Postgraduate Programme: Experimental Plant Biology
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INVESTIGATIONS ON THE HEAT STRESS TOLERANCE OF WINTER CEREALS

Main points of the PhD Thesis

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Introduction

Wheat is an extremely important agricultural crop, as it is able to adapt to a wide range of ecological conditions. However, its good genetic yield potential can only be exploited if the technology and environmental conditions are satisfactory. Extreme weather conditions, especially rainfall and temperature, have a substantial influence on the success of cultivation. Global climate change is increasingly affecting crop production. The mean temperature in Hungary is rising almost one and a half times faster than the mean temperature of the Earth as a whole. Sudden downpours and rapid rises in temperature may cause severe damage to wheat crops. Higher mean temperatures accelerate plant development, resulting in a shorter vegetation period. An increase in the number of extremely hot days during the cereal ripening period leads not only to considerable yield losses, but also to a great deterioration in the bread-making (technological) quality of the flour. High temperatures after the flowering of winter wheat result in poorer grain filling, consequently causing substantially lower yields.

The thesis discusses the results achieved in the Agricultural Research Institute of the Hungarian Academy of Sciences in research on the heat and drought tolerance of winter wheat subjected to various types of stress treatment in different stages of development and on the effect of these treatments on the physiological and yield traits of the crop.

Aims

Answers were sought to the following questions:

1. What effect does high temperature in the seedling and adult plant stages have on the yield of winter wheat varieties and on the quality components of wheat kernels?
2. Does heat stress applied in various stages of development have any effect on the antioxidant enzyme activity of wheat varieties? Are there any differences in stress tolerance in different stages of development? Is there any correlation between the changes observed in the activities of different antioxidant enzymes?
3. How do heat and drought stress, applied separately or in combination during the grain-filling period, influence the biomass accumulation and yield of cereals with different genetic backgrounds and the chemical composition of the kernels? What changes are caused by treatments during the grain-filling period in the physiological processes of the plants?
4. Can any differences be detected between the agronomic traits of lines from a doubled haploid population as the result of high temperature treatment in the early stage of embryo

development? What effect does heat stress in the early stage of embryo development exert on the germination ability of wheat kernels developing in stressed plants and on the initial growth vigour of the seedlings?

Materials and methods

Plant growth conditions

The experiments were carried out in plant growth chambers (Conviron PGV-36) in the Martonvásár phytotron between 2005 and 2010. The winter wheat varieties tested in the experiments were Plainsman V, Fatima 2, Mv Mambó, Mv Mariska, Maris Huntsman, Bánkúti 1201, Bezostaya 1, Mv Magma, Mv 15 and GK Öthalom (bread wheats), Franckenkorn (spelt wheat) and Mv Makaróni (durum wheat). In addition, a population consisting of 174 doubled haploid (DH) lines was developed, the parental varieties of which were Mv Magma and Plainsman V, which proved to be heat-tolerant and heat-sensitive, respectively, in preliminary experiments.

The treatments in the experiments were applied to these varieties in two phenophases: at the end of tillering or first node appearance (seedling stage, Zadoks 32, Tottman and Makepeace 1979) and in the grain-filling period, 12 days after heading (adult stage, Zadoks 75). In the case of the DH population, the 174 lines and the parental varieties were exposed to heat stress on the 6^{th} day after heading, during the early grain development stage.

Heat stress treatments

The stress treatments applied were adjusted to the stage of development: in the seedling stage (end of tillering, first node appearance) the stress involved a temperature of 30°C for 8 hours a day, with a minimum night temperature of 20°C, while in the adult stage the plants were exposed to 8 hours of 35°C, 38°C or 41°C, again with a minimum night temperature of 20°C. In both cases the treatment lasted for 15 days.

Drought stress and combined treatment

The soil moisture was adjusted in terms of the natural water capacity (NWC), which was taken to be the 100% water-saturated state. The value was set at 60–70% for control plants and 40–45% in the drought stress treatment. The soil moisture content was checked daily and the water deficit was replaced by watering. Both the control plants and those exposed to drought stress were kept at a day/night temperature of 24/20°C. In the case of the combined drought + heat stress treatment the plants were exposed both to water withholding and to a temperature of 35°C.

Determination of yield characters

After the plants reached harvest maturity, measurements were made on the number of tillers, the total aboveground biomass (g), the grain yield per plant (g) and the grain number, from which the thousand-kernel weight (g) was calculated. The harvest index was determined as suggested by Donald (1962).

Determination of grain quality

The protein content of the wholemeal was determined using a Kjeltec 1035 Autoanalyzer (Tecator, Sweden) from the dry matter content using a factor of 5.7. The Zeleny sedimentation value (Zeleny index) was obtained from wholemeal using a Perten Inframatic 8611 instrument (ICC 202, 159, MSZ 6367/17-89). A Mastersizer 2000 Ver. 5.22 instrument (Malvern Instruments Ltd, Australia) was used to determine the bulk density of B-type starch granules (< 7 µm), while the ratio of the protein components in the wheat grains was obtained using the SE-HPLC technique. The unextractable polymeric protein content (UPP) was determined using the method of Gupta et al. (1993), and measurements were also made on the glutenin/gliadin and albumin/globulin ratios.

Measurement of chlorophyll content and chlorophyll fluorescence induction (Fv/Fm)

The chlorophyll content was measured using a SPAD-502 instrument (Minolta, Japan), which records leaf transmittance in the red and near-infrared spectra and then calculates the SPAD index from these two values. Chlorophyll fluorescence induction parameters were recorded on fully-developed flag-leaves using a PAM-2000 instrument (Walz, Effeltrich, Germany). The maximum quantum efficiency of PSII (Fv/Fm) was recorded on dark-adapted plants.

Analysis of the antioxidant enzyme system

The antioxidant enzyme activity of plants exposed to heat stress in various stages of development was determined photometrically. Measurements were made on the activity of the glutathione reductase, glutathione-S-transferase, ascorbate peroxidase, catalase and guaiacol peroxidase enzymes (Janda et al. 2008).

Tests on germination ability

Germination tests were carried out on seeds originating from doubled haploid plants from the control and heat stress treatments. Measurements were made on the shoot and root length (cm) of the seedlings, and on the number of roots.

Statistical analysis

The data were evaluated using two-factor analysis of variance, and correlations between the traits were determined by means of correlation analysis.

Results

Extremely high temperature is one of the most frequent forms of abiotic stress, representing a great danger to crop production in Hungary, especially in combination with drought, and having a severely negative effect on cereal yields. This necessitates detailed research on the effect of heat stress on cereals. To this end, the stress tolerance of wheat varieties with different genetic backgrounds was studied under controlled conditions in a phytotron, where the environmental conditions could be adjusted as required. The main results were as follows:

Effect of heat stress in various phenophases

- High temperature (30°C) at the end of tillering or at first node appearance (young plant stage) significantly reduced the heading time of the wheat varieties examined and caused a large number of tillers to wither.
- Heat stress in the young plant stage was found to have a greater effect on yield parameters than when applied to adult plants. The thousand-kernel weight increased for kernels developing on plants exposed to stress in the young plant stage, while heat stress in the adult stage (12 days after heading) resulted in a substantial decrease in the thousand-kernel weight.
- A grain number reduction of over 50% was detected, averaged over the varieties, in response to heat stress in the seedling stage, causing a drastic loss of yield, while high temperatures during the grain-filling stage had little influence on the grain number in most wheat varieties.
- It was demonstrated that a temperature of 38°C during grain filling caused significantly greater damage to the yield, harvest index and thousand-kernel weight than treatment at 35°C. Increasing the temperature to 41°C, however, did not result in greater losses in these traits than the 38°C treatment.
- Among the protein components of the grains, lower values of UPP % and Glu/Gli ratio were found to indicate a deterioration in grain quality, despite the increase in protein content, observed especially when the temperature during grain filling was as high as

41°C. The quality deterioration was confirmed by the lower Zeleny index and the substantial reduction in grain yield.

- The increase in the relative protein content as the result of 30°C heat stress applied to young plants was found to be considerably greater than that induced by heat stress in the adult stage. However, the significant rise in the Zeleny index was associated with a significant reduction in the Glu/Gli ratio.

Role of antioxidant enzymes in heat tolerance

- The activity of the antioxidant enzymes glutathione-S-transferase, ascorbate peroxidase and catalase was found to be significantly enhanced by high temperature in most stress-treated wheat varieties regardless of the phenophase, suggesting that they play a role in defence against the negative effects of heat stress.
- The activity of the guaiacol peroxidase enzyme declined when wheat was exposed to high temperature in the young plant stage, indicating that GPx probably has no part in improving the resistance of wheat plants. In both phenophases tested, the activity of glutathione reductase exhibited the least sensitivity to heat stress.
- In response to heat stress in both young and adult plants, positive correlations were detected between the changes in the activity of individual antioxidant enzymes (APx–CAT, GR–CAT, APx–GR), confirming that the antioxidant enzymes exert a complex effect on each other in the defence response to heat stress.

Comparison of the effects of high temperature and drought

- It was found that in response to drought there was a much greater reduction in the chlorophyll content than after heat treatment. Compared to heat stress, the physiological changes caused by drought resulted in the more rapid decomposition of the leaf chlorophyll content, so the plants reached harvest maturity earlier than the control. The greatest and most rapid decomposition of chlorophyll pigment was induced by the combined stress.
- While heat stress did not result in substantial differences in the chlorophyll fluorescence induction (Fv/Fm) recorded in dark-adapted flag-leaves for most of the wheat varieties, the changes in the maximum quantum efficiency of PSII recorded after drought stress allowed the wheat varieties to be divided into tolerant and sensitive groups in terms of their response to drought.

- It was demonstrated that drought and drought + heat had a considerably greater effect on yield traits and quality than heat stress alone.
- The bulk density of < 7 µm B-type starch granules was found to be modified slightly by heat stress and to a far greater extent by drought and drought + heat.
- Among the heat, drought and combined heat + drought stress treatments, drought caused the most serious reduction in the UPP% and Glu/Gli ratios (% distribution of protein components), indicative of a deterioration in quality. The rise in the relative protein content occurring in response to drought stress was even more pronounced in the case of combined stress. High temperature also had an influence on the quality of the grain yield, but the deterioration was not as severe as in the case of drought.

Analysis of heat stress in a segregating wheat population

- Two separate experiments carried out to investigate the effect of heat stress at grain filling and during early embryo development on the yield traits and chlorophyll content of the wheat varieties Plainsman V and Mv Magma revealed that Mv Magma had better heat tolerance than Plainsman V.
- A genetic population was created in which substantial variability in heat tolerance was detected on the basis of yield traits and chlorophyll content, making it suitable for use in studies on the genetic background of heat tolerance.
- By determining the number of kernels per spikelet on the main spike, conclusions could be drawn on the ratio of pollinated flowers from which embryos developed. High temperature in the early embryo development stage was found to increase the number of aborted embryos.
- Correlation analysis on the yield parameters of a segregating population revealed a close positive correlation not only between various biomass and yield traits in response to heat stress, but also between these traits under control conditions. Relatively close positive correlations were also obtained between the values recorded under heat stress and control conditions for many yield traits.
- No substantial reduction was detected in the mean values of shoot length, root length and root number for seedlings developing from kernels on the main spike and other spike-bearing tillers of wheat plants exposed to high temperature. High temperature had

little influence on the germination vigour of the wheat kernels, though for a few lines the shoot and root length were reduced.

- Close positive correlations were observed between the shoot and root lengths of the seedlings and the grain yield obtained after exposure to 35°C, indicating better germination vigour for seed from lines that responded to heat stress with a less pronounced reduction in yield than for seed originating from lines more sensitive to heat stress.

Conclusions

The results proved that heat stress, which is experienced increasingly frequently in Hungary during the vegetation period, often in combination with drought, leads to substantial yield losses in cereals and to a deterioration in grain quality. Diverse response types were observed even among successfully cultivated varieties, and the analysis of how these were able to adapt to various stress conditions contributed to a more detailed understanding of the effects caused by extreme weather conditions. The genetic population created made it possible to investigate heat stress tolerance, and will also be suitable for future research on the genetic background of heat tolerance. The more detailed knowledge acquired on the environmental responses of the traits investigated in the experiments will be useful for the development and cultivation of new varieties adapted to altered environmental conditions.

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