al.*[8].

Wheat grain protein is deficient in lysine, methionine, threonine, tryptophane, isoleucine, leucine, phenylalanine and valine, and is predominantly rich in glutamic acid and its amide. Hence, PV of wheat grain protein is comparatively higher than HSPs and Rubisco (Table 1).

Nitrogen economy and N-use efficiency are altered under heat stress. Increasing soil N during the period of grain-filling at 20/15°C enhanced[26] grain weight, grain protein and grain protein %. However, at higher temperatures (30/25°C), these attributes were not enhanced by increasing N supply. High N at thermal stress reduced the duration of protein deposition in the grains due to inadequate supply of carbohydrates. In the absence of adequate N supply from the soil, 80% of grain N comes from remobilization from the leaves. Carbohydrates in wheat grain are derived from post-anthesis CO_2 assimilation, of which the flag-leaf contributes a large share[11]. Rubisco constitutes 60% of the soluble protein in the flag-leaf. In limiting soil N supply, Rubisco becomes the source of N for the developing grains. High turnover of Rubisco ten days

Table 3. Fertilizer requirement for wheat crop to produce grain yield of 4200 kg/ha

Nutrient	Per cent in grain	Harvested in grain (4200 kg/ha)	Per cent in straw	Harvested in straw (10,500 kg/ha)	Total grain + straw kg/ha
N	2.33	97.86	0.67	42.21	140.07
P	0.48	20.16	0.08	5.04	25.20
K	0.49	20.58	0.89	56.07	76.56

Assuming harvest index of 40.
Harvest index is defined as grain yield divided by biological yield × 100.

after anthesis has been reported[27]. Degradation of Rubisco further enhances flag-leaf senescence, contributing to reduction in the supply of assimilates to developing grains. Specific data on the turnover of the HSPs in wheat could not be found, though their degradation and resynthesis in other organisms is known. Therefore, competition for both C and N assimilates between Rubisco, HSPs and storage proteins in developing grains is expected during continuing heat stress.

Leaf area duration after anthesis is correlated to grain yield[11]. Induction of flag-leaf senescence could also be the reason for the reported[4] stagnation of yield in the advanced varietal trials of the AICWIP. In Table 3, the estimated N, P and K requirement to produce 4200 kg/ha yield of wheat to meet the projected demand are given. This shows that fertilizer applications will have to be considerably enhanced from the present usage of 126 kg/ha. The fertilizer requirement also points to the need for selection of genotypes with higher yield potential at these N levels in the breeding programmes.

The present yield gap between the mean productivity and that realized in the AICWIP trials is 50% (5400 – 2700 = 2700 kg/ha). The current yield gap in Punjab is (5400 – 4200 = 1200 kg/ha). While all efforts should be made to enhance the need-based fertilizer application and improve crop management to bridge the overall yield gap, productivity in the plant-breeding plots must also be enhanced. Soil fertility, availability of macro- and micronutrient, and organic matter to support microbial population should provide an environment conducive for full expression of the genetic yield potential.

With the predicted climate changes and global warming[5], terminal heat stress for the wheat crop is likely to increase in the near future. Heat tolerance would have an energetic cost as shown in the estimations presented. In energetic terms, plant breeding indirectly aims to enhance the energy flow through the cropping systems. External energy inputs in the form of irrigation, nutrients and pest-control measures help to capture a larger harvest of solar energy – a free resource. The various options for increasing grain productivity, energetic cost and N requirements have been examined earlier[24]. Increasing harvest index (HI) was found to be the best option from the energetics viewpoint. This option has been fully exploited and may not be able to provide the additional need of assimilates for heat stress tolerance. It would be necessary to maximize CO_2 fixation through early canopy development and enhance storage of soluble carbohydrates in the stem and leaf sheaths, that can meet the demand in the post-anthesis period. Genetic gains in grain yield of winter wheat cultivars . developed in the UK between 1972 and 1980 were largely due to increased HI, but the cultivars approved from 1983 onwards also show increased above-ground dry matter[28]. This is attributed to improved growth rate in the pre-anthesis period. Primitive 'Khapli' wheat (*Triticum dicoccoides*) grown in parts of Maharashtra and Karnataka have a large phytomass, and very low HI and grain yield compared to the modern wheat cultivars. Another possibility is by enhancing nitrogen use efficiency. This has been recently reported in rice by transferring alanine aminotransferase (AlaAT) c-DNA from barley into rice along with a root epidermal-specific promoter[29]. Transgenic plants had significantly higher biomass and seed yield compared to the control. Similar results have been reported in *Brassica napus*[30] from Arcadia Biosciences, a California-based company. Yield levels equivalent to the control with 50% less fertilizer nitrogen applications have been reported in *B. napus*. The reduced nitrogen technology seems to work for wheat and other crops[31].

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