

Productivity of a doubled haploid winter wheat population under heat stress

Research Article

Krisztina Balla^{1,*}, Ildikó Karsai¹, Tibor Kiss¹, Szilvia Bencze¹, Zoltán Bedő², Ottó Veisz¹

¹Agricultural Institute, Centre for Agricultural Research,
Hungarian Academy of Sciences,
H-2462 Martonvásár, Hungary

²Cereal Breeding Department, Agricultural Institute,
Centre for Agricultural Research, Hungarian Academy of Sciences,
H-2462 Martonvásár, Hungary

Received 08 June 2012; Accepted 29 July 2012

Abstract: Breeding of new winter wheat cultivars with good heat tolerance requires better understanding of the genetic background of heat tolerance. In the present work the effect of heat stress on the 6th day after heading was investigated in a doubled haploid (DH) population arising from a cross between heat-sensitive (Plainsman V) and heat-tolerant (Mv Magma) cultivars. Averaged over the population, heat stress was found to result in a significant reduction in biomass, grain yield and grain number per plant, and in thousand-kernel weight (TKW) and harvest index. High temperature had the greatest effect on the grain yield, with a drop of 36.2% compared with the control. This could be attributed jointly to significant reductions in the TKW of the main ear and in the grain number of the side tillers. The relationship between the yield parameters was confirmed by the positive correlations obtained for the lines in the population. The diverse levels of heat tolerance in the different lines were confirmed by the significant differences in the reduction in the chlorophyll content (SPAD index) of the flag-leaves and in yield parameters. The changes in yield components in stress condition, however, can be still the most effective tools for heat stress evaluation.

Keywords: Heat stress • Early grain filling • Grain yield • Wheat population

© Versita Sp. z o.o.

1. Introduction

Winter wheat is adversely influenced by the effects of high temperature stress in many wheat-producing areas [1,2]. High temperature is often accompanied by drought stress under field conditions, so breeding and selection for high yield under heat and drought stress are important objectives for breeders working in such environments. Increasing productivity under heat stress conditions requires the development and selection of wheat cultivars tolerant of high temperatures, which in turn necessitates detailed investigations under various stress conditions. High temperature tolerance can be determined by measuring various physiological parameters [3,4] and whole-plant productivity traits including yield components such as grain yield, biomass, grain number and thousand-kernel weight

(TKW). Although promising results have been obtained when using physiological screening to predict stress tolerance, the measurement of yield components under stress conditions is still the most effective way of identifying stress-tolerant wheat lines. The identification and measurement of traits conferring high temperature tolerance could contribute to understanding the genetic regulation of these traits and the genetic background of heat stress tolerance. Earliness, leaf rolling, early ground cover, shortness and stay-green are known to be associated with heat tolerance [5,6].

The damage caused by high temperature is greatly dependant on the developmental stage of the plants when subjected to the stress [7,8]. Heat stress at the beginning of flowering or during spikelet development reduces the number of potential grains. A temperature of 27°C at a slightly later developmental

* E-mail: balla.krisztina@agrar.mta.hu

stage, 50% anthesis, also resulted in a high proportion of sterile florets [9]. Heat stress during grain filling, on the other hand, influences the translocation of photosynthetic assimilates, starch synthesis, and the accumulation of starch in the grains, thus causing changes in grain quality and weight [10]. High temperature was also reported to cause reductions in the weight of individual grains [11–13]. The weight of mature grains was most sensitive to heat stress when this occurred early in grain filling, becoming progressively less sensitive in later stages [14]. The damage caused by heat stress depends not only on the timing, but also on the duration of the stress. Exposure to high temperatures for a period of eight days after anthesis caused no reduction either in grain number or weight or in the number of deformed grains [15].

The chlorophyll content in the leaves has also been used as a measure of heat tolerance, since it declines rapidly under heat stress conditions, so the plants turn yellow and reach harvest maturity far earlier than the control plants [16]. In plants exposed to stress during ripening, aging processes accelerate, thus shortening the period available for grain development and leading to considerable yield losses.

The development of a doubled haploid genetic mapping population segregating for heat stress tolerance was essential for studying the genetic background of tolerance and the association between physiological parameters and yield components under stress conditions. This required the use of genotypes exhibiting substantial differences in heat tolerance on the basis of physiological parameters (e.g. chlorophyll content) and yield components. The varieties selected as the parents of the genetic population used for the phytotron experiments were Plainsman V (heat-sensitive) and Mv Magma (heat-tolerant) [17]. The major aims of the study were (1) to determine the heat tolerance of individual lines in the doubled haploid Plainsman V×Mv Magma population during the early stages of grain filling, by recording agronomic and physiological traits, and (2) to explore the relationship between heat stress and the changes induced in various yield parameters.

2. Experimental Procedures

A population consisting of 174 doubled haploid (DH) lines was developed using the anther culture technique from the F_1 generation of a cross between parental lines found to be heat-tolerant (Mv Magma) and heat-sensitive (Plainsman V) in previous experiments [17]. Plainsman V is a hard-grained winter wheat with high protein content, while Mv Magma is a high quality bread

wheat with medium early ripening and excellent yield potential.

The experiment was carried out in 2010. The seeds were germinated and vernalised in peat blocks. On 15th December 2009, after 45 days of vernalisation at 4°C with low light intensity and short day length, six plants of each line were transferred to individual pots holding approximately 1.5 kg of a 3:2:1 mixture of garden soil, compost and sand. The plants were then placed randomly in the greenhouse, where they were grown at controlled temperatures with daily watering and a twice-weekly supply of nutrients (Volldünger Solution, Linz, Austria, in tap-water) until the start of the stress treatment. Heading was recorded daily, and three plants of each DH line that headed at approximately the same time were exposed to heat stress for 15 days, starting on the 6th day after heading, in the early stages of grain development (Zadoks 71–73 according to Tottman and Makepeace [18]). The treatment, involving a night temperature of 20°C and a day length of 14 hours, for 8 hours of which the plants were exposed to a temperature of 35°C and a light intensity of 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$, was carried out in the heat stress chamber (Conviron PGV-36) in the Martonvásár phytotron [19]. The remaining three plants were raised under normal conditions (19–25°C) in the greenhouse. The natural light conditions in the greenhouse were supplemented with 8 hours artificial lighting with an intensity of 170 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Thus the light intensity of the greenhouse was similar to the light intensity of the controlled growth chamber. The photosynthetic photon flux density was measured at the canopy at three points in the growth chamber of the phytotron and in the greenhouse using a LI-COR LI189 quantum radio-photometer (LI-COR Biosciences, Lincoln, NE, USA).

After the heat stress treatment, the plants were returned to the greenhouse and raised with the control plants till maturity. The yellowing of the flag-leaves was recorded by measuring the chlorophyll content with a SPAD-502 meter (Minolta, Japan). Three readings were taken along the middle section of the leaf and the mean value, expressed in SPAD units, was used for analysis. Chlorophyll measurements were performed once after the heat stress treatment, on day 15.

When harvest maturity was reached, the yield, biomass, TKW, grain number and harvest index of control and heat-stressed plants were recorded. The harvest index (%) was calculated as grain yield/biological yield × 100, as reported by Donald [20].

The data were statistically evaluated using two-way analysis of variance (ANOVA) with replications, using the Microsoft Excel 2000 program. Correlation analysis was performed for each treatment separately (control, heat

stress, control vs. heat stress) on the yield parameters of the individual lines (mean value of six plants) of the genetic population using Microsoft Excel 2000 program.

3. Results

3.1 Changes in the yield parameters of the doubled haploid population in response to heat stress

The 15-day heat stress during early embryo development caused considerable changes, averaged over the lines in the DH population. Significant reductions were observed in the biomass per plant, the harvest index, the grain number per plant, the average TKW and the grain yield per plant (Table 1). There was also a significant decline in the upper and lower values of the range for all the parameters. However, when the grain number, TKW and grain weight of the main spike were analysed, significant reductions were only detected for the TKW and the grain weight. The upper and lower values of the range for these parameters again exhibited a significant decline. As heat stress only caused a slight reduction in the number of productive tillers (averaged over the plants), this suggests that the grain number decreased to a greater extent in the side spikes than in the main spike, while the TKW declined to a lesser extent. The significant deviations in the chlorophyll content of the flag-leaf were also indicative of differences in the heat tolerance of individual lines (Table 1). The data revealed that some lines were capable of remaining almost as green as the control plants despite the heat treatment (Figure 1A). The variability in the responses of the

lines was also illustrated by the frequency distribution. Averaged over the population there was a 58.5% reduction in the chlorophyll content, but the frequency distribution curve differed significantly from the normal distribution, the greatest frequency (23% of the lines) being observed for a drop in chlorophyll content of 25.5%. The shape of the frequency distribution curve suggests that several dominant major genes may be responsible for the development of the stay-green trait.

Heat stress in the early phases of grain-filling induced very varied responses in biomass and grain number of individual lines of the population. On average, high temperature caused a 24.5% reduction in the biomass, and this was the value recorded most frequently, due to the normal distribution of the curve (Figure 1B). In the case of grain number per plant, the mean reduction, averaged over the population, was 25.7%, compared with the control. Considerable differences were observed between the lines, with the two extremes being 2.7 and 75% reduction, but the curve exhibited normal distribution and these extreme values only occurred with low frequency (7.6 and 0.8%, respectively) (Figure 1D). The normal distribution suggested that the changes in grain number induced by heat stress were determined by the additive effect of several minor genes.

Heat stress had the least effect on the harvest index, calculated from the plant yield parameters (Figure 1C), and on the mean TKW per plant (Figure 1E). The harvest index decreased by an average of 16.5% and the TKW by 13.9%. In both cases the largest frequency group (26.5% of the lines for harvest index and 35% for TKW) had values close to the mean. Decreases of 40-50-60% in the TKW were only observed for a very small number

Parameter	Control		Heat stress		LSD _{5%}
	Mean	Range	Mean	Range	
Chlorophyll content	41.8	31.9–51.7	24.4*	2.8*–46.1*	1.08
Biomass/plant (g)	6.95	3.7–10.2	5.3*	2.7*–7.8*	0.16
No. of productive tillers	3.4	1.7–6	3.1*	1.6–6.5*	0.12
Harvest index/plant	39.0	18.6–59.4	32.6*	9.7*–55.4*	0.87
Main spike grain weight (g)	1.4	0.4–2.3	1.1*	0.3*–1.8*	0.03
Main spike No. of grains	39.6	9.5–63.0	38.8	10.0–63.0	0.9
Main spike TKW (g)	36.5	21.7–49.5	27.4*	14.4*–36.3*	0.43
Grain No./plant	116.8	43.6–190.0	86.7*	17.5*–156.0*	3.4
TKW/plant (g)	29.5	17.4–41.7	25.4*	14.2*–36.6*	0.47
Grain yield/plant (g)	3.5	1.3–5.7	2.2*	0.5*–3.9*	0.1

Table 1. Effect of heat stress on yield components and physiological parameters of the doubled haploid wheat population.

TKW: Thousand-kernel weight, LSD: Least significant difference

*Significantly different from the control at the $P \leq 0.05$ probability level.

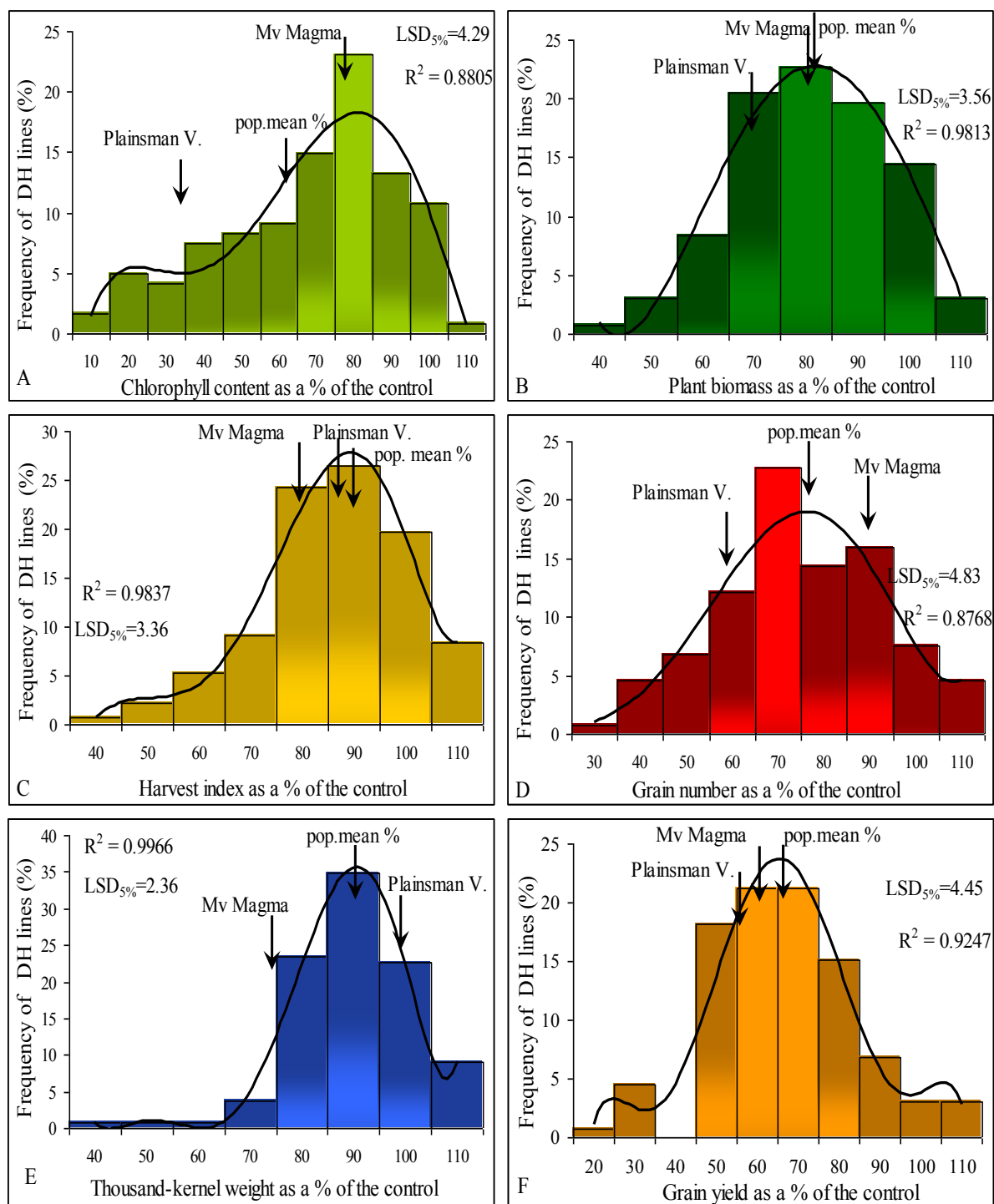


Figure 1. Frequency distribution (%) of the lines in the doubled haploid population on the basis of chlorophyll content (A), biomass (B), harvest index (C), grain number (D), thousand-kernel weight (E) and grain yield (F) in response to heat stress in the early stages of embryo development, as a % of the control

of lines. Reductions of less than 20% were recorded for around 81% of the whole population for TKW, and for 70% in the case of harvest index.

Heat stress caused a considerable loss of grain yield per plant, with a mean value of 36.2%, which was characteristic of around 42% of the lines (Figure 1F).

The lines most sensitive to heat stress responded with a yield loss of 80.5%, while a few heat-tolerant lines were found with yield losses of only 5.8%.

3.2 Analysis of correlations between yield parameters

Positive correlations were detected between the majority of yield components for the lines in the population, the only exception being the negative correlation found between grain number and TKW in the control treatment (Table 2). The closest correlations, such as those between biomass and grain number, biomass and grain yield, harvest index and yield, and grain number and grain yield, could be observed not only under control conditions, but also after heat stress. The correlation between biomass and yield components was closer under control conditions, while that observed between the harvest index and the yield components was closer in the heat stress treatment. This was also true of the correlation between grain number and grain yield. The correlation between chlorophyll content and yield components was only significant in the case of the TKW under control conditions.

When correlations were sought between the values of the individual parameters under control and heat stress conditions, close positive correlations were detected in the case of harvest index, grain number, TKW and grain yield, while weaker correlations were found for the biomass and chlorophyll content.

4. Discussion

Previous studies on the yield parameters and chlorophyll content of various wheat cultivars indicated that Mv Magma had better heat stress tolerance than Plainsman V [17]. When a DH population arising from a cross between these two cultivars was subjected to heat stress in the early stages of grain development, significant differences in heat tolerance were observed between the lines, which was confirmed by differences in yield and biomass production. The random inbred lines (RIL) developed by Blum *et al.* [21] also responded to heat stress with significantly different yield losses.

The beginning of heat stress was timed to coincide with the desired developmental stage in the main spike, when the spikes on the side tillers were still in

Parameters	Control	Heat stress	Control vs. Heat stress
Biomass-Grain number	0.77***	0.71***	-
Biomass-TKW	0.01	0.18	-
Biomass-Grain yield	0.82***	0.79***	-
HI-Grain number	0.58***	0.76***	-
HI-TKW	0.21*	0.22*	-
HI-Grain yield	0.72***	0.83***	-
Grain number-TKW	-0.34***	-0.18	-
Grain number-Grain yield	0.88***	0.91***	-
TKW-Grain yield	0.12	0.21*	-
Chlorophyll content-Grain number	0.02	-0.07	-
Chlorophyll content-TKW	0.28**	0.05	-
Chlorophyll content-Grain yield	0.17	-0.07	-
Chlorophyll content	-	-	0.19*
Biomass	-	-	0.22*
Harvest index	-	-	0.52***
Grain number	-	-	0.49***
TKW	-	-	0.35***
Grain yield	-	-	0.40***

Table 2. Correlations between the yield parameters of the wheat DH population in the control and heat stress treatments. The yield parameter data from the control experiment was also correlated with the same parameters originating from the heat stress, in order to evaluate the associations between the yielding ability of a given line under control and heat stress condition.

HI- Harvest index, TKW- Thousand-kernel weight.

*, **, *** denote significant relationships at $P < 0.05$, $P < 0.01$ and $P < 0.001$ probability level.

an earlier stage of development, which could explain the differences in the responses recorded for the main and side spikes. The main spikes were in the early embryo development stage, so the grain number did not change substantially after heat stress, while there was a significant drop in the TKW. The side spikes, however, were subjected to heat stress before or during flowering, resulting in a significant reduction in fertilisation and the number of grains developing, but having much less influence on the TKW. It was demonstrated by Mohammadi *et al.* [22] that 3-day treatment at a temperature of 35/30°C ten days after flowering caused a substantial decrease in the weight of the grains and the spike, while no change was observed in the grain number, which could be explained by the time elapsing between heading and heat treatment.

For the lines in the present population the relationships between various yield parameters (grain number and yield, biomass and yield, harvest index and yield, harvest index and grain number, biomass and grain number) were confirmed by the positive correlations obtained in the correlation analysis. The closest of these correlations ($P \leq 0.001$) could be detected in the population not only under control conditions, but also after exposure to heat stress in the early stages of embryo development. In plants subjected to a temperature of 35°C much closer positive correlations were found between harvest index and grain number, harvest index and grain yield, and grain number and grain yield than under control conditions. The varied responses to heat stress observed in the lines of the population could be attributed partly to the diverse physiological behaviour of the parental genotypes. Studies on evapotranspiration revealed that when exposed to heat stress Mv Magma responded with enhanced evapotranspiration, making it better able to cope with the high temperature than Plainsman V (unpublished data).

When the values for the various parameters were compared under control and heat stress conditions, weaker correlations were obtained for the biomass and chlorophyll content, and closer positive correlations for the harvest index, grain number, TKW and grain yield. Studies on the heat tolerance of RIL plants confirmed the close correlations between the various yield components (e.g. grain weight and spike weight, or grain number and spike weight), which were considered by the authors to be suitable for testing the heat tolerance of populations [22].

This study included an analysis of the content of chlorophyll, an important pigment, in the wheat leaves. Of the two parents used to create the DH population, Mv Magma was found to maintain a high chlorophyll content

for a long period under both control and heat stress conditions (stay-green trait). The changes in chlorophyll content observed in the lines of the population after heat stress, and the frequency distribution of these changes, suggested that one or a small number of dominant major genes could be responsible for the development of the stay-green trait. As reported [23] that stay-green was associated with post-anthesis drought resistance, giving plants resistance to premature senescence at various levels of soil moisture stress. Stay-green plants produce higher, stable yields with a high chlorophyll content [24]. The relative water content in the leaves of stay-green lines is much higher than that of non-stay-green lines, indicating that a better stalk transportation system functions under stress conditions. Three QTLs were found to affect the chlorophyll content under post-anthesis drought stress, the intervals of which completely overlapped the stay-green QTL intervals [23]. The leaf chlorophyll content exhibited a significant correlation with the stay-green rating, suggesting that the measurement of chlorophyll content using a chlorophyll meter and the manifestation of the stay-green trait could be useful in screening for drought and heat tolerance in wheat [2,23,25,26]. In the present work, the significant differences in the chlorophyll content of the flag-leaves confirmed the fact that some lines were more tolerant than others. Other author [26] examined the heat and drought tolerance of numerous wheat varieties, considerable differences were observed in the extent to which the chlorophyll content decreased, as also found in the present work. A similar decline in the chlorophyll content was reported in response to heat stress by Balouchi [2]. By contrast, when in another study [27] a barley variety with low chlorophyll content were examined, authors found that the light green colour reduced the absorbance of the leaves, thus protecting the plants from the damaging warming effect of strong sunshine and influencing the heat regulation of the leaves. The present correlation analysis, however, revealed that of all the yield components, only the TKW was significantly correlated with the chlorophyll content under control conditions, while after exposure to heat stress the chlorophyll content did not exhibit a correlation with any of the yield components. There was, however, a correlation between leaf colour and chlorophyll content, as expected, since chlorophyll loss is the main factor responsible for alterations in leaf colour [28]. In contrast to the present results, this author found that the chlorophyll content was positively correlated with the biomass and grain weight per plant. The present data also revealed a positive correlation between the chlorophyll content and the grain weight, but this was not-significant even under control conditions.

The significant genetic variation detected in the present work in the heat stress responses of the wheat lines in the genetic population suggests that the DH population could be a useful tool for future studies on the genetic background of heat tolerance.

Acknowledgements

This paper was funded from the DROPS Project (EU-FP7 No. 244374).

References

- [1] Porter D.R., Nguyen H.T., Burke J.J., Genetic control of acquired high temperature tolerance in winter wheat, *Euphytica*, 1995, 83, 153-157
- [2] Balouchi H.R., Screening wheat parents of mapping population for heat and drought tolerance, detection of wheat genetic variation, *Int. J. Biol. Life Sci.*, 2010, 6, 56-66
- [3] Moffat J.M., Sears G., Cox T.S., Paulsen G.M., Wheat high temperature tolerance during reproductive growth. I. Evaluation by chlorophyll fluorescence, *Crop Sci.*, 1990, 30, 881-885
- [4] Ibrahim A.M.H., Quick J.S., Heritability of heat tolerance in winter and spring wheat, *Crop Sci.*, 2001, 41, 1401-1405
- [5] Blum A., Nguyen C.Y., The effect of plant size on wheat response to agent of drought stress. II. Water deficit, heat and ABA, *Plant Physiol.*, 1997, 24, 35-41
- [6] Fokar M., Nguyen T., Blum A., Heat tolerance in spring wheat. II. Grain filling, *Euphytica*, 1998, 104, 9-15
- [7] Paulsen G.M., High temperature responses of crop plants, In: Boote K.J., Sinclair T.R., Paulsen G.M. (Eds.), *Physiology and Determination of Crop Yield*, American Society of Agronomy, Madison, 1994
- [8] Porter J.R., Gawith M., Temperatures and the growth and development of wheat: a review, *Eur. J. Agron.*, 1999, 10, 23-36
- [9] Wheeler T.R., Batts G.R., Ellis R.H., Hadley P., Morison J.I.L., Growth and yield of winter wheat (*Triticum aestivum* L.) crops in response to CO₂ and temperature, *J. Agric. Sci. Camb.*, 1996, 127, 37-48
- [10] Bhullar S.S., Jenner C.F., Differential responses to high temperature of starch and nitrogen accumulation in the grain of four cultivars of wheat, *Aust. J. Plant Physiol.*, 1985, 12, 363-375
- [11] Randall P.J., Moss H.J., Some effects of temperature regime during grain filling on wheat quality, *Aust. J. Agr. Res.*, 1990, 41, 603-617
- [12] Wardlaw I.F., The effect of high temperature on kernel development in wheat: variability related to pre-heading and post-anthesis conditions, *Aust. J. Plant Physiol.*, 1994, 21, 731-739
- [13] Stone P.J., Nicolas M.E., 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.*, 1995, 22, 935-944
- [14] Stone P.J., Nicolas M.E., Effect of timing of heat stress during grain filling on two wheat varieties differing in heat tolerance. I. Grain growth, *Aust. J. Plant Physiol.*, 1995, 22, 927-934
- [15] Stone P.J., Nicolas M.E., A survey of the effects of high temperature during grain-filling on yield and quality of 75 wheat cultivars, *Aust. J. Agric. Res.*, 1995, 46, 475-492
- [16] Hoel B.O., Solhaug K.A., Effect of irradiance on chlorophyll estimation with the Minolta SPAD-502 leaf chlorophyll meter, *Ann. Bot.*, 1998, 82, 389-392
- [17] Balla K., Bencze S., Janda T., Veisz O., Analysis of heat stress tolerance in winter wheat, *Acta Agron. Hung.*, 2009, 57, 437-444
- [18] Tottman D.R., Makepeace R.J., An explanation of the decimal code for the growth stages of cereals, with illustrations, *Ann. Appl. Biol.*, 1979, 93, 221-234
- [19] Tischner T., Rajkainé Végh K., Kőszegi B., Effect of growth medium on the growth of cereals in the phytotron, *Acta Agron. Hung.*, 1997, 45, 187-193
- [20] Donald C.M., In search of yield, *J. Austr. Inst. Agr. Sci.*, 1962, 28, 171-178
- [21] Blum A., Klueva N., Nguyen H.T., Wheat cellular thermotolerance is related to yield under heat stress, *Euphytica*, 2001, 117, 117-123
- [22] Mohammadi V., Qannadha M.R., Zali A.A., Yazdi-Samadi B., Effect of post anthesis heat stress on head traits of wheat, *J. Agr. Biol.*, 2004, 6, 42-44
- [23] Xu W., Subudhi P.K., Crasta O.R., Rosenow D.T., Mullet J.E., Nguyen H.T., Molecular mapping of QTLs conferring stay-green in grain sorghum (*Sorghum bicolor* L. Moench), *Genome*, 2000, 43, 461-469
- [24] Thomas H., Smart C.M., Crops that stay-green, *Ann. Appl. Biol.*, 1993, 123, 193-233
- [25] Ristic Z., Bukovnik U., Vara Prasad P.V., Correlation between heat stability of thylakoid membranes

- and loss of chlorophyll in winter wheat under heat stress, *Crop Sci.*, 2007, 47, 2067-2073
- [26] Mohammadi M, Karimizadeh R.A., Naghavi M.R., Selection of bread wheat genotypes against heat and drought tolerance based on chlorophyll content and stem reserves, *J. Agr. Soc. Sci.*, 2009, 5, 119-122
- [27] Havaux M., Tardy F., Loss of chlorophyll with limited reduction of photosynthesis as an adaptive response of Syrian barley landrace to high light and heat stress, *Aust. J. Plant Physiol.*, 1999, 26, 569-578
- [28] Zaharieva M., Gaulin E., Havaux M., Acevedo E., Monneveux P., Drought and heat responses in the wild wheat relative *Aegilops Roth*: potential interest wheat improvement, *Crop Sci.*, 2001, 41, 1321-1329