Heat-shock effects on photosynthesis and sink–source dynamics in wheat (*Triticum aestivum* L.)

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Received 8 January 2007; accepted 5 July 2007

Abstract

To assess the mechanisms causing genotypic differences in heat tolerance of wheat (*Triticum aestivum* L.), physiological responses to a heat shock in a vegetative ('end of tillering') or a reproductive ('early grain filling') stage were studied. Three cultivars – Lavett, Ciano-79 and Attila – differing in adaptation to heat were grown in a glasshouse at a day/night temperature regime of 15/10 °C and a 12-h daylength from sowing to 'end of tillering' and next at two day/night regimes of 25/20 and 18/13 °C under natural daylength. The heat-shock treatment consisted of an exposure of plants to temperatures raised gradually over a time-span of 12 hours to above 30 °C with a maximum of 38 °C during three hours at midday for three days either at the 'end of tillering' or at 'grain filling'. A heat shock at the 'end of tillering' strongly reduced the rate of leaf photosynthesis. A similar heat shock during 'grain filling' decreased both rate of photosynthesis (source) and grain growth (sink). The rate of leaf photosynthesis was decreased by 40 to 70%, depending on cultivar and developmental stage. Photosynthesis fully recovered within 4 days after the heat-shock treatment was ended. The effects of the heat shock on biomass yield were more pronounced for treatments at 'early grain filling' than at 'end of tillering'. However, the impact of a 3-day heat shock on biomass yield was less than the effects of the pre- and post-treatment growing temperature.

Additional keywords: heat tolerance, stomatal conductance, fluorescence, senescence

Introduction

Heat and drought are the main abiotic constraints on the yield of cereals (Araus *et al.*, 2002). Heat stress has become an increasingly important factor in limiting wheat yields (Porter & Gawith, 1999; Viswanathan & Renu Khanna-Chopra, 2001). Generally, temperatures during reproductive growth of wheat are relatively high, exceeding 30 °C during grain growth and reducing wheat yield and quality in major wheat growing regions (Wardlaw & Wrigley, 1994; Gibson & Paulsen, 1999). To improve yield in heat- and drought-stressed environments, breeding for specific aspects of heat tolerance is widely believed to be an achievable goal (Aggarwal *et al.*, 1994; Gutiérrez-Rodríguez *et al.*, 2000; Reynolds *et al.*, 2000). Improvements concern processes like photosynthesis and transpiration (Monneveux *et al.*, 2003), optimized storage remobilization (Blum, 1998; Calderini *et al.*, 1999) and heat-tolerant endosperm cell division or grain filling (Stone & Nicolas, 1995).

Wheat is affected very often by relatively short periods (3–5 days) of heat stress. Especially when occurring during grain filling, heat stress exerts a large impact on grain quality not only of wheat but also of barley (Blumenthal *et al.*, 1991; Wardlaw *et al.*, 2002). However, the effect of such short heat events on yield is not a simple reflection of genetic plant attributes, because physiological responses to heat stress vary for different developmental stages (Slafer & Rawson, 1994; Asseng *et al.*, 2002). Besides, physiological responses are strongly affected by feedback control exerted by interacting processes (Slafer *et al.*, 1996). To achieve improvements for a wider range of agro-ecological conditions, a better understanding is required of the regulation of carbon and nitrogen fluxes, including feed forward and feedback controls of associated processes. Photosynthesis is a tangible target for research; it represents both the driving force for carbon fluxes related to CO_2 fixation and assimilation and for chlorophyll fluorescence related to photosynthetic light use (Schreiber, 1986; Schreiber *et al.*, 1994). As to grain growth there is substantial evidence that sink capacity is a major limiting factor for crop productivity; a physiological stress-related decrease in sink activity directly affects grain yield (Reynolds *et al.*, 2005).

The study aims at quantifying the response of photosynthesis and related growth processes on heat stress for contrasting wheat genotypes grown at a low or high temperature regime. To compare the relative impact on source and sink processes, heat stress was either imposed at the 'end of tillering' or during 'early grain filling'. The study's objective was a better understanding of the mechanisms controlling heat tolerance in wheat genotypes, based on an evaluation of photosynthetic and storage processes embedded in whole-plant responses. The effect of heat shocks was studied within the framework of dynamic interactions between sink and source.

Materials and methods

Plant material and controlled environment conditions

Three spring wheat (*Triticum aestivum* L.) cultivars – Lavett, Ciano-79 and Attila – were used that are known for their differences in sensitivity to heat stress. Lavett, which is adapted to a temperate climate, was selected because of its characteristics as described in the 77th Recommended List of Varieties of Field Crops (Anon., 2001). The two other cultivars were selected from a stock of CIMMYT wheat cultivars for warmer environments. Based on studies of Yang *et al.* (2002), Ciano-79 and Attila can be classified as heat-sensitive and heat-tolerant, respectively.

The plants were grown in naturally lit, climate-controlled greenhouses of the Plant Sciences Group, Wageningen University and Research Centre. Supplemental light was provided by 400 W SON-T Agro Philips lamps (0.5 lamp m^{-2}), which were switched on during daytime when solar radiation dropped below 400 W m^{-2}, and switched off when solar radiation exceeded 500 W m^{-2}. The fraction of photosynthetically active radiation (PAR) absorbed by the plants was 0.60.

Wheat seeds were sown on 26 March 2003 in 5-litre pots filled with a standard potting soil and 1 g osmo-coat (N–P–K contents: 15–11–13) per litre soil. Plant density was standardized to 12 plants per pot. After germination, the plants were vernalized for 7 days at a night temperature of 4 °C. A total of 144 pots was placed in a greenhouse with a day/night temperature regime of 15/10 °C and a 12-h daylength. The pots were watered regularly using an automatic drip irrigation system to ensure that soil moisture content was kept at an optimum level. After four weeks the 144 pots were divided into two groups; pots were placed in compartments of the greenhouse with a day/night temperature regime of either 18/13 °C (96 pots) or 25/20 °C (48 pots) at a daylength of 14 hours. The number of days from sowing until maturity for the cultivars Lavett, Ciano-79 and Attila was 88, 94 and 96, respectively, when grown at 25/20 °C and 121, 128 and 130, respectively, when grown at 18/13 °C.

From the ‘end of tillering’ onwards four pots per treatment were used for determining total dry weight and dry weights of the following organs: stem, peduncle, flag leaf, other leaves and ear at the stage of the heat-shock treatment. All remaining pots were harvested when grain moisture content had dropped below 15%. Total dry weight and grain weight were determined after oven-drying at 80 °C for 24 hours.

Heat-shock treatments

The heat-shock treatments were applied at the end of tillering (stage 26; Zadoks *et al.*, 1974) or in the early grain-filling phase (stages 71–75). Regarding the latter this meant 10 and 14 days after the beginning of anthesis for the plants grown at 25/20 °C and 18/13 °C, respectively. Pots were transferred from the greenhouse to a controlled environment chamber with a daylength of 16 hours, a diurnal temperature regime of 38/20 °C and a relative humidity of 70/85% for day and night, respectively. Light intensity at flag leaf level was 425 µmol m^{-2} s^{-1}; the light was provided by 400 W SON-T Agro Philips lamps and 400 W HPI-T Philips lamps (3.5 lamps m^{-2}).

Heat shocks were applied following a gradual transition from the night to the maximum day temperature (Figure 1). The treatment consisted of a 12-h daily exposure for 3 consecutive days to temperatures above 30 °C with a maximum of 38 °C during 3 hours in a climate-controlled environment. During the heat treatment the pots were placed in a cooling water bath to keep the soil temperature at 25 °C. After the 3-day heat treatment pots were placed again in the greenhouse under the same conditions that preceded the treatment.

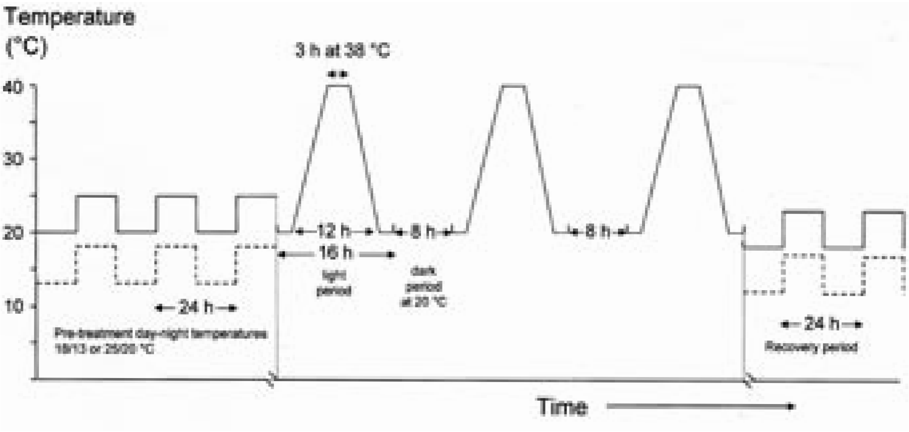


Figure 1. Course of temperatures before, during and after the heat-shock treatments. Plants were grown in the greenhouse at 18/13 or 25/20 °C before and after the heat-shock treatment in a climate-controlled compartment.

Measurements

Photosynthesis–light response curves and fluorescence quenching characteristics were measured simultaneously with a LICOR-6400-40 at PAR values ranging from 100 to 350, 500 and 1200 μmol m^{-2} s^{-1} after dark adaptation for 10 minutes. Data on stomatal conductance were derived from the measured transpiration rate and the measured vapour pressure deficit of the leaves. Photosynthesis measurements were carried out during the 3-day heat shock treatment on whole plants when at the main shoot the sixth leaf was present as well as at early grain filling on flag leaves. The measurements were also carried out during the recovery period. The photosynthesis measurements on individual leaves were done in fourfold for each cultivar and treatment.

Statistical analysis

Statistical analyses were done with GenStat for the effects of heat shock and cultivars on photosynthetic processes and biomass yield. Effects of the chronic growth temperatures of 25/20 °C and 18/13 °C on plants grown in different compartments of the greenhouse were not statistically analysed because of lack of replicates.

Results

Heat-shock effects on photosynthesis

Under non-stress conditions the rate of leaf photosynthesis at the end of tillering was significantly ($P < 0.001$) higher for Ciano and Attila than for Lavett (Figure 2A).

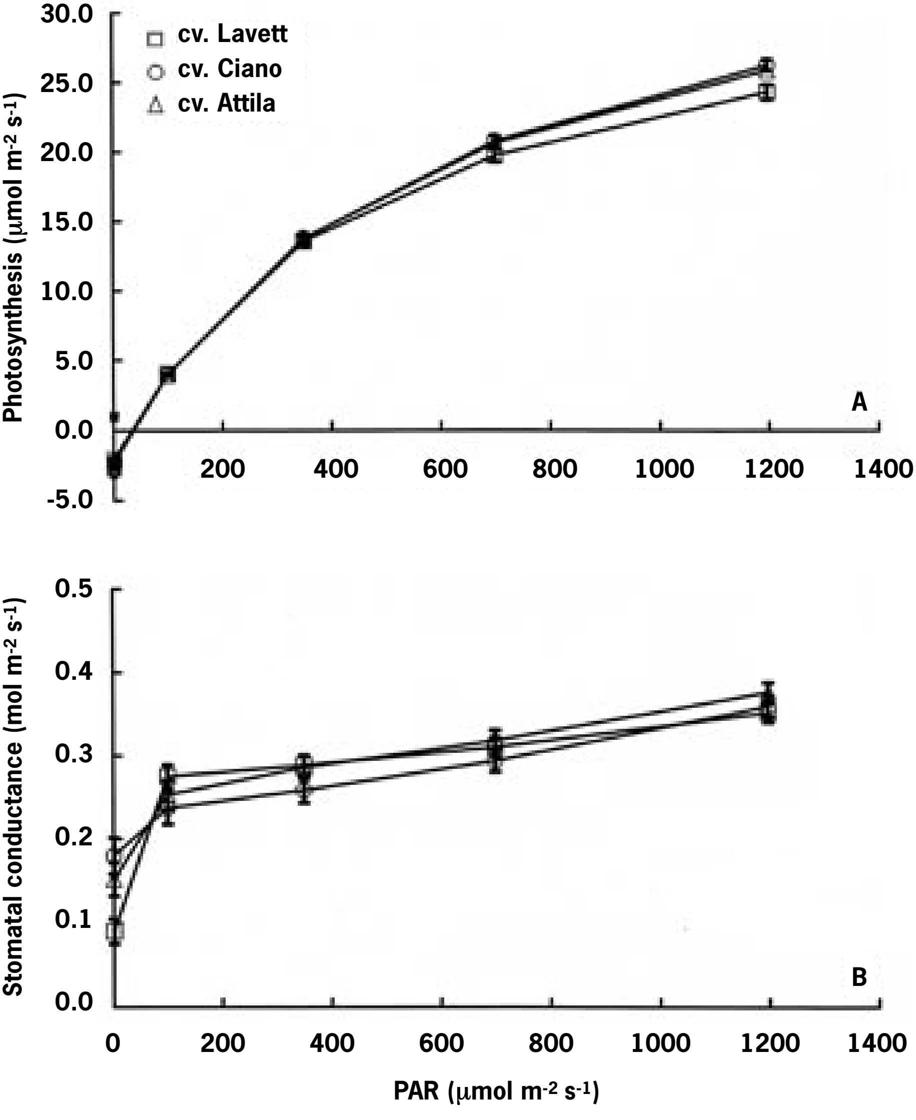
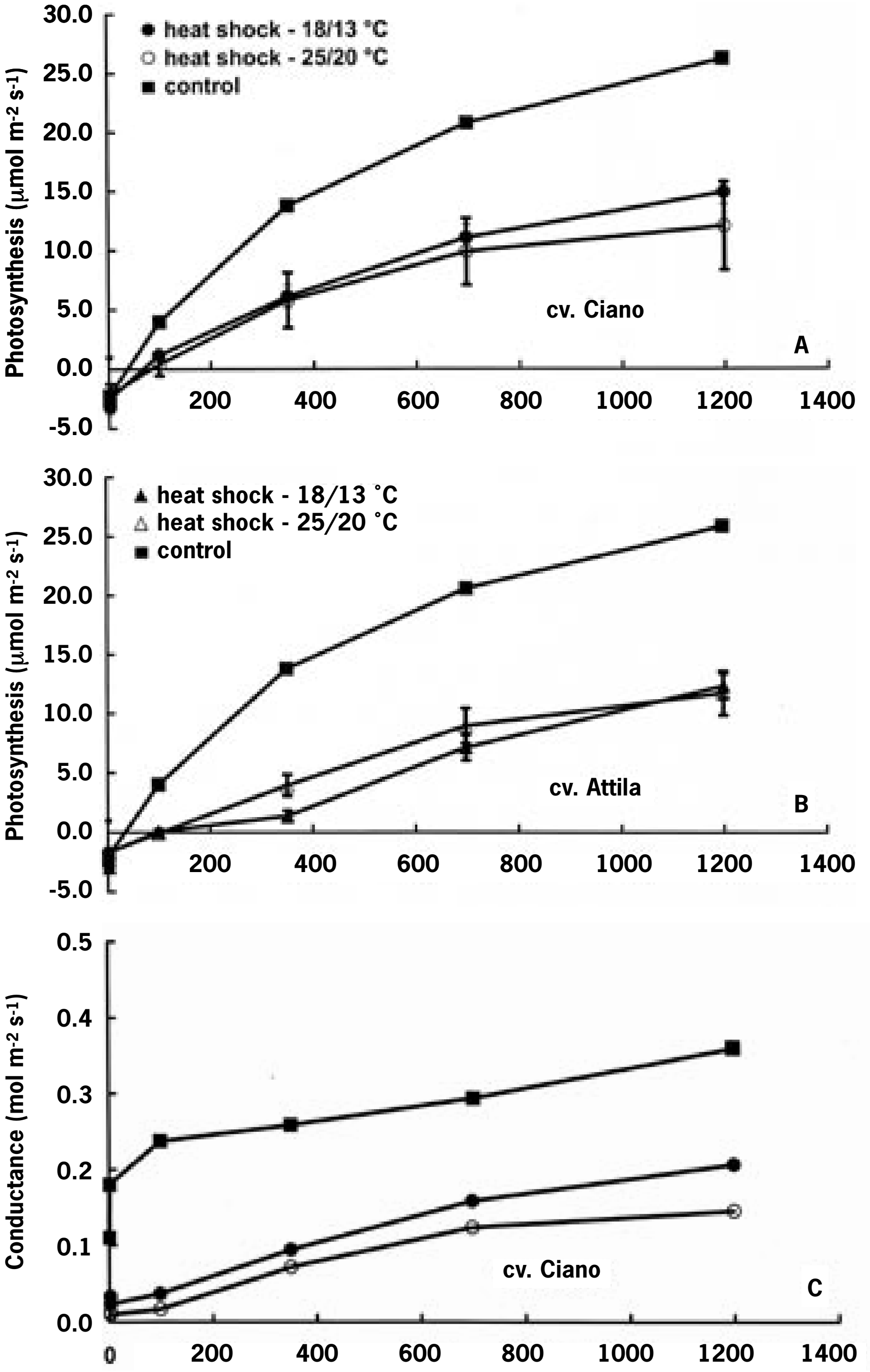


Figure 2. The relationship between photosynthetically active radiation (PAR) and rate of photosynthesis (A) and between PAR and stomatal conductance (B) at the end of tillering for mature leaves of three wheat cultivars.

However, differences between cultivars in light-dependent stomatal conductance were not statistically significant (Figure 2B). On average, the 3-day heat-shock treatment at the end of tillering decreased the maximum rate of net photosynthesis (A_{max}) by 50 to 60% in Ciano and Attila, respectively (Figures 3A, B). No heat-shock treatments were applied to the cultivar Lavett in the vegetative stage. Heat-shock effects on A_{max} were


30.0
25.0
20.0
15.0
10.0
5.0
0.0
-5.0
Photosynthesis (µmol m-2 s-1)
● heat shock - 18/13 °C
○ heat shock - 25/20 °C
■ control
cv. Ciano
A
200
400
600
800
1000
1200
1400
30.0
25.0
20.0
15.0
10.0
5.0
0.0
-5.0
Photosynthesis (µmol m-2 s-1)
▲ heat shock - 18/13 °C
△ heat shock - 25/20 °C
■ control
cv. Attila
B
200
400
600
800
1000
1200
1400
0.5
0.4
0.3
0.2
0.1
0.0
Conductance (mol m-2 s-1)
cv. Ciano
C
0
200
400
600
800
1000
1200
1400

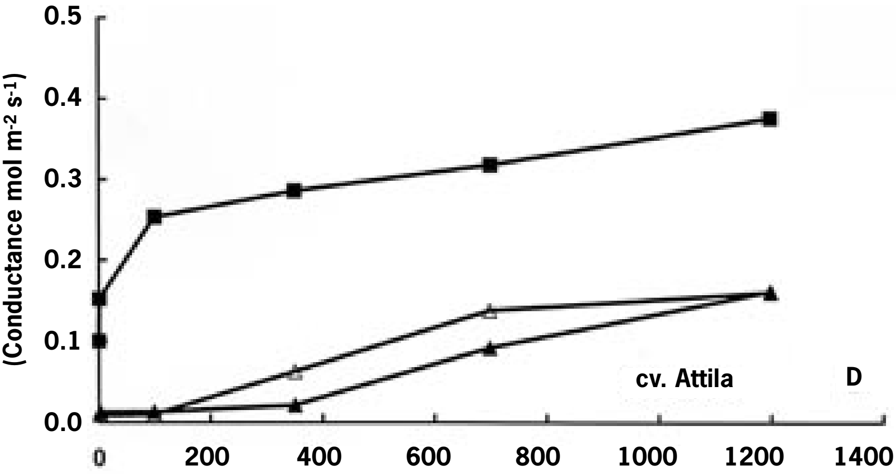
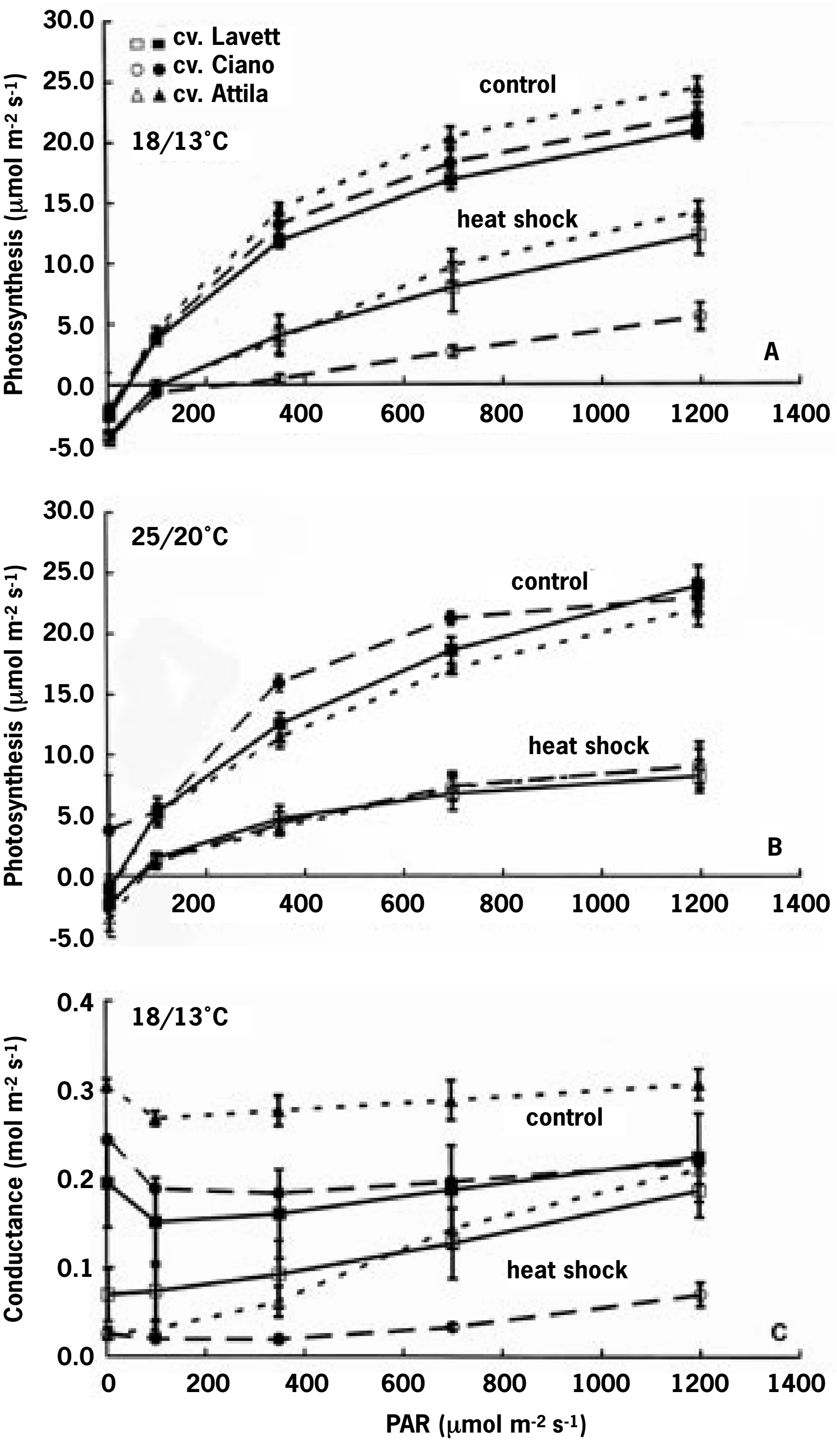


Figure 3. The effect of a heat shock at the end of tillering on the relationship between photosynthetically active radiation (PAR) and rate of photosynthesis (A, B) and on the relationship between PAR and stomatal conductance (C, D) for mature leaves of two wheat cultivars grown at temperature regimes of 18/13 or 25/20 °C.

closely associated with stomatal behaviour (Figures 3C, D). As a result of the heat shock, stomatal conductance under steady-state conditions in daylight decreased. This effect was already apparent after two days. Prolonging the heat stress until three days had no further impact. The effect of the pre-treatment growth temperatures on the heat-shock effect was negligible.

The heat shock at early grain filling induced a decline in rate of net photosynthesis for all three cultivars (Figures 4A, B). Also the differences between cultivars were more pronounced. For the cultivars Lavett and Attila grown at 18/13 °C both the photosynthetic efficiency and the rate of photosynthesis at light saturation (A_{max}) decreased by about 40%, but for the heat-sensitive cultivar Ciano the reduction reached up to about 75% (Figure 4A). The photosynthetic response to the heat shock depended on the pre-treatment growth temperature and on genotypic traits. The cultivar Attila was more tolerant to the heat shock when grown at 18/13 °C (Figure 4A) and the cultivar Ciano was more tolerant when grown at 25/20 °C (Figure 4B). In accordance with the observations at the end of tillering, the effects were closely correlated with stomatal behaviour (Figures 4C, D). Photosynthesis and stomatal conductance after the heat-shock treatment showed a linear relationship (Figure 5A), which was not present before the heat shock. Apparently, after the heat shock, photosynthetic rates became mainly dominated by stomatal limitations and not by intrinsic photosynthesis processes, which play a major role in limiting photosynthesis before the heat stress. This hypothesis is confirmed by the observation that the rate of photosynthesis was linearly related to stomatal conductance (Figure 5A) and that the internal CO_2 concentration was reduced by the heat-shock treatment (Figure 5B), indicating a predominant role of stomatal limitation. Stomatal closure associated with a decrease of the internal CO_2


cv. Lavett
cv. Ciano
cv. Attila
18/13°C
control
heat shock
A
Photosynthesis (μmol m-2 s-1)
30.0
25.0
20.0
15.0
10.0
5.0
0.0
-5.0
200
400
600
800
1000
1200
1400
25/20°C
control
heat shock
B
18/13°C
control
heat shock
C
Conductance (mol m-2 s-1)
0.4
0.3
0.2
0.1
0.0
0
PAR (μmol m-2 s-1)

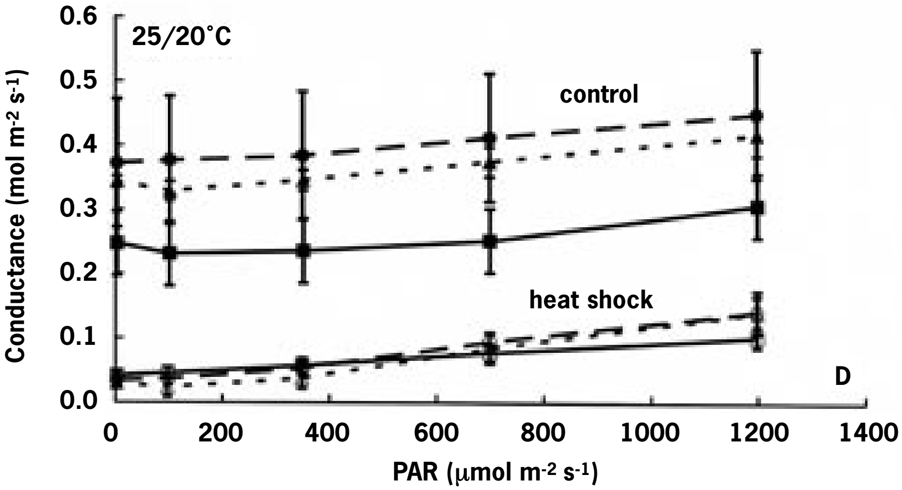


Figure 4. The effect of a heat shock at early grain filling on the relationship between photosynthetically active radiation (PAR) and rate of photosynthesis (A, B) and on the relationship between PAR and stomatal conductance (C, D) for flag leaves of three wheat cultivars grown at temperature regimes of 18/13 or 25/20 °C.

concentration will enhance photorespiration. Indirect evidence for this hypothesis comes from the shift in the relationship between the electron flow rate and the rate of photosynthesis (Figure 5C). It is shown that the ratio of electron flow per CO_2 molecule assimilated was increased by 10 to 15% as a consequence of the heat shock. This percentage represents the increase in non-linear electron pathways such as photorespiration, water to water cycle and non-linear electron flow in photosystem 1. In addition to these alternative electron transport sinks, part of the decrease in photosynthesis is due to down-regulation of photosynthesis by non-photochemical quenching of fluorescence (Npq) during the heat shock. Since CO_2 is the substrate for photosynthesis, the need to dissipate surplus of energy will increase. Under field conditions it is of major importance that plants are capable of recovering from a heat shock. In our experiment photosynthesis recovered completely from the heat shock within 4 days (Figure 6A). After full recovery the A_{max} value in cultivar Attila was higher (5.7 μmol m^{-2} s^{-1}) in the heat-shock treatment than in the control. In general, A_{max} values after recovery were slightly higher than before the heat shock and also the fluorescence component (Npq) was overcompensated. The apparent overcompensation may be attributed to the stomatal conductance, which recovered to a value that was significantly higher than before the heat stress (Figure 6B). The observed recovery of photosynthesis matched the accompanying full recovery of stomatal conductance.

More insight into the recovery mechanism can be derived from a comparison of electron transport rates and CO_2 assimilation rates before and after the heat-shock treatments. From the differences between the electron transport rates and the CO_2 assimilation rates it can be derived that heat creates a condition in which more electrons are required for each CO_2 molecule that is reduced, causing lower

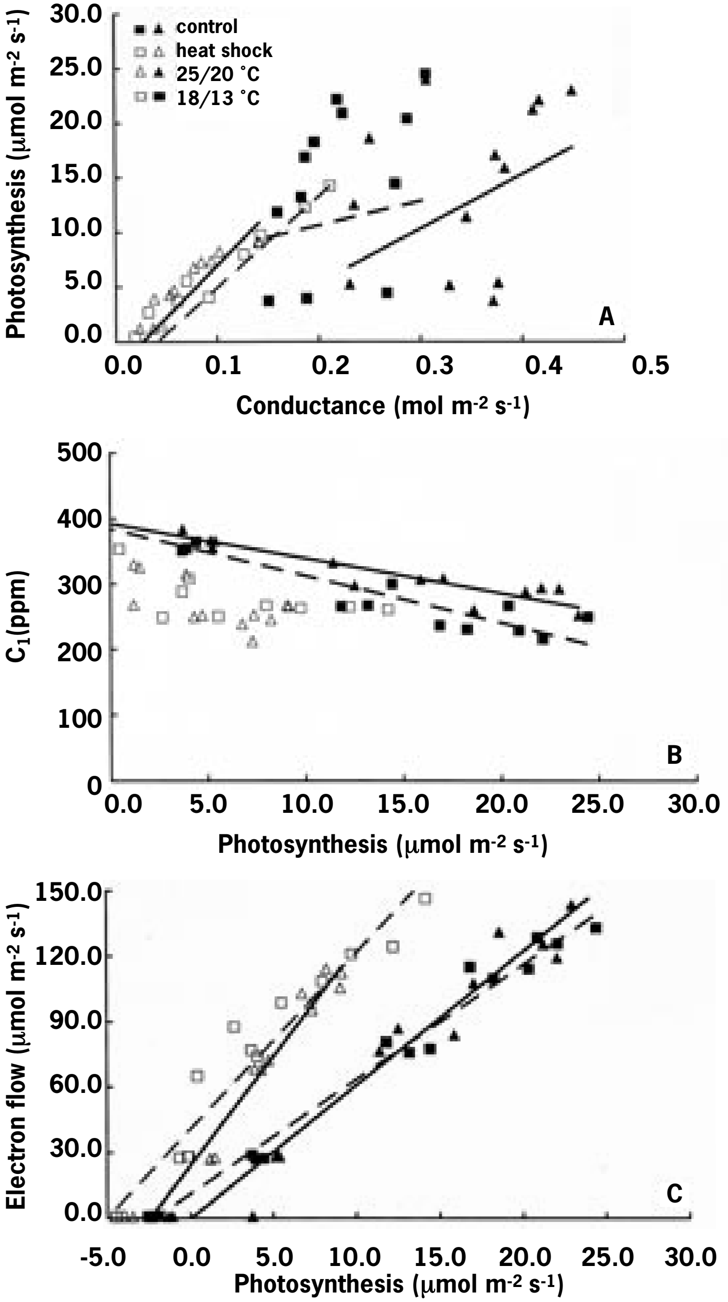


Figure 5. The effect of a heat shock at early grain filling on the relationship between stomatal conductance and photosynthesis (A), the relationship between internal CO$_2$ concentration (C$_i$) and photosynthesis (B), and the relationship between electron flow rate and photosynthesis (C) for leaves grown at two temperature regimes.

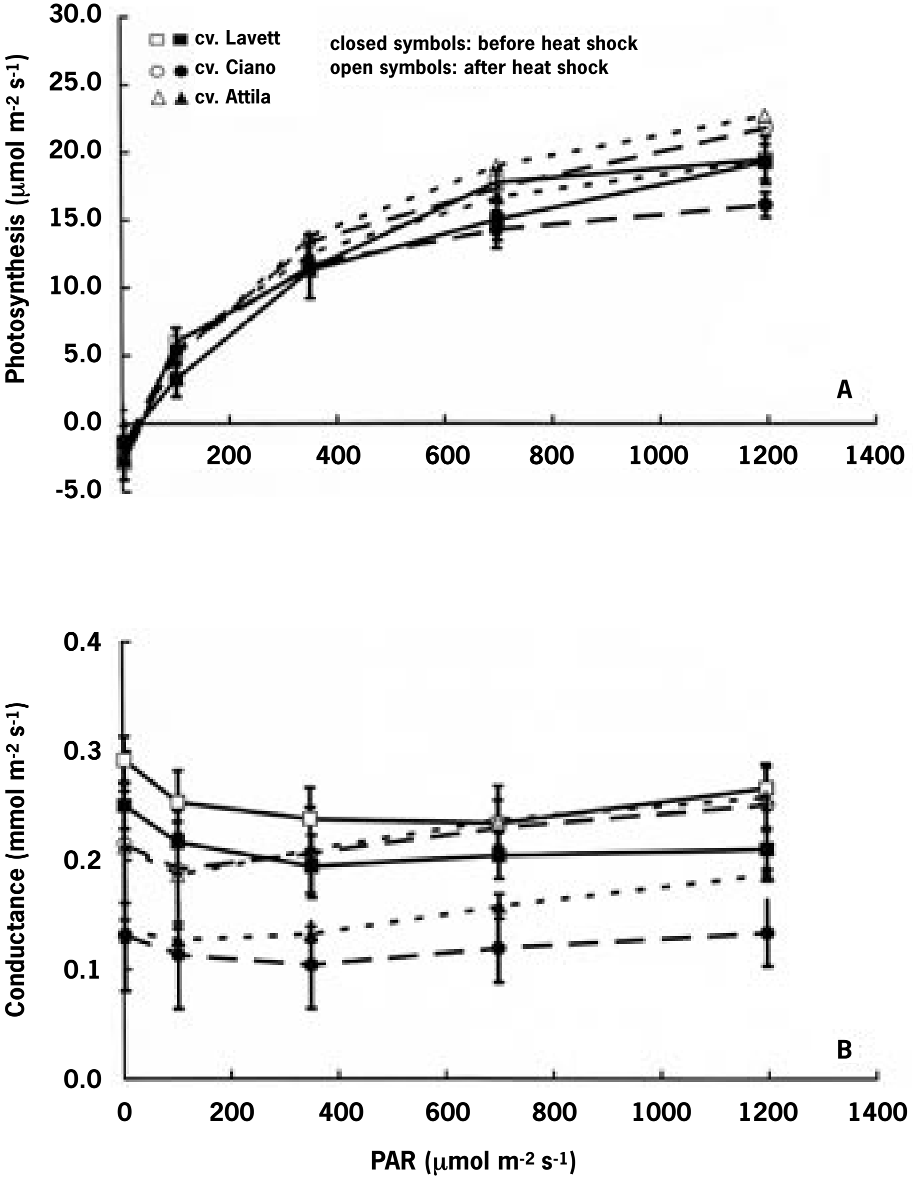


Figure 6. The relationship between photosynthetically active radiation (PAR) and rate of photosynthesis (A) and between PAR and stomatal conductance (B) at early grain filling before a heat shock treatment and after a 4-day recovery from the heat shock, for three wheat cultivars grown at a temperature regime of 18/13 °C.

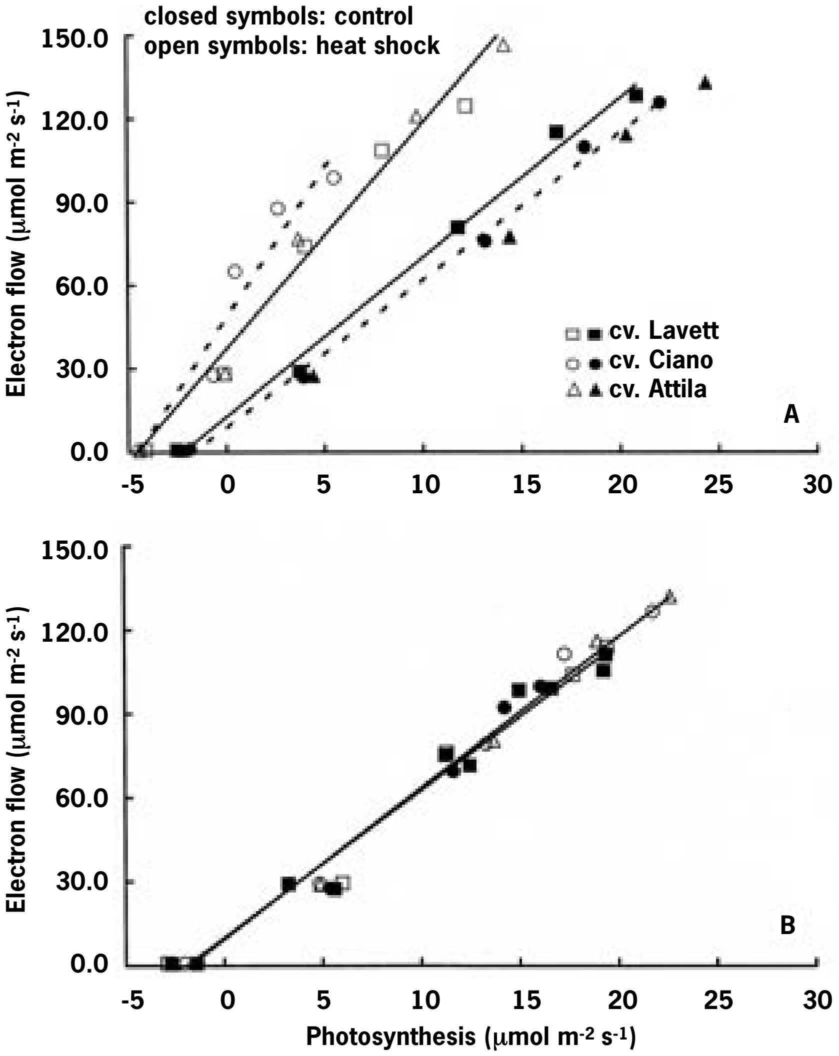


Figure 7. The relationship between electron flow and rate of photosynthesis, after the heat shock at the end of tillering (A) and after a 4-day recovery from the heat shock (B), for three wheat cultivars grown at temperature regimes of 18/13 or 25/20 °C.

photosynthetic efficiencies (Figures 7A, B). A heat sensitive genotype that is capable of mitigating the effects of low internal CO_2 concentrations by increasing the alternative electron flow, overcomes the threat of irreversible photodamage and is also capable of a full recovery. The heat sensitive cultivar Ciano is a good example of this strategy. From the results obtained it is evident that short heat pulses had an immediate but quickly reversible effect on photosynthesis.

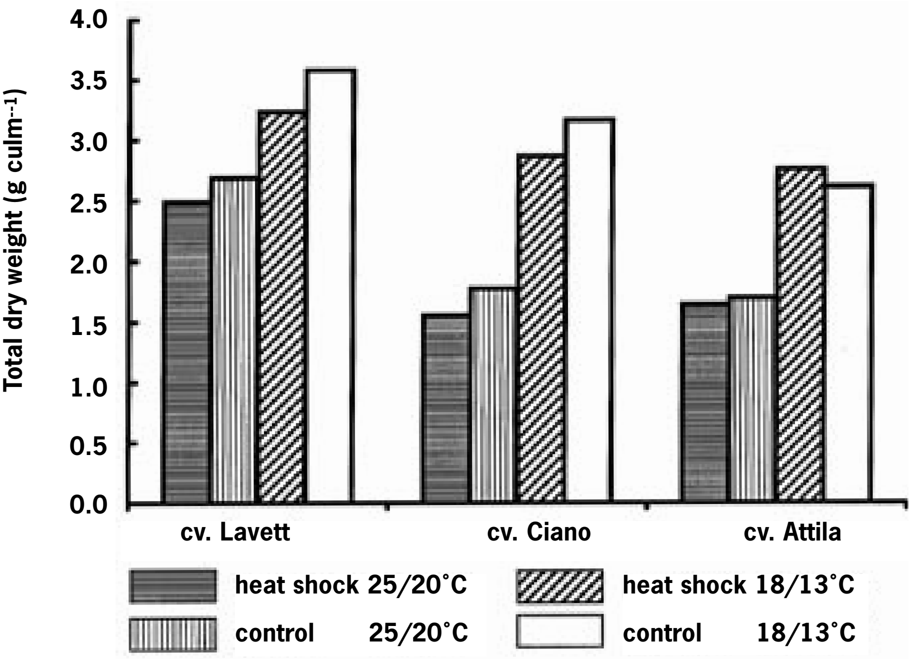


Figure 8. Effects of two growth temperatures, 18/13 or 25/20 °C, and a heat shock at early grain filling on total dry weight at the final harvest for three wheat cultivars.

Heat shock and its effects on biomass

The effects of the heat shock on biomass yield were more pronounced when the shock had been applied at early grain filling. The reversible effects of a 3-day heat shock on photosynthesis and fluorescence on biomass yield were less than the effects of high temperatures maintained during the growing season. Reductions in biomass yield ranging from 0.2 to 0.4 g per culm were found for the cultivars Lavett and Ciano (Figure 8). However, for the heat-tolerant cultivar Attila there was no yield response to the heat shock; the effect was absent or even slightly positive for plants grown at 18/13 °C.

Discussion

Genotypic variation in traits and tolerance to a heat shock

Heat tolerance is an important objective in plant breeding. A generic conceptual model of a core-set of traits for adaptation of wheat genotypes to dry as well as hot, irrigated environments was presented by Reynolds & Trethowan (2007). The model was developed by physiologists and breeders at CIMMYT and is used to assist with breeding decisions permitting a strategic approach. The conceptual platform can also be used to assess whether drought and other abiotic stresses such as heat share common physiological bases. The traits associated with adaptation to drought and hot, irrigated environments are classified in four groups: early growth, access to water by roots, water or radiation use efficiency, and photoprotection.

In our study we focused on traits that determine tolerance to extreme temperatures during a relatively short period. We found that a repeated heat shock during three days decreased leaf photosynthesis temporarily by 50 to 80%. The sensitivity of the plants to heat stress and its variation between cultivars were much higher in the generative than in the vegetative stage. A prolonged decline of the rate of photosynthesis would certainly affect plant productivity; however, in our experiment a rapid and full recovery of leaf photosynthesis took place within 4 days after the heat shock. At that moment, the effects of heat spells on grain yield would be relatively small when determined by photosynthesis only. However, heat shocks not only affect temporarily the photosynthesis rate, but also lead to significantly lower grain yields. Harding *et al.* (1990) hypothesized that both a diminished source and sink activity may be equally important in reducing productivity. Spiertz *et al.* (2006) reported that grain weight and grain number of plants grown at the lower temperature regime (18/13 °C) showed a stronger response to a heat shock than plants grown at the higher regime (25/20 °C). The reduction in grain weight as a result of the 3-day heat shock ranged from 13.4% at 25/20 °C to 16.9% at 18/13 °C. The yield reductions due to the heat shock were largest for the temperate cultivar Lavett; the yield of the heat-tolerant cultivar Attila did not respond to the heat shock. This indicates that a 3-day heat shock during the reproductive stage may lead to grain yield losses that are more determined by genotypic variation in storage processes (sink) than by photosynthesis-linked processes (source).

Evaluation of heat-shock effects on photosynthesis, stomatal conductance and fluorescence

Understanding the response of photosynthesis to changing environmental conditions is of great importance to predict the effects of climate change on plant productivity. Heat stress affects photosynthesis primarily through stomatal closure. This seems the crucial factor that determines the genotypic expression upon heat shock. Higher wheat yields in cultivars released by CIMMYT between 1962 and 1988 have been associated with a higher stomatal conductance, increasing internal CO_2 concentrations (Ci), and also Ci-independent photosynthetic capacity (Fischer *et al.*, 1998). Stomatal conductance regulates both photosynthesis and the hydrological status of the plant. Physiological data from replicated yield trials at two sowing dates demonstrated a clear genetic association of yield with photosynthesis and the related parameters for stomatal conductance (Gutierrez-Rodrigues *et al.*, 2000).

We hypothesized that stomatal closure is a primary response to a heat shock, leading to an increase in energy dissipation through non-photochemical quenching and a switch to non-assimilatory electron flow. The greater resistance of the stomata to CO_2 diffusion results in a reduction of the CO_2 concentration inside the leaf and so in a lowered rate of photosynthesis. The results indeed showed a decrease of the ratio in intercellular and ambient CO_2 concentration (Ci/Ca). So photosynthesis is less inhibited than transpiration, because the concentration gradient for CO_2 over the stomatal opening increases, while that for water vapour remains the same. This results in a higher water use efficiency, which is profitable when heat and drought stress occur simultaneously. With combined heat and drought stress, not only stomatal closure and energy dissipation occur but also irreversible damage to the photosystem II takes place (Schapendonk *et al.*, 1989). This might be caused by a drought-related decline of the cooling capacity of the leaves, leading to a heat-induced decrease of the natural sink for electron-flow coupled energy. So the damage may be caused by free radicals of O_2 that replace the natural electron acceptors. Thus, quantification of photosynthetic regulation in response to environmental stresses requires a model that incorporates various electron transport pathways. The recent review of Allen (2003) gives an updated insight into the interplay of the linear chain, cyclic and other nonlinear pathways of electron flow. Use of a generalized steady-state model for the description of various electron transport pathways might contribute to new insights into photosynthetic response to stress (Yin *et al.*, 2004). Recent evidence shows also that inhibition of net photosynthesis correlates with a decease in the activation state of Rubisco in both C3 and C4 plants and that this decrease in the amount of active Rubisco can fully account for the temperature response of net photosynthesis (Salvucci & Crafts-Brandner, 2004). A likely cause of reduced Rubisco activation at high temperature is the low temperature optimum of the activase and its thermal lability. Accurate predictions of plant growth require valid models of photosynthesis that are biochemically based.

Need for an integrated assessment of sink–source relationships

Is it necessary to test genotypes in different environments in order to predict heat tolerance? From the results presented it is clear that heat does affect yield at various levels in both source- and sink-related processes. Genotypic differences in heat tolerance were more pronounced in grain yield than in biomass yield. This finding is confirmed by the results reported by Tahir & Nakata (2005); they found significant differences among 18 genotypes in reduction of grain yield, grain weight, grain-filling duration and harvest index. Heat stress did not only reduce grain yield but also N remobilization whereas it increased the remobilization of total non-structural carbohydrates.

It is not a simple task to obtain a clear view on the way these processses are interacting over time, sometimes under multiple stresses such as heat and drought. So it will remain essential to test newly developed genotypes under conditions that prevail during crop growth (Mittler, 2006). Alternatively, to overcome the difficulty of testing numerous genotypes it seems worthwhile to carry out a selective screening based on photosynthetic performance following short heat treatments. From the three genetically different cultivars used in this experiment it became very clear that the effect of a temporary reduction in rate of photosynthesis on yield was relatively small. To answer the question of how heat tolerance in wheat can be improved, implicitely requires that interactions between source- and sink-related processes under heat stress are resolved. Only then the opportunity will be created to improve the genetic basis for heat tolerance.

In our study the genetic variation in photosynthetic properties for heat tolerance could best be assessed in a generative stage (early grain filing). It became evident that photosynthesis measurements in the vegetative stage (end of tillering) are not a reliable indicator for the prediction of yield, because the responses in photosynthesis are quickly reversible and the magnitude of the genotypic responses to a heat shock differs from those in the grain-filling stage. The underlying mechanisms in the vegetative stage and the generative stage may be similar but the relative expression varies considerably. In the literature, evidence is lacking that a sequence of heat shocks will show a different ranking in heat tolerance of genotypes. The marked differences between photosynthetic responses to the heat shock in different developmental stages indicate that a fast detection of photosynthetic capacity in an early stage is not a reliable option. This finding is a drawback for fast screening of young plant material. Screening in a post-anthesis stage still offers prospects if it is fast and simple. Gas exchange measurements however are relatively slow, expensive and time-consuming. Our findings indicate that fluorescence measurements may provide a tool that meets the requirements, because heat stress has a direct effect on alternative electron flows and associated fluorescence quenching parameters.

Acknowledgements

We wish to thank CIMMYT for providing the seed of the wheat cultivars Attila and Ciano and the staff of UNIFARM (Wageningen University and Research Centre) for taking care of the experiment in the controlled environment facilities. The active participation of Mrs Francis Hoogerwerf in the initial phase of the experiment, the comments of Drs Matthew Reynolds (CIMMYT) and Xinyou Yin (Wageningen University) and the technical support of Mrs Gon Van Laar are gratefully acknowledged.

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