Quality of Winter Wheat in Relation to Heat and Drought Shock after Anthesis

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Abstract

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Raw material quality, which is influenced not only by the protein content, insoluble protein polymers, and glutenin-to-gliadin ratio but also by the starch granule size, is very important for the quality of bakery products. This study investigated the effect of high temperature and drought (during grain-filling) on the quality and components yield of five winter wheat varieties. Drought and drought + heat were found to have a much greater influence on the yield and quality than heat stress alone. Averaged over the varieties, the yield losses were 57% after drought, 76% after drought + heat, and only 31% after heat stresses. The reductions in the unextractable polymeric protein fraction and glutenin-to-gliadin ratio indicated a poorer grain yield quality, despite the higher protein content. Quality deterioration was observed after drought or drought + heat, while high temperatures alone resulted in no change or in a better ratio of protein components. A significant negative correlation was observed between starch granule size and relative protein content after drought, demonstrating that this parameter contributes, together with protein, to the baking quality of the flour.

Keywords: starch granule size; protein content; glutenin-to-gliadin ratio; high temperature

Wheat varieties with satisfactory genetic traits are essential for the production of high quality wheat. Certain quality parameters vary over a very wide range in individual breeding lines. This broad genetic variability is demonstrated by the fact that the protein content ranges from 10–17%, the gluten content from 20–45%, the grain hardness from 10–85, the falling number from 60–450, and the farinograph index from C2 to A1. These differences in quality also depend on the soil, nutrient supplies, standard of plant protection, and other agronomic factors. The weather conditions during the growing season, especially the rainfall quantity and temperature, have a substantial influence on the plant metabolic processes, and thus on wheat quality.

Stress during the grain-filling stage may have an even greater effect on wheat, as it may cause reduced grain-filling (Wardlaw & Moncur 1995), accelerated cell death, and an earlier attainment of the harvest maturity (Altenbach *et al.* 2003), which may result in substantial changes in the protein composition of the grains and in the size distribution of starch granules.

The yield losses are caused by a reduction in the starch content, since over 65% of cereal kernels is composed of starch (Jenner 1994; Barnabás *et al*. 2008). Starch accumulation is correlated with

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the sucrose content of the kernels and with the activity of sucrose synthase and other enzymes with an important role in starch synthesis (Yan *et al.* 2008). This suggests that a low sucrose content and a decline in the enzymatic activity involved in starch synthesis are responsible for the reduction of starch accumulation. According to Labuschagne *et al.* (2009), the lower ratio of starch in the endosperm in response to high temperature can be attributed to the heat inactivation of starch synthase, the key enzyme in the starch biosynthesis reaction pathway (Denyer *et al.* 1994). The functional properties of starch, especially the water absorption ability of starch or flour and the flexibility of the dough, are dependent on the amylose-to-amylopectin ratio and the size distribution of the starch granules (Labuschagne *et al.* 2009).

Considerable changes in the size and shape of starch granules are also observed when starch is incorporated into the endosperm during ripening (Bechtel *et al.* 1990; Ellis *et al.* 1998; Stoddard 1999; Hurkman *et al.* 2003).

It was demonstrated that even a low light intensity may have an effect on various types of starch granules, resulting in a sudden decline in the grain yield and in the starch content of the kernels. B-type starch granules (≤ 9.9 μm) proved to be much more sensitive to shading and high temperature during the grain-filling period than A-type granules (> 9.9 μm) (Blumenthal *et al.* 1995; Cai *et al.* 2008; Li *et al.* 2008, 2010). Various authors (Panozzo & Eagles 1998; Viswanathan & Khanna-chopra 2001; Chinnusamy & Khanna-Chopra 2003; Hurkman *et al.* 2003; Zhao *et al.* 2008) reported that a high temperature after flowering reduced the starch content and had a significant influence on the starch granule size distribution in the wheat kernels. The number of B-type granules decreased in response to high temperature while that of A-type granules rose. In some of the varieties tested, drought after flowering also reduced the % volume and surface area of B-type granules (< 10 μm) (Zhang *et al.* 2010). Flour properties are closely associated with the starch granule size distribution (Soh *et al.* 2006), large (A-type) granules leading to lower maximum viscosity in comparison with small (B-type) granules. These differences mean that the two types of starch granules are differently utilised in processed foods and non-food products.

Protein accumulation also changes in response to stress. Instead of the proteins that play an active role in the biosynthesis and metabolism, storage proteins and those involved in protecting the plants against biotic and abiotic stresses are accumulated preferentially. Specific protein responses depend on whether or not high temperature occurs during the early or middle phases of grain-filling (Hurkman *et al.* 2009). The protein content in flour increases significantly with bread wheat as the result of the heat stress (Bencze *et al.* 2004; Balla & Veisz 2007; Labuschange *et al.* 2009), a phenomenon also noted by other authors (Wrigley *et al.* 1994; Corbellini *et al.* 1997; Daniel & Triboï 2000; Hrušková & Švec 2009). The application of N after flowering may indirectly increase the protein content of the kernels without reducing the yield, while the increased grain protein content caused by heat and drought stress after flowering may be associated with yield losses, due to a reduction in the starch production (Bhullar & Jenner 1985; Cassman *et al.* 1992; Fowler 2003).

The protein composition, which is the most decisive factor in breadmaking quality, is greatly influenced by various types of stress. The gluten proteins, glutenin and gliadin, are responsible for the flexibility and extensibility of the dough. Although albumins-globulins have only a slight influence on the dough quality, they are important from the nutritional point of view, due to their high contents of essential amino acids. Gupta *et al.* (1992) and Triboï *et al.* (2003) reported an increase in the gliadin proportion and a reduction in the glutenin proportion as the protein content of the flour increased. The ratio of albumins-globulins did not increase proportionately with the protein content in response to a rise in temperature (Gupta *et al.* 1992; Wieser & Seilemeier 1998; Triboï *et al.* 2003). It was demonstrated by Zhao *et al.* (2009) that protein components are very sensitive to drought stress during the later phenophases of grain-filling. The deterioration in the dough quality could be attributed to the decline in the glutenin-to-gliadin ratio and in the percentage of very large glutenin polymers in response to a high temperature (Blumenthal *et al.* 1994, 1995; Bencze *et al.* 2004; Balla & Veisz 2007).

In Hungary, high temperature and drought occur most frequently after heading, during the grain-filling period, causing stress that influences the quality of the wheat kernels. One of the main aims of the present work was thus to analyse the changes in the nutrient content, quality and quantity of the yield of a wide range of wheat varieties under various environmental conditions, including high temperature and drought stress during ripening. The measurements involved the protein content of the kernels, proportion of protein components, and the size of starch granules produced from stressed plants.

MATERIAL AND METHODS

Plant material and growing conditions. Five winter wheat cultivars with a wide range of genetic backgrounds were chosen for the quality analysis: Plainsman V. (USA), Fatima 2 (Hungary), Mv Mambó (Hungary), Mv Mariska (Hungary), and Bezostaya 1 (Russia). The hard red wheat cv. Plainsman V. has a high protein content and good drought tolerance. The mid-early maturing cv. Fatima 2 is known for its excellent yield potential and the good milling and baking quality of its flour, which can be utilised for baking bread of excellent quality. The early maturing bread wheat cv. Mv Mambó is characterised by excellent adaptability, high yields, and good quality. The hard bread wheat cv. Mv Mariska has extra early maturity, with high yields and good quality. Cv. Bezostaya 1 has above-average yield potential, excellent adaptability, hard endosperm structure, and excellent rheological properties.

The studies were carried out in a Conviron PGV-36 climate chamber in the Martonvásár phytotron to investigate the effect of high temperature and drought stress on the quality components of the grain yield.

Pots with a volume of 3500 cm^3, containing a known quantity of a 3:2:1 ratio of soil, Vegasca, and sand, were planted each with four germinated wheat seeds. After 6 weeks of vernalisation at 4°C, the plants were grown at controlled temperature using the T2NY2 climate program (Tischner *et al*. 1997) until the start of the stress treatment.

The experiment consisted of four treatments: control (C), heat stress (H), drought stress (D), and drought + heat stress (D + H). For each cultivar, there were a total of 16 pots, 4 pots per treatment, with 4 plants per pot. The treatment began on the 12^{th} day after heading (Zadoks-75) and was continued for 15 days. The temperature was programmed at 24/20°C (day/night) in the control chamber (Tischner *et al*. 1997) and at 35/20°C (with 8 h at 35°C) for the heat stress treatment. The soil moisture was adjusted to 60–70% of natural water content (NWC, taken as 100%) for the control treatment and 40–45% for the drought stress treatment. Water was added on a weight basis. The light intensity was adjusted to 350 μmol/m^2/s during the stress treatments.

The data were statistically evaluated using two-way analysis of variance (ANOVA) (Láng *et al*. 2001). Correlation analyses were done between the yield parameters on the five genotypes in each treatment.

Methods. The measurements were made on the protein content of the grain, the proportions of the protein components, changes in the proportion of starch granules with a size of less than 7 μm, and other parameters characteristic of grain quality (e.g. Zeleny Index). A Perten Inframatic 8611 instrument (Perten Instruments AB, Huddinge, Sweden) was used to determine the changes in the moisture content and water absorption of the kernels. After harvest maturity was reached, the grain yield per plant, kernel number, thousand-kernel weight, and harvest index (HI) were determined. Harvest Index (%) was calculated according to (grain yield/biological yield) × 100 based on Donald (1962).

Wholemeal samples for the measurements of quality parameters were prepared using a Perten 3100 Laboratory Mill (Perten Instruments AB, Huddinge, Sweden).

NIR analysis. Wholemeal samples from the stress-treated plants were analysed in three replications using a Perten Inframatic 8611 instrument (ICC 202; ICC 159). This instrument works on the principle of near infrared reflectance (NIR) spectroscopy at the wavelengths of 1680 nm and 2230 nm. When suitably calibrated using standard methods and measurements, the light absorption of the illuminated samples can be used to measure traits such as the moisture content (ICC 110/1), protein content (ICC 105/2), Zeleny index (ICC 116/1), and water absorption ability (ICC 115/1) rapidly on small samples. The Zeleny index, which indicates the sedimentation of the flour in a lactic acid solution, provides a reliable prediction of the loaf volume, where low values are indicative of more intense sedimentation, leading to denser loaves with smaller volumes.

Determination of protein content. The protein content of the wholemeal was determined in two replications of 1 g samples using a Tecator Kjeltec 1035 Autoanalyzer (Tecator, Höganäs, Sweden), according to the Kjeldahl method (ICC 105/2) applying a factor of N × 5.7.

Determination of the quantitative proportions of protein components. The changes in the quantitative ratios of the protein components were determined in wheat kernels taken from the plants exposed to high temperature and drought. The measurements were made in three replications using 1 g samples. The total glutenin, gliadin, and albumin-globulin contents were determined using the SE-HPLC technique, i.e. the proteins were separated on a size basis using a modification of the method outlined by Batey *et al.* (1991). A Phenomenex BIOSEP-SEC 4000 column was used for the analysis and the proteins were detected at 210 nm. The unextractable polymeric protein fraction (UPP%) was determined using the method of Gupta *et al.* (1993) relative to the total polymeric protein fraction. The measurements were also made of glutenin-to-gliadin ratio and the albumin-globulin %, which was calculated in relation to the total polymeric protein fraction.

Starch isolation and determination of granule size distribution. The samples for the analysis of the changes in starch granule size were prepared in the Martonvásár laboratory and the volume % of starch granules with a size of less than 7 µm were measured using a Mastersizer 2000 Ver. 5.22 instrument (Serial No.: MAL100292, Malvern Instruments Ltd., accessory name: Hydro 2000S) at CSIRO (Canberra).

A small-scale isolation of wheat starch was carried out on whole seed as follows: In the first step, kernels were placed between plastic sheeting and crushed with pliers, aiming to open the seed, but not to pulverise it, as this might damage the starch. The crushed grains were placed in 2 ml Eppendorf tubes and steeped in 1 ml NaCl (0.5M) for at least 4 min on ice. The steeping process enables gluten to become more cohesive, while the ice minimises the enzyme activity. In the next step, the seeds were pulped with a pestle to form a gluten ball, after which the starch solution was decanted. This was continued, adding further 1 ml quantities of NaCl solution, pulping and decanting the starch solution, until the solution was clear (usually a minimum of 6 times). The starch solution was centrifuged in a 10 ml tube (5 min, 5000 rpm) and the supernatant was discarded. The starch pellet was dispersed in 5 ml acetic acid (0.1M), centrifuged, and the supernatant was discarded. After repeating this step, the starch pellet was washed twice in 5 ml water and finally freeze-dried.

RESULTS AND DISCUSSION

The experiments carried out in the plant growth chambers and in the field have proved that the temperature and available water quantity play a substantial role in the quantity and quality of wheat grains (Gibson & Paulsen 1999; Bencze *et al.* 2004; Balla & Veisz 2007; Mikulíková *et al.* 2009; Zhao *et al.* 2009). The various stress treatments applied had significant effects on the yield parameters and also influenced the quality of the varieties tested.

The present results indicated that extreme weather conditions, such as high temperature and water deficiency, were capable of influencing the quantity and quality of grains. The differences between the varieties were not influenced by the heading date or maturity date, as all the varieties were exposed to stress in the same phenophase. While high temperature is generally considered to have a decisive effect on the quantity and quality of grains, the present analysis indicated that the drought and drought+heat stresses caused much more drastic changes than the heat stress alone (Table 1). Averaged over the varieties, for instance, the yield losses amounted to 57% in the case of drought stress, and to 76% when drought stress was combined with heat stress, while heat stress alone resulted in a yield loss of only 31%. Similar

Table 1. Changes in wheat yield parameters (grain yield, kernel number, harvest index, thousand-kernel weight) in response to heat, drought and drought + heat, averaged over the varieties

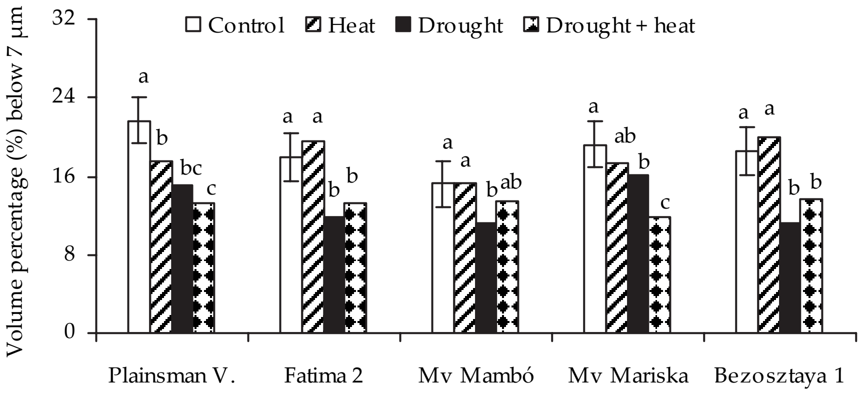
Parameter	Grain yield/plant (g)	Kernel number/plant (pc)	Harvest Index (%)	1000-kernel weight (g)
Control	2.99	88.49	39.28	35.30
Heat	2.05	76.04	34.11	27.61
Drought	1.29	70.86	26.57	19.68
Drought + heat	0.72	66.30	17.87	11.57
$LSD_{5\%}$	0.22	8.77	1.84	1.63

$LSD_{5\%}$ represents the minimal difference between the genotypes which is statistically significant at the $P \leq 0.05$ level results were found for the thousand-kernel weight, which was reduced by 44% by drought and by 67% by the combined stress. This significant effect could also be detected in the case of kernel number and harvest index (Table 1). Although the change in the kernel number in response to stress was not as drastic, it was nevertheless significant. This can be explained by the fact that the treatment was begun 12 days after heading, when flowering was completed and seeds were beginning to form. In the cvs Plainsman V., Fatima 2, and Mv Mambó, a significant decline occurred in the kernel number in response to heat stress, contrary to Mohammadi *et al.* (2004) having reported no significant change in the kernel number of a population arising from a cross between heat-tolerant and heat-sensitive wheat varieties after the exposure to high temperature. However, Gibson and Paulsen (1999) found losses of 78% in the yield and 63% in the kernel number with plants exposed to 35/20°C heat stress 10 days after flowering (compared with 20/20°C). Even when applied 15 days after flowering, heat stress still caused a yield loss of 18% a though, at this stage, it had no influence on the kernel number. By contrast, in the present experiments heat stress led to a 31% yield loss when applied 12 days after heading. High temperature during the grain-filling period had a negative effect on the grain yield, which could be attributed to the drastic reduction in the kernel weight (Gibson & Paulsen 1999; Tahir & Nakata 2005).

The yield reduction observed when developing cereal grains were exposed to high temperature could also be attributed to the lower final starch content. It would appear that the starch synthesis slows down or ceases completely as a result of heat stress (Denyer *et al.* 1994). Enzymes lose their activity rapidly at high temperature, but they are sometimes capable of partial recovery when returned to cooler conditions (Jenner 1994). Labuschagne *et al.* (2009) also reported that elevated temperature resulted in a lower kernel weight due to the reduced starch accumulation.

The shape and size distribution of starch granules changes significantly as the cereal kernels develop. The present results proved that the size distribution of the starch granules was significantly influenced by the environmental conditions. While heat stress had only a moderate influence on the volume percentage of B-type starch granules (below 7 μm), drought or drought + heat had a much greater effect (Figure 1). This could be explained by the fact that the B-type granules started to develop during the stress period. The A-type granules (10–35 μm in diameter) start to develop 4–5 days after flowering and this process lasts to the end of the endosperm cell division phase. By contrast, B-type starch granules (1–10 μm) do not appear until 12–14 days after flowering and their growth continues until 21 days after anthesis (Bechtel *et al.* 1990; Hurkman *et al.* 2003). Li *et al.* (2010) reported a decrease in the number of B-type granules and an increase in that of A-type granules.

Cv. Plainsman V. was the only variety where the size of the starch granules was significantly reduced by heat stress, which had no significant effect on the other varieties (Figure 1). The drought caused the smallest reduction in cv. Mv Mariska (16%) and the greatest reduction in cv. Bezostaya 1 (39.3%). The granule size of cvs Plainsman V. and Mv Mariska was more severely influenced by drought + heat stress than by drought alone, while in the case of cvs Fatima 2 and Bezostaya 1 water withdrawal proved to be the most severe stress factor (Figure 1). Zhang *et al.* (2010) reported that water deficiency after flowering reduced the volume percentage and surface area of B-type granules



$LSD_{5\%}$ represents the minimal difference between the genotypes which is statistically significant at the $P \leq 0.05$ level

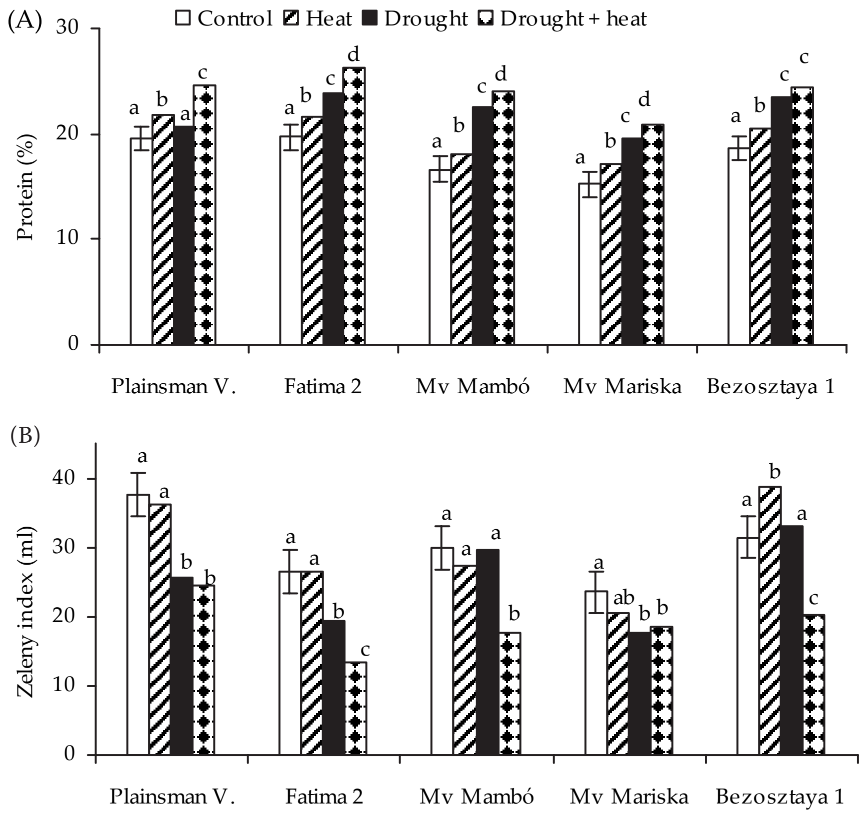
a, b, c – different letters denote significant differences

Figure 1. Changes in the starch granule size of the tested varieties in response to stress (heat, drought, drought + heat)

(diameter < 10 μm) in some cultivars, while for one variety there was an increase in the rat of wheat. The amylose content exhibited a negative correlation with the volume percentage of starch granules measuring less than 10 μm and a positive correlation with those greater than 15 μm. This explains the fact that small starch granules have a low amylose content, while that of large granules is high. These results suggested that granules with a high amylose content are less affected by drought stress than those with a low amylose content (Zhang *et al.* 2010). According to Singh *et al.* (2008) the effect of drought stress on the starch granule size distribution in wheat kernels was genotype-dependent.

In response to the stress treatments, in addition to the reduction in the starch accumulation an increase in the protein content also occurred, which could be explained by the decline in the grain size, i.e. thousand-kernel weight. The greatest significant increase in protein content was observed for all the varieties in the case of combined drought and heat stress (34.4% on average). A relative increase in protein (10.5% on average) could also be observed in the grain of the heat-stressed plants (Figure 2A), but this was generally not as severe as in the drought-stressed plants (23.2%). Other authors also noted an increase in this parameter at high temperature (Ciaffi *et al.* 1996; Stone *et al.* 1997; Bencze *et al.* 2004). This increase in protein content, however, was not associated with a better grain quality, as indicated by the decrease in the Zeleny index (Figure 2B) and the deterioration in the gluten protein composition (Figure 3). There was also a drastic reduction in yield (Table 1). The more the grain protein content rose in response to stress, the more the Zeleny index declined. The Zeleny index of the drought-stressed kernels dropped by an average of 15.8% and that of the kernels exposed to the combined stress by 36.5%. The Zeleny index is an important parameter for the breadmaking industry, its lower values resulting in denser loaves with smaller volumes.

Substantial differences were observed between the varieties (Figure 2). Cv. Mv Mambó proved to have the best heat tolerance, with a thousand-kernel weight closest to that of the control and the lowest (9.2%) increase in protein content (Figure 2A). Its Zeleny index (Figure 2B) was not much lower than that of the control, suggesting it would give good loaf volume. In the case of cv. Mv Mariska, although this variety exhibited the greatest relative increase in the protein content (12.2%) when exposed to temperatures of 35°C, the Zeleny index



Bar: differences significant at the $P \leq 0.05$ level

a, b, c – different letters denote significant differences

Figure 2. Changes in relative grain protein content (A) and Zeleny index (B) in response to heat, drought and combined drought + heat stress

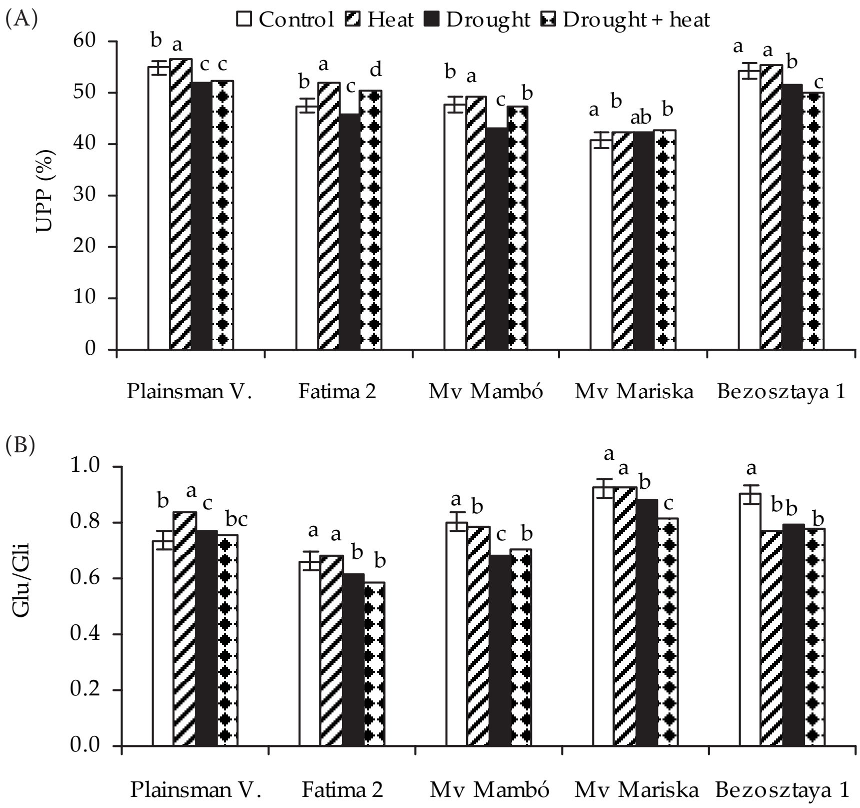


Figure 3. Changes in the unextractable polymeric protein content (UPP%) (A) and glutenin-to-gliadin (Glu/Gli) ratio (B) in response to heat, drought and combined drought + heat stress

Bar: differences significant at the P ≤ 0.05 level

a, b, c – different letters denote significant differences

dropped to the greatest extent in comparison with both the control and the other varieties, so the loaf volume was likely to be small. The cv. Bezostaya 1 exhibited not only an increase in the protein content (Figure 2A) but also a significant increase in the Zeleny index (Figure 2B), which could indicate good breadmaking properties.

In response to drought, the relative protein content (Figure 2A) rose to the smallest extent (5.4%) in cv. Plainsman V., with a moderate decline in the thousand-kernel weight, but the decrease in the Zeleny index (Figure 2B) was one of the most severe. The greatest increase in protein content (35.3%) and the smallest reduction in the Zeleny index (1.2%) were recorded for cv. Mv Mambó under water-deficient conditions.

Cv. Mv Mambó also exhibited the greatest increase in the protein content when exposed to combined drought and heat, while the smallest increase was recorded for cv. Plainsman V. However, the moderate decline in the Zeleny index with these two varieties suggested a deterioration in the yield quality (Figure 2B). Temperatures of over 35°C during the grain-filling period are capable of causing a substantial decline in the grain yield quality. Wheat varieties exposed to 40°C 10 h a day for 3 days exhibited significant changes, such as a significant rise in protein content and a significant decrease in thousand-kernel weight (Blumenthal *et al.* 1995).

Research has shown that not only the starch granule size, but also the protein components may be very sensitive to extreme weather conditions (Zhao *et al.* 2009). The development and accumulation of storage proteins in the kernels is of key importance during the post-flowering period. The stress that affects the developing proteins may be a decisive factor for grain quality. The accumulation of albumins and globulins continues for approx. 20 days after flowering, after which a constant level is generally reached. The accumulation of storage proteins begins approx. 6 days after flowering and continues until the end of the grain-filling period (Gupta *et al.* 1996; Stone & Nicolas 1996; Panozzo *et al.* 2001).

The present results indicated that the various stress treatments all influenced the proportions of the protein components (glutenins, gliadins, albumins-globulins) during grain filling (Figure 3). The decreasing values of the unextractable polymeric protein fraction (UPP%) (Figure 3A) and the glutenin-to-gliadin ratio (Glu/Gli) (Figure 3B) were indicative in most cases of a weakening of the grain quality despite the increase in the protein content (Figure 2A). The changes resulting in quality deterioration were observed mainly in the drought and drought + heat treatments in the present work, while high temperature did not influence the proportion of protein components, or in some cases even improved it. Although a rise in UPP% was detected in a few varieties, there was no significant change in the relevant Glu/Gli ratio, with very few exceptions. Cv. Plainsman V. was the only variety to exhibit a significant increase in both UPP% (Figure 3A) and the Glu/Gli ratio (Figure 3B), indicating that the quality may be improved under heat stress. In the other varieties, with the exception of cv. Bezostaya 1, the significant rise in UPP% after heat stress was not accompanied by a significant change in Glu/Gli. The quality analysis thus confirmed that drought, even alone, caused a greater stress than heat. When combined, the stress effect was greater than that of either factor alone. Water deficiency led to the quality deterioration in all the varieties, with declining values of both UPP% and Glu/Gli. In most cases the change in both values was significant. The situation was more complex in the case of combined stress, the quality-reducing effect of which was perceptible mainly in a decline in the Glu/Gli ratio (Figure 3B). Among the storage proteins, the albumin-globulin % did not change consistently in response to combined stress (Figure 4), rising in some cases and decreasing in others, and with two varieties the differences were not significant.

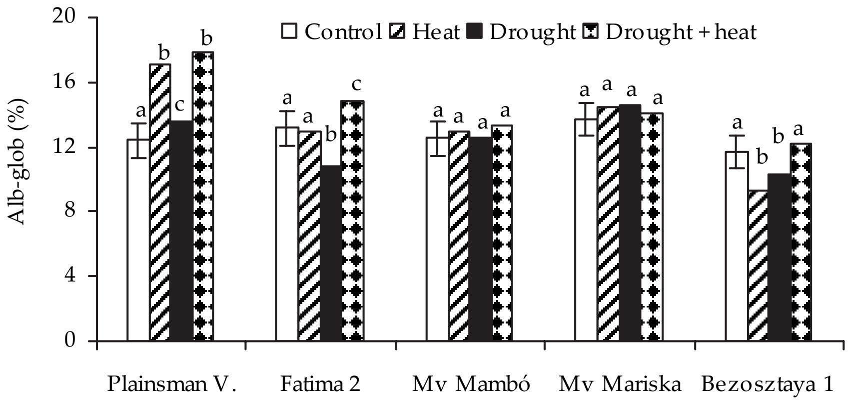
The accumulation of the various protein fractions was not synchronised, suggesting that the protein composition of the kernels changes as they develop. High temperature or drought may, however, shorten the grain-filling period, thus influencing the balance between the protein fractions (Jamieson *et al.* 2001).

It was found by Blumenthal *et al.* (1995) that the deterioration in dough properties could be attributed to the reductions in the Glu/Gli ratio and percentage of very large glutenin polymers in response to heat stress. The Glu-Dld allele (glutenin subunits 5 and 10) may be useful in breeding for tolerance, as it improves both of these parameters.

Other authors reported that in some varieties the flour, dough, and technological quality parameters changed in response to a brief period of heat stress (> 35°C) and this appeared to be correlated in some cases with an increase in Glu/Gli (Blumenthal *et al.* 1991) and reduction in the percentage of very large glutenin polymers (Wardlaw *et al.* 2002). Moderately high (25–32°C) temperatures, however, had a positive effect on dough properties (Wrigley *et al.* 1994) and led to modifications in the composition of the gliadin fraction (Daniel & Triboï 2002).

The stress treatments also influenced the moisture and water absorption ability of the cereal kernels (Figures 5 and 6). The more severe the stress, the lower the moisture content of the kernels (Figure 5A), while the water absorption ability increased (Figure 5B). High temperature caused the least reduction in the moisture content (1.5% on average), followed by water withholding (2.8%) and the combined treatment (9.9%). A comparison of the varieties demonstrated that cv. Mv Mariska suffered the greatest loss of grain moisture when treated at 35°C, while cv. Mv Mambó exhibited a significant water loss in response to drought and the combined stress. The most significant change in water absorption ability was induced by drought + heat treatment (Figure 5B). The grain yield of cv. Mv Mambó, which suffered the greatest moisture loss, responded to the most severe treatment with the greatest water absorption ability. Various storage proteins are also capable of influencing water absorption. It would appear that the gluten proteins and starches in wheat compete for water, thus causing changes in the dough mixing properties (Rakszegi *et al.* 2006).

A negative correlation was detected between the yield and protein content of the five wheat varieties stressed during grain filling. In the case of heat



Bar: differences significant at the $P \leq 0.05$ level

a, b, c – different letters denote significant differences

Figure 4. Changes in the albumin-globulin % in the yield in response to heat, drought and combined drought + heat stress

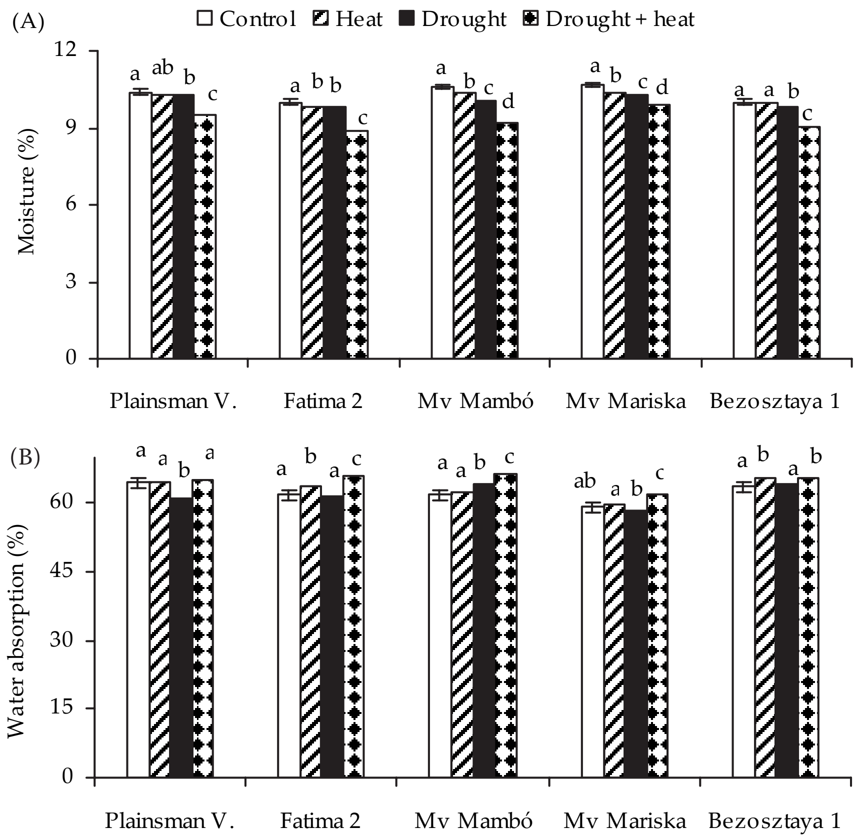


Figure 5. Changes in the moisture content (A) and water absorption ability (B) of the yield in response to heat, drought and combined drought + heat stress

Bar: differences significant at the $P \leq 0.05$ level

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stress, the correlation was relatively close ($r = -0.839^+$) (Figure 6A), while it was even higher in the case of combined drought + heat ($r = -0.875^+$) (Figure 6B) for a sample number of $n = 5$ (critical r value 0.8783 at $P = 5\%$, 0.8054 at $P = 10\%$). A negative correlation between the yield and protein content was also reported by other authors (Pleijel *et al.* 1999; Fowler 2003).

A significant negative correlation ($r = -0.92^*$) was demonstrated between the starch granule size and the relative grain protein content (Figure 7) in the drought treatment with all five varieties (critical r value 0.8783 at $P = 5\%$, 0.8054 at $P = 10\%$). A positive correlation was obtained between these parameters in the case of heat ($r = 0.597$) and drought + heat ($r = 0.80$) for a sample number of $n = 5$, which was fairly close, but not significant. In contrast, Park *et al.* (2009) reported an inverse correlation between the protein content and B-type starch granules. It seems that starch granule size distribution is a unique property that affects the physicochemical properties of wheat, flour, and breadmaking properties in conjunction with its counterpart, protein (Park *et al.* 2009).

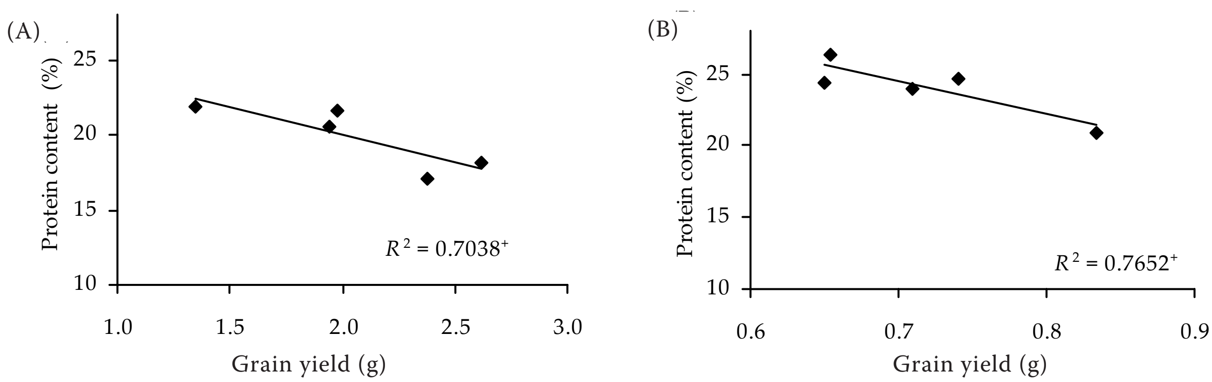

Figure 6. Correlation between the yield and relative protein content of the five tested varieties in the case of heat stress (35°C) (A) and drought + heat stress (B) (critical r value 0.8054 at $P = 10\%$, 0.8783 at $P = 5\%$, 0.9587 at $P = 1\%$). Each data point represents a different variety

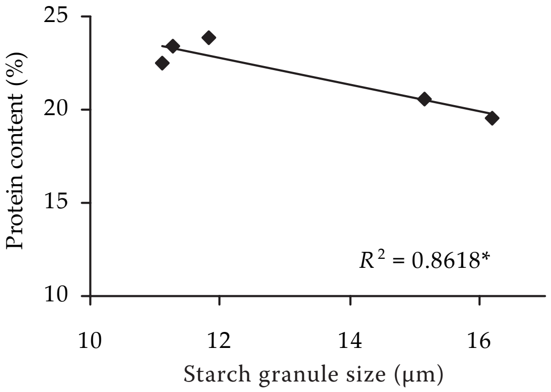

Figure 7. Correlation between the starch granule size and relative protein content of the five tested varieties in the case of drought (critical r value 0.8783 at P = 5%, 0.9587 at P = 1%). Each data point represents a different variety

Gibson & Paulsen (1999) underlined the importance of the date when the stress affected the wheat plants (number of days after flowering), as this had a great influence on the extent and duration of grain filling. At the same time, the synergistic interactions indicated that heat stress starting 12 days after flowering had a much smaller influence on the yield quantity and quality than drought stress or combined drought and heat.

In summary, it can be stated that the varieties tested had diverse responses to the stress treatment during the grain-filling period. Cv. Plainsman V. exhibited a significant increase in UPP% and Glu/Gli ratio in response to heat stress despite showing the greatest reduction in the yield and thousand-kernel weight. The Zeleny index did not decline in spite of the rise in protein content. This suggested that there was no deterioration in quality at the high temperature treatment, and that the parameters indicative of improved quality (e.g. higher protein content) could probably be attributed to the drastic drops in yield and thousand-kernel weight. This effect of high temperature on grain quality was observed with several varieties. The significant negative correlation (r = –0.92*) between starch granule size and relative grain protein content in the case of drought may indicate an important interaction between starch granules and proteins in determining the breadmaking properties of flour.

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