

## Grain Filling Duration and Temperature Pattern Influence on the Performance of Wheat Genotypes under Late Planting

D. GARG<sup>1</sup>, S. SAREEN<sup>1</sup>, S. DALAL<sup>2</sup>, R. TIWARI<sup>1</sup> and R. SINGH<sup>1\*</sup>

<sup>1</sup>Directorate of Wheat Research, Karnal-132001, India

<sup>2</sup>Kurukshetra University, Kurukshetra, India

(Received 8 June 2012; accepted 19 October 2012;  
Communicated by J. Johnson)

Terminal heat referred to as increase in temperature during grain filling, is one of the important stress factors for wheat production and is responsible for decline in wheat production in many environments worldwide. In order to meet the challenges of high temperature ahead of global warming, concerted efforts are needed to evaluate wheat genotypes for heat tolerance and develop genotypes suitable for such stressed environments. Twenty-seven advanced wheat genotypes developed for stress and normal environments by different research centres were evaluated during 2009–10 and 2010–11 under timely sown (normal) and late sown (heat stress) environments. Analysis of variance revealed that the genotypes differed significantly in grain filling duration (GFD), grain growth rate (GGR) and thousand-grain weight (TGW). Out of 27 genotypes, 16 were found to be tolerant for thousand-grain weight under late planting (heat stress) during 2009–10 but only 12 were tolerant during 2010–11. Many of the genotypes registered more reduction in thousand-grain weight during 2010–11 as compared to 2009–10; the temperatures during 2009–10 were higher. The differences in grain filling duration under two conditions during both seasons as well as difference in temperatures during first half of grain filling explain the reduction pattern in the genotypes. GFD had significant negative correlation with temperatures during post heading period and the difference in GFD under two environments had positive correlation with these temperatures. The reduction in GFD had regression of 33.3% on reduction in GGR and reduction in GGR had regression of 41.6% on reduction in TGW genotypes AKW 1071, DBW 17, HS 277, K 7903, K 9107, NW 1014 and RAJ 3765 had less sensitivity to stress environments during both years.

**Keywords:** grain filling duration, thousand-grain weight, heat stress, grain growth rate, wheat (*Triticum aestivum* L.)

### Introduction

Heat stress is responsible for decline in wheat production in many environments around the world covering 36 m ha (Hays et al. 2007). Current estimates indicate that wheat crop grown on around 13.5 m ha in India is affected by heat stress (Joshi et al. 2007). In South East Asia, a significant wheat growing area is affected by heat stress and majority of this lies in Eastern Gangetic Plains, central and peninsular parts of India. Intergovernmental

\* Corresponding author; E-mail: rajenderkhokhar@yahoo.com

Panel on Climate Change projected that temperature increase by the end of this century is expected to be in the range 1.8 to 4.0°C (IPCC 2007). Due to global warming, by 2020 in south Asia the Rabi season will face an increase of 1.08°C and 1.54°C increase in minimum and maximum temperature and by 2050 an increase of 2.54 and 3.18°C in minimum and maximum temperatures has been predicted. In many regions of the world, including the parts of India, Pakistan, United States, Australia and Mexico, wheat crops are exposed to high temperatures during grain filling period and thus adversely affecting the plant growth, yield and grain quality. High temperature shortens the duration of grain fill and decrease the time to apoptosis and harvest maturity (Altenbach et al. 2003). Yield loss due to moderately high temperature is associated with shortening of grain growth period (Bagga and Rawson 1977; Stone and Nicolas 1995a). In order to meet the challenges of high temperature ahead of global warming, concerted efforts are being made to evaluate germplasm for heat tolerance and identify and develop genotypes suitable for such stressed environments. The crop is exposed to high temperature stress by late planting. However, the stress intensity varies from season to season under field conditions. Our objectives were to study genotypes performance during both crop seasons and stress environments and to determine the role of grain filling and temperature in reduction pattern of genotypes.

## Materials and Methods

### *Plant materials*

Twenty-seven advanced breeding lines and cultivars of wheat (*Triticum aestivum* L.) developed at various centres under All India Coordinated Wheat Improvement Program for different agro-climatic conditions constituted the plant material for present study (Table 2).

Table 1. Anova of TGW, GFD, GGR during 2009–10 and 2010–11

Source of variation	DF	2009–10			2010–11		
		TGW	GFD	GGR	TGW	GFD	GGR
Genotype	26	40.38**	49.49**	0.043**	51.47**	22.25**	0.04**
Environment	1	433.69**	680.01**	0.02	149.1**	286.72**	0.02
Genotype × Environment	26	10.57	8.34	0.02	16.88*	19.91*	0.01
Residual	27	7.58	8.06	0.01	8.45	8.98	0.01
Total (corrected)	107	24.19	23.56	0.02	23.90	17.14	0.02

\* significant at  $P < 0.05$ ; \*\* significant at  $P < 0.01$

Field trials was conducted during two consecutive crop seasons 2009–10 and 2010–11 under timely (normal) and late (heat stress) conditions at Directorate of Wheat Research (ICAR), Karnal (Haryana), India. The experiment was laid out in randomized complete block design (RCBD) with two replications. The plot area was 1.2 m<sup>2</sup> and seed rate was 100 kg/ha. Irrigation was applied as per required while fertilizer application were fol-

*Table 2.* Reduction (%) in TGW, GFD and GGR and Heat susceptibility index of 27 genotypes during 2009–10 and 2010–11

Genotype	2009–10				2010–11			
	TGW	GFD	GGR	HSI	TGW	GFD	GGR	HSI
AKW 1071	6.8	-1.4	8.2	0.7	-0.87	8.07	-9.73	-0.2
DBW 14	5.0	10.2	-4.8	0.5	12.15	13.07	-1.05	2.2
DBW 17	8.9	0.0	8.8	0.9	-0.41	4.29	-4.91	-0.1
HD 2329	8.3	18.7	-13.0	0.9	13.39	9.25	4.55	2.4
HD 2687	4.8	2.9	1.9	0.5	6.27	3.95	2.41	1.1
HD 2733	11.9	4.3	7.6	1.2	9.20	-30.67	23.29	1.6
HD 2833	12.4	8.1	4.6	1.3	17.90	25.77	-10.57	3.2
HS 277	3.9	18.1	-17.3	0.4	-7.17	-9.30	2.49	-1.3
HUW 510	0.9	11.5	-12.3	0.1	14.61	16.67	-2.78	2.6
K 7903	3.6	15.5	-14.0	0.4	4.22	22.50	-23.53	0.7
K 9107	4.1	5.4	-1.3	0.4	-7.80	8.73	-18.27	-1.4
K 9465	3.3	11.1	-8.7	0.3	8.23	14.23	-7.42	1.5
K 9644	12.0	20.5	-11.1	1.2	-4.55	5.63	-9.81	-0.8
NIAW 34	12.9	18.0	-6.6	1.3	9.07	8.57	0.55	1.6
NW 1014	-6.6	13.6	-21.9	-0.7	3.04	15.27	-14.45	0.5
PBW 175	26.3	5.7	21.9	2.7	16.21	1.33	14.99	2.9
PBW 502	8.7	18.5	-9.9	0.9	11.22	7.87	3.70	2.0
RAJ 3765	7.3	12.6	-5.8	0.8	-2.00	13.56	-18.21	-0.4
RAJ 4014	13.7	15.2	-1.8	1.4	18.66	15.29	3.90	3.3
RAJ 4083	6.9	20.5	-17.2	0.7	10.09	9.14	1.07	1.8
UP 2425	5.6	13.3	-7.2	0.6	18.48	3.02	15.88	3.3
VL 616	21.8	7.1	15.7	2.3	-14.40	-0.67	-13.64	-2.6
VL 804	10.7	14.3	-4.2	1.1	10.94	5.13	6.08	1.9
WH 147	21.8	12.9	10.7	2.3	-2.84	5.96	-9.41	-0.5
WH 533	13.2	11.3	2.6	1.4	-18.02	-1.37	-16.23	-3.2
WH 542	20.7	12.8	11.8	2.1	3.54	12.03	-9.49	0.6
WH 730	6.4	23.9	-23.0	0.7	7.77	7.93	-0.20	1.4

lowed as per recommended agronomic packages and practices. Daily mean maximum and mean minimum temperatures were recorded for characterization of environments. Mean minimum and maximum temperatures before and after heading were calculated by taking into consideration the minimum number of days to heading and maximum number of days to maturity during each year. The observations were recorded for phenology and yield related traits of all the genotypes. The investigated traits were days to heading (DH), days to anthesis (DA), days to maturity (DM), grain yield (YLD), number of grains per spike (GN), grain weight per spike (GW), thousand-grain weight (TGW) (g), grain filling duration (GFD). Phenological traits were recorded at 75% condition. Grain filling duration was calculated as the period from days to anthesis to days to physiological maturity. The five main shoot spikes sampled from each plot were hand threshed to obtain grain number and grain weight/spike.

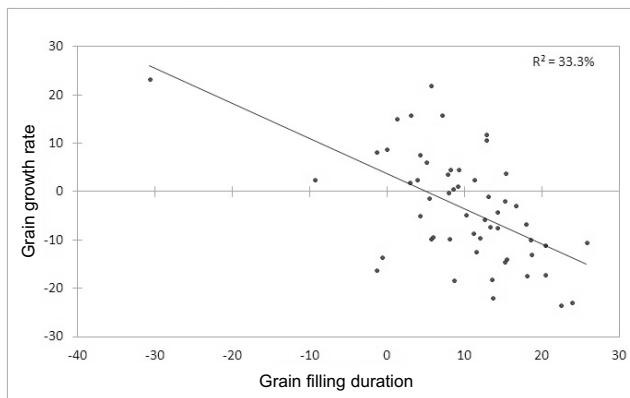
Data was subjected to statistical analysis using SAS computer software.

*Table 3.* Correlation between grain filling duration under normal and stress environments and temperatures during grain filling period

Temperature	Heading to anthesis					
	2009/10		2010/11		2009/10	2010/11
	GFD1	GFD2	GFD3	GFD4	GFDd1	GFDd2
Maximum	-0.80**	-0.13	-0.46*	-0.76**	0.19	-0.46*
Minimum	-0.71**	0.28	-0.32	-0.47**	0.07	-0.37*
Average	-0.76**	0.07	-0.43*	-0.68**	0.13	-0.47**
Anthesis day						
Maximum	-0.08	-0.83**	-0.17	-0.60**	0.39*	-0.46*
Minimum	0.12	-0.68**	0.10	-0.53**	0.00	-0.46**
Average	0.05	-0.81**	-0.03	-0.59**	0.18	-0.49**
One week after anthesis						
Maximum	-0.22	-0.96**	-0.74**	-0.67**	0.53**	0.01
Minimum	-0.13	-0.96**	-0.85**	-0.54**	0.48**	-0.04
Average	-0.17	-0.97**	-0.85**	-0.62**	0.51**	-0.02
Two weeks after anthesis						
Maximum	-0.80**	-0.95**	-0.93**	-0.63**	0.81**	0.83**
Minimum	-0.59**	-0.86**	-0.80**	-0.45*	0.52**	0.36
Average	-0.73	-0.94**	-0.90**	-0.56**	0.74**	0.73**
Three weeks after anthesis						
Maximum	-0.82**	-0.96**	-0.96**	-0.63**	0.49**	0.89**
Minimum	-0.72**	0.29	-0.82**	0.09	0.12	0.73**
Average	-0.78**	-0.92**	-0.92**	-0.22	0.34	0.85**
Four weeks after anthesis						
Maximum	-0.66**	-0.95**	-0.87**	-0.75**	0.58**	0.75**
Minimum	-0.57**	-0.88**	-0.81**	-0.20	0.58**	0.23
Average	-0.64**	-0.94**	-0.88**	-0.43*	0.59**	0.49**

\* significant at  $P < 0.05$ ; \*\* significant at  $P < 0.01$

GFD 1&3 = normal sowing; GFD 2&4 = late (stress) sowing



*Figure 1a.* Regression of reduction in grain filling duration on grain growth rate

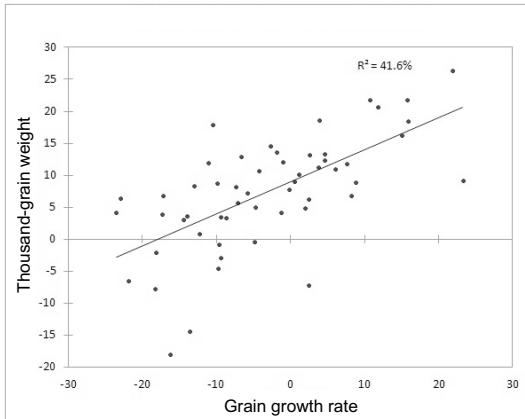


Figure 1b. Regression of reduction in grain growth rate on thousand-grain weight

## Results

The mean average temperature during two crop seasons was 16.5°C and 15.6°C under timely sown conditions and 17.3°C and 16.4°C under late sown conditions. During post heading period, the maximum temperature ranged from 18.8 to 37.5°C and 25.0 to 33.2°C and minimum temperature from 4.7°C to 19.6°C and 9.5°C to 20.5°C under timely sown conditions during two crop seasons. Under late sown conditions the maximum temperature ranged from 24.6 to 40.6°C and 20.0 to 34.0°C and minimum temperature from 10.6 to 19.8°C and 6.2 to 20.5°C, respectively. On average late sown temperature were higher by 3.7 and 2.4°C during 2009-10 and by 3.1 and 1.7°C during 2010-11. The daily weather data revealed that the heat stress ( $>30^\circ\text{C}$ ) initiated after 15 days of heading during 2009-10 crop season and after 21 days during 2010-11 crop season.

Analysis of variance revealed that the genotypes differed significantly in grain filling duration (GFD), grain growth rate (GGR) and thousand-grain weight (TGW) (Table 1). The mean values of GFD ( $35.4 \pm 0.39$  and  $37.9 \pm 0.41$  days), GGR ( $1.06 \pm 0.01$  and  $1.04 \pm 0.01$  mg/day) and TGW ( $37.4 \pm 2.76$  and  $39.3 \pm 0.4$  g) under heat stress (late sown) were significantly different from that under normal (timely sown) conditions, indicating significant influence of heat stress (late planting) on these traits. GFD under normal and stress conditions ranged from 30 to 43 days and 25 to 36 days during 2009-10 and 28 to 45 days and 30 to 36 days during 2010-11. The average reduction in GFD under stress conditions was 3 and 2 days, respectively, during two seasons. Maximum reduction of 9 days was recorded during both seasons. Four genotypes registered more than 7 days reduction in GFD during 2009-10 and two genotypes during 2010-11. The average grain growth rate (GGR) was higher under late sown environments than timely sown environments during both crop seasons. There was about 3% increase in grain growth rate under late sown environments. The grain growth rate among genotypes ranged from 0.78 to 1.29 and 0.67 to 1.31 mg/day under timely sown environments and 0.86 to 1.32 and 0.83 to 1.26 mg/day under late sown environments. Ten genotypes suffered significant reduction in grain

growth rate. The average TGW was 41 g under normal conditions and 37 and 39 g under stress conditions during both years. There was 9.7 and 5.6% reduction in TGW under heat stress conditions of two crop seasons. Genotypes PBW 175, RAJ 4014, VL 616, WH 147 and WH 542 were significantly affected in TGW during 2009–10 crop season where as genotypes HD 2833, HUW 510, PBW 175, RAJ 4014 and UP 2425 were significantly affected during 2010–11 crop season. However, sixteen genotypes had less than 1.0 heat susceptibility index (HSI) during 2009–10 and only twelve had HSI less than 1.0 during 2010–11 (Table 2). These genotypes were considered as tolerant.

### Discussion

Wheat is a cool season crop and grows with an optimum temperature regime of 15–18°C during the grain filling stage (Wardlaw and Wrigley 1994; Stone 2001) but daily high temperature of 25–35°C or greater is common across many regions of the world where wheat is grown which affect the crop (Stone 2001). High temperature restricts the growth, biomass production and productivity (Boyer 1982; Lobell and Asner 2003; Peng et al. 2004), and reduces the grain mass and quality of the harvested products (Stone and Nicolas 1995a, b). Yield loss due to moderately high temperature estimated as 4 percent for each degree centigrade above the optimum in wheat is associated with shortening of grain growth period (Bagga and Rawson 1977; Stone and Nicolas 1995a). An average temperature of 15°C during grain formation is considered optimum for maximum grain weight (Chowdhary and Wardlaw 1978; Fischer 1986). Different genotypes show different response for grain weight under high temperature after anthesis (Ahmad et al. 1989). Tahir and Nakata (2005) reported 20% to 40% reduction in main stem grain weight of 18 wheat genotypes. The optimum kernel weight maintained under stress is a good criterion for measure of heat tolerance (Tyagi et al. 2003). During the present study the reduction in TGW under heat stress conditions was detected. The reduction was higher (10%) during crop season 2009–10 (heat intensity 0.097) and comparatively low (6%) during crop season 2010–11 (heat intensity 0.056). Sixteen genotypes were tolerant for TGW during 2009–10 and only twelve during 2010–11 (Table 2); although temperatures during 2009–10 were higher as compared to during 2010–11. Of twenty-seven genotypes investigated, 14 did not conform to their tolerance/susceptible status in second year (Table 2). Five of these genotypes, namely K9644, WH 533, WH 542, VL 616 and WH 147 were susceptible under high stress i.e. crop season 2009–10 (heat intensity 0.097) and tolerant under low stress, i.e. crop season 2010–11 (heat intensity 0.056). These genotypes had higher reduction in grain filling duration under high stress conditions. Thousand-grain weight is reduced more in warmer environments (Mohammadi 2012). Rest of the genotypes, HUW 510, HD 2329, K 9465, WH 730, DBW 14, RAJ 4083, UP 2425, PBW 502 and HD 2687 were tolerant under high stress conditions and susceptible under low stress conditions. Six of these genotypes, RAJ 4083, UP 2425, PBW 502, HD 2329, HD 2687 and WH 730 had higher reduction in GFD under high stress conditions. Still these genotypes had higher reduction in TGW under low stress conditions and less reduction in GFD. The reduction in GFD due to heat stress affect the grain weight as well as grain yield. GFD

was also reduced by 3 and 2 days, respectively, under stress conditions (average temperature was higher by 3.1 and 2.4°C, respectively). GFD under timely sown environments had significant negative correlation with temperatures during heading to anthesis as well as weekly temperatures from 2 weeks after anthesis to 4 weeks after anthesis. However GFD under late sown environments had significant negative correlation with temperatures on anthesis day as well as weekly temperatures from one week after anthesis to 4 weeks after anthesis (Table 3). The difference in GFD under two environments had positive correlation with these temperatures. Grain-filling duration is determined predominantly by temperature (Sofield et al. 1977; Slafer and Rawson 1994) and its decreases at warmer temperatures (Sofield et al. 1977; Wardlaw et al. 1980; Al-Khatib and Paulsen 1984; Jenner 1991). The duration of grain filling decreases with increase in every 1°C above the optimal growing temperature (Streck 2005). According to Wiegand and Cuellar (1981) thousand-grain weight is dependent on grain filling duration, when temperature is in excess of about 15°C.

Genotypes RAJ 4083, UP 2425, PBW 502, HD 2329, HD 2687 and WH 730 had higher reduction in GFD less reduction in TGW under high stress conditions. The grain growth rate in these genotypes was comparatively less under low stress conditions, which resulted into more reduction in TGW under these conditions. The grain growth rate is also influenced by temperature as well as GFD. Grain growth rate is negatively correlated with grain filling duration under both, normal as well as stress environments. The reduction in GFD has regression of 33.3% on reduction in GGR and reduction in GGR has regression of 41.6% on reduction in TGW (Figs 1a, b). In wheat high temperature (>30°C) after anthesis, decrease the rate of grain-filling and decrease the quality (Al-Khatib and Paulsen 1984; Randall and Moss 1990; Stone et al. 1995). Grain weight is reduced by high temperatures (Wardlaw et al. 1980; Johnson and Kanemasu 1983), as mediated by a reduction in both the duration and rate (Sofield et al. 1977; Wardlaw et al. 1980) of grain filling. Genotypes tend to increase grain growth rate to compensate for reduction in duration and to maintain grain weight under high temperature stress conditions.

### Acknowledgement

The authors acknowledge the financial support from ICAR for NPTC: Functional Genomics project.

### References

- Ahmad, S., Ahmad, N., Ahmad, R., Hamid, M. 1989. Effect of high temperature stress on wheat reproductive growth. *J. Agric. Res.* **27**:307–313.
- Al-Khatib, K., Paulsen, G.M. 1984. Mode of high temperature injury to wheat during grain development. *Plant Physiol.* **90**:1041–1048.
- Altenbach, S.B., DuPont, F., Kothari, K., Chand, R., Johnson, E., Lieu, D. 2003. Temperature, water and fertilizer influence the timing of key events during grain development in US spring wheat. *J. Cereal Sci.* **37**:9–20.
- Bagga, A.K., Rawson, H.M. 1977. Contrasting responses of morphologically similar wheat cultivars to temperatures appropriate to warm climates with hot summers: A study in controlled environment. *Aust. J. Plant Physiol.* **4**:877–887.

- Boyer, J.S. 1982. Plant productivity and the environment. *Science* **218**:443–448.
- Chowdhary, S.I., Wardlaw, I.F. 1978. The effect of temperature on kernel development in cereals. *Aust. J. Agric. Res.* **29**:205–223.
- Fischer, R.A. 1986. Physiological limitations to producing wheat in semitropical and tropical environments and possible selection criteria. In: Proc. Int. Sym. Wheat for Tropical Environments. CIMMYT/UNDP, Mexico, pp. 209–230.
- Hays, D., Mason, E., HwaDo, J., Menz, M., Reynolds, M. 2007. Expression of quantitative trait loci mapping heat tolerance during reproductive development in wheat (*T. aestivum*). In: Buck, H.T., Nisi, J.E., Salomón, N. (eds), *Wheat Production in Stressed Environments*. Springer, Amsterdam, Netherlands, pp. 373–382.
- IPCC 2007. Summary for Policymakers. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (eds), *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK and New York, NY, USA.
- Jenner, C.F. 1991. Effects of exposure of wheat ears to high temperature on dry matter accumulation and carbohydrate metabolism in the grain of two cultivars. I. Immediate responses. *Aust. J. Plant Physiol.* **18**:165–177.
- Johnson, R.C., Kanemasu, E.T. 1983. Yield and development of winter wheat at elevated temperatures. *Agron. J.* **75**:561–565.
- Joshi, A.K., Mishra, B., Chatrath, R., Ortiz Ferrara, G., Singh, R.P. 2007. Wheat improvement in India: Present status, emerging challenges and future prospects. *Euphytica* **157**:431–446.
- Lobell, D.B., Asner, G.P. 2003. Climate and management contributions to recent trends in U.S. agricultural yields. *Science* **299**:1032.
- Mohammadi, M. 2012. Effects of kernel weight and source-limitation on wheat grain yield under heat stress. *African J. Biotechnol.* **11**:2931–2937.
- Peng, S., Huang, J., Sheehy, J.E., Laza, R.C., Visperas, R.M., Zhong, X., Centeno, G.S., Khush, G.S., Cassman, K.G. 2004. Rice yields decline with higher night temperature from global warming. *Proc. Natl Acad. Sci. USA* **101**:9971–9975.
- Randall, P.J., Moss, H.J. 1990. Some effects of temperature regime during grain filling on wheat quality. *Aust. J. Agric. Res.* **41**:603–617.
- Slafer, G.A., Rawson, H.M. 1994. Sensitivity of wheat phasic development to major environmental factors: a re-examination of some assumptions made by physiologists and modellers. *Aust. J. Plant Physiol.* **21**:393–426.
- Sofield, I., Evans, L.T., Cook, M.G., Wardlaw, I.F. 1977. Factors influencing the rate and duration of grain filling in wheat. *Aust. J. Plant Physiol.* **4**:785–797.
- Stone, P. 2001. The effects of heat stress on cereal yield and quality. In: Basra, A.S. (ed.), *Crop Responses and Adaptation to Temperature Stress*. Food Products Press, Binghamton, NY, USA, pp. 243–291.
- Stone, P.J., Nicolas, M.E. 1995a. Effect of timing of heat stress during grain filling on two wheat varieties differing in heat tolerance. I. Grain growth. *Aust. J. Plant Physiol.* **22**:927–934.
- Stone, P.J., Nicolas, M.E. 1995b. Comparison of sudden heat stress with gradual exposure to high temperature during grain filling in two wheat varieties differing in heat tolerance. I. Grain growth. *Aust. J. Plant Physiol.* **22**:935–944.
- Stone, P.J., Savin, R., Wardlaw, I.F., Nicolas, M.E. 1995. The influence of recovery temperature on the effects of brief heat shock on wheat. I. Grain growth. *Aust. J. Plant Physiol.* **22**:945–954.
- Streck, N.A. 2005. Climate change and agroecosystems: The effect of elevated atmospheric CO<sub>2</sub> and temperature on crop growth, development and yield. *Ciencia Rural* **35**:730–740.
- Tahir, I.S.A., Nakata, N. 2005. Remobilization of nitrogen and carbohydrate from stems of bread wheat in response to heat stress during grain filling. *J. Agron. Crop Sci.* **191**:106–115.
- Tyagi, P.K., Pannu, R.K., Sharma, K.D., Chaudhary, B.D., Singh, D.P. 2003. Response of different wheat cultivars to terminal heat stress. Tests of Agrochemicals and Cultivars **24**:20–21.
- Wardlaw, I.F., Sofield, I., Carrwright, P.M. 1980. Factors limiting the rate of dry matter accumulation in the grain of wheat grown at high temperature. *Aust. J. Plant Physiol.* **7**:387–400.
- Wardlaw, I.F., Wrigley, C.W. 1994. Heat tolerance in temperate cereals: An overview. *Aust. J. Plant Physiol.* **21**:695–703.
- Wiegand, C.L., Cuellar, J.A. 1981. Duration of grain filling and kernel weight of wheat as affected by temperature. *Crop Sci.* **21**:95–101.