The effect of heat stress on some main spike traits in 12 wheat cultivars at anthesis and mid-grain filling stage

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Abstract: High temperature decreases winter wheat grain yield by reducing the grain number and grain weight. The effect of heat stress on spike grain distribution and weight of individual grains within spike and spikelets was less studied. Our aim is to identify influence of high temperatures during different phenological stages on spike grain distribution and weight and to explore genotypic variation of the studied wheat cultivars. Within this study, a controlled experiment was conducted with 12 different winter wheat cultivars under heat stress at anthesis and mid-grain filling stage. The results showed that spike grain weight, thousand-grain weight and grain number per spike decreased moderately in treatments with individual heat stress at anthesis and mid-grain filling period, respectively, which decreased severely in the multiple heat stressed plants at both stages compared with the control treatment. Heat stress decreased number of spikelets with grains. Grain weight at the G1, G2 and G3 positions had a positive relationship with spike grain weight. Among the studied Serbian wheat cultivars Subotičanka and Renesansa were identified as the most heat tolerant and sensitive, respectively. Heat tolerance of the studied cultivars should be based on the cultivar capacity to retain higher grain weight, and to maintain production of distal spikelet grains.

Keywords: cereal; drought; extreme heat; flowering; global warming; *Triticum aestivum* L.

The grain yield potential of small grain cereals is determined by the number of grains per unit area and grain weight, and changes in one or both of these components have a notable effect on final grain yield (Prado et al. 2017). Grain number could be shown as a result of different numerical subcomponents, including number of plants per unit area, spikes per plant, spikelets per spike and grains per spikelet. At the plant level, there is a hierarchical structure in grain yield formation, where grain number per unit area shows higher plasticity than grain weight. Also, due to a fine regulation in grain yield formation, an increase in one subcomponent is followed by reduction in the other ones (Slafer et al. 2014). A wheat spike comprises a variable number of spikelet (20–28), and each spikelet contains a several florets (1–5). Between different spikelets, grains varied regarding developmental stage, weight, number and fruiting efficiency (Ferrante et al. 2015).

During the past decades, there was a significant trend of temperature increase followed by more frequent extreme heat in Europe. Prediction of further global warming showed that incidents of heat waves and droughts would increase during wheat anthesis and grain filling period (Olesen et al. 2011), emphasising the importance of heat tolerance improvement. Under predicted climate conditions in Europe, heat stress, rather than drought, will increase vulnerability of wheat production (Semenov and Shewry 2011), as the probability of extreme heat events is likely to

increase at least by 30%, most affecting late-maturing cultivars (Trnka et al. 2014). The effect of increased temperatures on wheat development depends on timing, duration and level of heat stress (Wahid et al. 2007). In wheat, heat stress led to changes in Rubisco activity and photorespiration, photosynthetic capacity leaf senescence, water relations, ethylene production, grain growth and development and grain quality (Akter and Islam 2017). During the growing season, increased temperature and heat waves decrease wheat grain yield by reducing the grain number per unit area and grain weight (García et al. 2015), while the most negative influence of high temperature occurs during anthesis and grain filling (Arisnabarreta and Miralles 2008).

Although the influence of high temperature on grain weight and number is well documented, the effect of heat stress on grain distribution and weight of individual grains within spike and spikelets in wheat at different phenological stages was less investigated. The aim was to identify the effect of heat stress during different phenological stages on grain distribution and weight and to explore genotypic variation of the studied wheat cultivars. This study will not only help understand the changes in spike traits as response to different heat stress but will also provide valuable information for breeding heat tolerant wheat.

MATERIAL AND METHODS

The experiment was conducted at the Department of Food Science, Aarhus University, Denmark. The plant material consisted of ten wheat cultivars (Renesansa, Subotičanka, NS Javorka, Pobeda, Avangarda, Simonida, NS Obala, NS 40S, NS Mila and NS Rani otkos) from Serbia and two cultivars with known heat tolerance (Paragon – sensitive cultivar from UK and Gladius – tolerant cultivar from Australia). A completely randomised pot experiment with five pots/replications per treatment was conducted during the December 2018 to mid-June 2019 under greenhouse conditions combining natural and supplementary light ($160 \pm 20 \ \mu mol/m^2/s$ photosynthetic photon flux density), $48 \pm 5\%$ air relative humidity (RH) and average temperature of 25/22 °C for 16/8 day/night. Two seeds of the 12 cultivars were sown in plastic pots (9 cm height, 11 cm diameter) filled with a commercial peat based potting substrate (Pindstrup Færdigblanding 2, Pindstrup Mosebrug A/S, Ryomgaard, Denmark). Seedlings were thinned to one plant per pot when plants reached three fully developed leaves (BBCH 13),

approximately 14 days after sowing, and transferred to a cold chamber (4–6 °C, 8 h day length) for 6 weeks for vernalisation. Pots with vernalised seedlings were returned to glasshouse and grown until anthesis phase BBCH 65. The plants at anthesis were transferred to climate chambers (MB-Teknik) with 14 h photoperiod, photon flux density of 500 $\mu mol/m^2/s$ (LED FL300 Sunlight, Fionia Lighting), 65% RH, and 400 ppm CO_2 concentration. The day length was set from 5:00 to 19:00 in both chambers. Plants were watered with a nutrient solution (190 ppm N; 35 ppm P; 275 ppm K, pH 6.0) three times per day.

Different temperature regimes were established with five replicates per treatment. The four treatments included (1) control treatment (Con), 16/18 °C (day/night temperature); (2) heat stress at anthesis (H-A), 35/25 °C for seven days; (3) heat stress at mid-grain filling (H-GF) (14 days after anthesis), 38/28 °C for seven days; (4) combined heat stress treatment (H-A + GF), 35/25 °C for seven days at anthesis and 38/28 °C for seven days at mid-grain filling. After heat stress treatments, all replicates were moved back to the glasshouse and grown until the ripening stage.

Spikes were harvested individually from each pot and placed in paper bags. Thousand-grain weight (calculated as the ratio of the spike grain weight and grain number per spike), grain weight per spike and the number of grains per main spike were determined for all 12 cultivars. Based on the thousand-grain weight, grain weight per spike and the number of grains per main spike, the most sensitive Serbian cv. Renesansa, the most tolerant cv. Subotičanka and the two cultivars with known stress tolerance were selected to describe changes in individual weight of the first three grains within spikelet. To determine the distribution of individual grain weight across different grain positions, the main spike per plants were selected at maturity. Spikelet and grain position are shown in a schematic diagram (Figure 1). From the spike, each spikelet was separated from the base to the tip of the spike. Each grain within the spikelet was identified, numbered from most basal (G1) to the most distal position (G4) and weighed separately after drying for two days at 60 °C.

Differences in main spike and grain traits were analysed with a two-way analysis of variance (ANOVA) using a generalised linear model along with treatments, cultivars replications within treatments, and their interactions were applied. All factors were treated as random effects. One-way ANOVA was used to analyse differences between treatments for
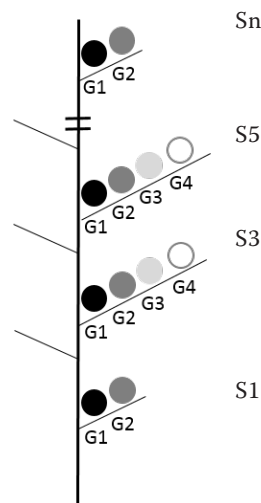
Figure 1. Schematic diagram showing positions of individual grains in different spikelets; S – spikelet; G – grain; 1 is the most basal position; adapted from Acreche and Slafer (2006)

each cultivar, and differences between cultivars for each individual heat treatment. Differences among means were tested using the Tukey's test ($P < 0.05$). Traits associations were analysed by correlation analysis using the Infostat (Di Renzo et al. 2011).

RESULTS AND DISCUSSION

Spike grain weight, grain number per spike and thousand-grain weight. Table 1 showed a significant influence of the cultivar (C), treatment (T) and cultivar by treatment (C × T) interaction on spike grain weight (SGW), grain number per spike (GNS) and thousand-grain weight (TGW). Compared across treatments, SGW, TGW and GNS showed a general pattern with the highest values in the control, moderate reduction in treatments with heat stress at anthesis and mid-grain filling period and the highest reduction in heat stressed plants at both stages.

Table 1. Average spike grain weight (SGW), grain number per spike (GNS) and thousand-grain weight (TGW) under control conditions (Con), heat stress at anthesis (H-A), heat stress at mid grain filling (H-GF) and heat stress at anthesis and mid grain filling (H-A + GF)

Cultivar	SGW (g)				GNS				TGW (g)			
	Con	H-A	H-A + GF	H-GF	Con	H-A	H-A + GF	H-GF	Con	H-A	H-A + GF	H-GF
Renesansa	1.92^{deA}	0.93^{eBC}	0.83^{c-eC}	1.09^{eB}	46.0^{fA}	42.4^{c-eB}	39.6^{cdC}	44.2^{deB}	41.8^{abA}	21.9^{dB}	21.0^{cC}	24.6^{eB}
Subotičanka	1.69^{efA}	1.36^{b-dB}	1.04^{a-cC}	1.26^{deBC}	49.2^{efA}	39.0^{eB}	39.0^{c-eB}	42.8^{eB}	34.8^{b-dA}	34.9^{abA}	26.8^{abB}	29.5^{b-eB}
NS Javorka	1.50^{fA}	1.23^{deB}	0.68^{deC}	1.05^{eB}	44.4^{fA}	33.6^{fB}	35.8^{deB}	41.2^{eB}	33.8^{cdA}	36.6^{aA}	19.0^{cC}	25.5^{deB}
Pobeda	2.30^{bcA}	1.61^{abcB}	1.04^{a-cD}	1.40^{cdC}	49.4^{efA}	45.2^{bcB}	37.2^{deC}	40.4^{eC}	46.6^{aA}	35.6^{aB}	28.0^{aC}	34.8^{abB}
Avangarda	2.44^{abA}	1.75^{aB}	1.14^{aC}	1.57^{bcB}	60.0^{bcA}	53.4^{aB}	48.8^{aC}	55.4^{a-cB}	40.7^{abcA}	32.8^{abB}	23.3^{a-cC}	28.4^{c-eCB}
Simonida	2.35^{abcA}	1.62^{abB}	0.96^{a-cC}	1.74^{bB}	57.6^{cdA}	52.2^{aA}	43.4^{bcB}	55.0^{a-cA}	40.9^{a-cA}	31.1^{abcB}	22.3^{bcC}	31.6^{a-cB}
NS Obala	2.26^{bcA}	1.61^{a-cB}	0.97^{a-cD}	1.37^{cdC}	56.6^{cdA}	48.8^{abBC}	46.6^{abC}	50.2^{cdB}	39.9^{a-cA}	33.0^{abB}	21.0^{cD}	27.3^{c-eC}
NS 40S	2.41^{abA}	1.80^{aB}	1.07^{abC}	1.72^{bB}	53.8^{deA}	52.8^{aA}	46.0^{abB}	53.4^{a-cA}	44.8^{aA}	34.1^{abB}	23.5^{a-cC}	32.3^{a-cB}
NS Mila	2.64^{aA}	1.69^{aC}	0.96^{abcD}	2.04^{aB}	57.8^{cdA}	48.8^{abB}	43.4^{bcC}	57.6^{abA}	45.7^{aA}	34.6^{abB}	22.2^{bcC}	35.5^{aB}
NS Rani otkos	1.93^{deA}	1.31^{cdB}	0.92^{abcC}	1.56^{bcB}	64.2^{bA}	45.4^{bcC}	46.0^{abC}	52.2^{bcB}	30.1^{deA}	28.9^{bcA}	20.0^{cB}	29.9^{a-eA}
Paragon	2.03^{cdA}	1.14^{deB}	0.64^{eC}	1.03^{eB}	83.2^{aA}	45.0^{b-dC}	34.8^{eD}	59.4^{aB}	24.5^{eA}	25.2^{cdA}	18.4^{cC}	17.4^{fC}
Gladius	1.76^{d-fA}	1.27^{dB}	0.87^{b-dC}	1.27^{deB}	44.0^{fA}	39.8^{deB}	38.8^{c-eB}	41.6^{eB}	40.1^{a-cA}	31.9^{abB}	22.4^{bcC}	30.5^{a-dB}
Average	2.10^{A}	1.44^{B}	0.93^{C}	1.42^{B}	55.5^{A}	45.5^{C}	41.6^{D}	49.5^{B}	38.7^{A}	31.7^{B}	22.3^{D}	28.9^{C}

Analysis of variance

	SW		SGN		TGW	
	df	P-value	df	P-value	df	P-value
Cultivar (C)	11	< 0.01	11	< 0.01	11	< 0.01
Treatment (T)	3	< 0.01	3	< 0.01	3	< 0.01
C × T	33	< 0.01	33	< 0.01	33	< 0.01

Differences between cultivars were analysed using the Tukey's test. Different lower case letters within column denote statistically significant differences ($P < 0.05$) between cultivars. Different upper case letters indicate statistically significant differences ($P < 0.05$) between control and heat treatments

Compared to the control, SGW was reduced by 31% across cultivars in H-A treatment, 32% in H-GF treatment and 56% in H-A + GF treatment (Table 1). High temperature during anthesis and grain filling stage decreased both grain number and grain weight (Pradhan et al. 2012), resulting in the SGW reduction. The cultivars Paragon (44%) and Renesansa (52%) showed the largest reduction in SGW in H-A, while the lowest response to H-A treatment was observed in cvs. Subotičanka (20%) and NS Javorka (18%). The effect of H-GF treatment notably varied between cultivars with the highest reduction in cv. Paragon (48%) and the smallest reduction in cv. NS Rani otkos (19%). The values of SGW strongly decreased in all cultivars at H-A + GF treatment with variation between 38% (cv. Subotičanka) and 68% (cv. Paragon).

A decrease in GNS was 18% for H-A, 11% for H-GF and 25% for H-A + GF. Heat stress at anthesis resulted in florets sterility and grain abortion, while post-anthesis heat stress led to grain yield abortion in wheat (Barlow et al. 2015). Under H-A treatment, the highest decrease of GNS was observed in cv. Paragon (46%), while the lowest reduction of GNS was shown in cv. Gladius (10%). Among Serbian cultivars, reduction in GNS varied between 2% and 29% under H-A treatment. Heat stress at mid-grain filling period did not result in significant changes in GNS in cvs. Simonida, NS Mila, NS 40S, whereas the highest response in GNS was recorded in cv. Paragon. The combined heat stress treatments at both stages (H-A + GF), decreased GNS from 12% in cv. Gladius to 58% in cv. Paragon. Similarly, Mahrookashani et al. (2017) reported notable genotypic variation (14–28%) in grain number under heat conditions.

Furthermore, TGW declined by 18, 25 and 42% in the H-A, H-GF and H-A + GF treatments, respectively. Reduction in GW due to temperature increase during grain filling period was found to be mainly as a result of decrease in leaf chlorophyll content and maximum quantum yield (Pradhan et al. 2012), grain filling duration and a stay-green ability (Shirdelmoghanloo et al. 2016). Moreover, a temperature increase during pre-anthesis and early grain filling decreased GW due to high temperature negative effect on source of assimilates, growing florets and early grain development (Ugarte et al. 2007, Talukder et al. 2014). Cultivar Pobeda was characterised by the highest TGW (46.6 mg) under control conditions. The cultivars had different response to high temperature during anthesis. No reduction in grain weight was observed in one third of the cultivars (Subotičanka, Javorka, NS Rani otkos and Paragon), while the highest reduction was recorded in cv. Renesansa. The heat stress during the mid-grain filling period decreased TGW from 1% (cv. NS Rani otkos) to 41% (cv. Renesansa). Notably, the most negative effect was observed under conditions of combined heat stress. Under H-A + GF, the highest reduction was reported in cv. NS Mila, while cv. Subotičanka showed the lowest TGW decrease. The reduction of GW due to heat stress was in accordance with Yang et al. (2002) who observed a decrease of GW from 15.6 mg to 35.3 mg.

Moreover, according to the Table 1, the cultivars with higher GNS and low TGW under control conditions showed higher GNS and lower TGW reduction due to heat stress than the cultivars with low GNS and high TGW. Increased GNS in the studied cultivars is mainly a result of higher number of fertile spikelets and distal grains per spikelet. The effect of high temperature led to decrease in the number of distal grains (G3) and basal and proximal spikeletes (Savin et al. 1999), resulting in higher reduction in cultivars with higher GNS. On the other hand, the cultivars with higher GNS had lower TGW and under heat stress, these cultivars mainly lost distal grains characterised by the lowest grain weight, resulting in lower final TGW reduction.

Changes in single grain traits and relationship to SGW. Analysis of the average individual grain weight across all spikelet positions and within each of the spikelet showed a clear pattern of changes in individual weight (Figure 2). Weight of G1 and G2 was consistently heavier in all treatments and spikelets than that of G3. Moreover, grains in central spikelets had higher weight than in apical or basal ones. In wheat, the middle spikelet tend to have more and heavier grains than the basal and top spikelet (Li et al. 2016)

Heat treatments decreased the number of spikelets with grains (mainly apical and basal), and the most negative influence was found under H-A + GF. The combined stress resulted in the lowest weight of individual grains at all spikelet positions. Tolerant cultivars Subotičanka and Gladius consistently had higher grain weight under H-A and H-GF treatments at the G1, G2, and G3 grain positions. Under the combined treatments, grain weight of cv. Gladius was close to cv. Renesansa, but still higher than in cv. Paragon. Efficient carbohydrates flow to the florets during floral development and grain filling period is necessary to provide grain development and growth. Abortion of grains in distal position of the spike and spikelet due to H-GF and H-A + GF treatment could
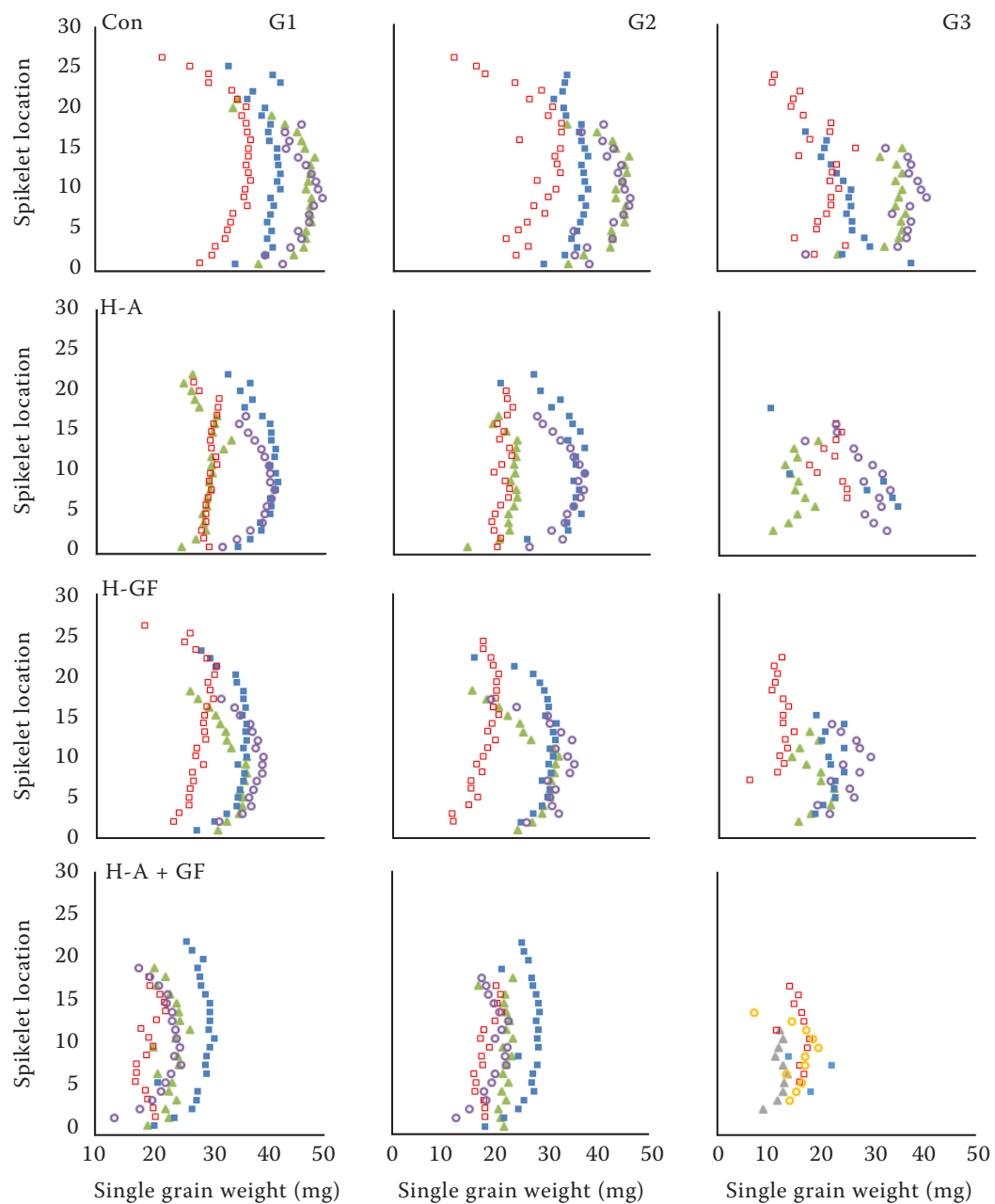
Figure 2. Average grain weight at G1 (the first column); G2 (the second column) and G3 (the third column) of four wheat cultivars (Renesansa – closed green triangle; Subotičanka – closed blue rectangle; Paragon – open red rectangle; Gladius – open purple circle) grown under control (the first row), heat stress at anthesis (the second row), heat stress at mid-grain filling period (the third row) and heat stress at anthesis and mid-grain filling period (the fourth row)

be a result of the restricted assimilate transportation (Arisnabarreta and Miralles 2008).

Grain weight at G1, G2 and G3 positions had a significant relationship with SGW under all treatments (Table 2). Moreover, NG at G3 under H-A, G2 under H-A + GF and G3 under H-GF was positively related with SGW. Therefore, heat tolerance of the cultivars should be based on the cultivar capacity to retain higher grain weight of G1, G2 and G3, and to maintain grains of distal spikelets (G3).

In conclusion, the results showed a significant effect of H-A and H-GF on the analysed grain, and a significant variation in cultivars reaction to heat stress. H-A was more related to decrease in GNS,

Table 2. Pearson correlations between spike grain weight (SGW) with grain number per spike (NGS), grain weight (GW) and grain number (NG) at G1, G2 and G3, and thousand-grain weight (TGW) under control conditions (Con), heat stress at anthesis (H-A), heat stress at mid-grain filling (H-GF) and heat stress at anthesis and mid grain filling (H-A + GF)

Trait	Treatment	NGS	GW1	GW2	GW3	NG1	NG2	NG3	TGW
SGW	Con	0.32	0.63*	0.66*	0.72**	0.12	0.17	0.23	0.57
	H-A	0.73**	0.68*	0.68*	0.81**	0.38	0.39	0.63*	0.65*
	H-A + GF	0.70*	0.78**	0.84**	0.70*	0.34	0.59*	0.40	0.75**
	H-GF	0.53	0.85**	0.79**	0.78*	0.18	0.40	0.58*	0.76**

*$P < 0.05$; **$P < 0.01$.

while TGW reduction was more pronounced under heat stress at H-GF. Based on the spike traits, cultivars Subotičanka and Renesansa were the most heat tolerant and sensitive, respectively. A negative effect of multiple heat stress at anthesis and mid-grain filling stage was stronger than at single stages, although their degree varied both across cultivars and analysed spikes.

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