FINAL PROJECT REPORT

High temperature is one of the main abiotic stress factors occurring in increasingly greater frequencies in Hungary, which have gained growing attention in the last years due to economic-related problems. In the present work, special care has been taken in the research of high temperatures because this stress factor alone can cause significant damages to crop safety and yield. For maintaining food security, it is necessary to increase the heat tolerance capacity in wheat. Therefore, the specific aim of this research project was to identify different heat tolerance sources and to create genetic populations, in which the high heat tolerance properties are incorporated. These populations are the basis to develop new varieties with good adaptability and performances under heat stress conditions.

Topic 1. *Studies for comparative investigation of the effect of different duration of heat stress in various developmental stages of wheat varieties with different genetic origin.*

One hundred wheat varieties of different genetic background were involved in phytotron experiments under controlled growth conditions in order to investigate the heat stress response of the genotypes. The effect of the duration of heat stress in various developmental stages were examined in three independent experiments in which the same standard plant rising protocol were applied. The three developmental phases studied were the booting stage (**ZD49**), the heading stage (**ZD59**) and early milk developmental stage (the 6th day after heading) (**ZD72**). Various lengths of heat stress treatments (36°C) were applied lasting for 5 (H5), 10 (H10) or 15 (H15) days in all three phenophases. Every plant received heat stress treatment in the same specific developmental stages examined within the given experiment. The plants were raised till the given phenophase in the greenhouse, the application of the heat stress were carried out in the growth chambers of the phytotron (Conviron PGV-36). After the heat stress treatment, the plants were returned to the greenhouse and raised with the control plants till maturity. The effects of heat stress treatments were examined on the same sets of morphological properties and yield related components in all the three experiments. Physiological properties such as determination of chlorophyll content, photosynthetic efficiency were also measured, on a single occasion after heat stress treatment in each experiment.

Results of the different duration of heat stress experiments in wheat

The experiments extended the determination of the development and the examination of some physiological parameters and yield components of the winter wheat varieties under control and stressed conditions. Different heat tolerance sources of the wheat varieties of different genetic origin were identified across the three heat stress experiments. This research provided the basis for the article which was published in PLOS ONE in 2019 (K. Balla, I. Karsai, P. Bónis, T. Kiss, Z. Berki, Á. Horváth, M. Mayer, Sz. Bencze, O. Veisz (2019): Heat stress responses in a large set of winter wheat cultivars (*Triticum aestivum* L.) depend on the timing and duration of stress. PLOS ONE 14 (9): e0222639. IF: 2.74 (2019) https://doi.org/10.1371/journal.pone.0222639). Since 2019, 34 independent citations have been received for this article.

The results showed that the phenological timing of heat significantly influenced the thousand-kernel weight and reproductive tiller number. The duration of heat stress was the most significant component in determining both seed number and seed weight, as well as the grain yield consequently, explaining 51.6% of its phenotypic variance. Irrespective to the developmental phase, the yield-related traits gradually deteriorated with the increasing duration of heat, but even a 5-day heat stress was sufficient to cause significant reductions. ZD59 was significantly more sensitive to heat than either ZD49 or ZD72. The photosynthetic activity of the flag leaf was mostly determined by heat stress duration. No significant associations were noted between physiological parameters and heat stress response as measured by grain yield.

Significant differences were observed between the wheat genotypes in heat stress responses, which varied greatly with developmental phase (Figure 1.). As grain yield is the strongest and final indicator of stress tolerance, we run various multi-variate analyses on the data matrix of all 12 treatments and 101 genotypes, in order to evaluate the heat-stress reactions of the wheat genotypes. We have created a heat map (Figure 1.), which showed the grain yield average per plant. This heat map expressed the difference between the individual genotype and the main grain yield average of each treatment. (The rows indicated the individual varieties. The columns indicated the heat stress treatments and developmental stages). Based on the cluster analysis, we could identify eight clusters of wheat genotype (at 32% of the largest distance on the dendrogram). Different numbers of varieties belonged to the eight clusters. Clusters 1, 3 and 4, which had the lowest numbers of members (5, 5, and 9), represented the most extreme groups in terms of the yield formation. The only exception was Cluster 7, which was the largest group with 37 genotypes. Its members showed greater dispersion in their grain yield under longer heat stress periods at ZD59. Strong associations between the heat stress responses and the geographic origin of wheat genotypes was not found. The majority of Cluster 1 and Cluster 3 were of west-European region (origin), while most of the cultivars in Cluster 4 came from China and Southern Europe. Based on the heat map of grain yield, Clusters 1, 2, and 3 were the best groups across all the control and heat stress treatments. In contrast, the members of Cluster 4 gave the lowest grain yield irrespective of the treatment. This followed by Cluster 5, while Clusters 6, 7 and 8 were intermediate in their reactions.

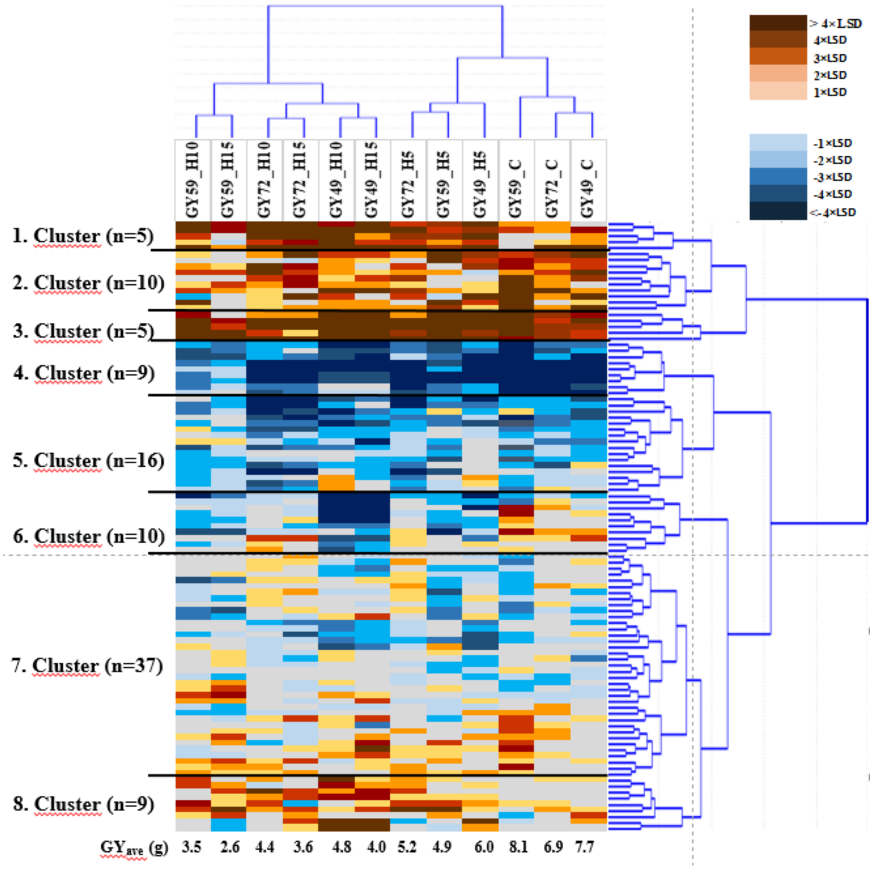


Figure 1. Heat map of average grain yield/plant (g) across whet genotypes and treatments. The main GY average of each treatment is represented at the bottom of each column. *C: Control, H5-H10-H15: Heat stress lasting for 5, 10 and 15 days, (Zadoks) 49: Booting stage, 59: Heading stage, 72: Early milk development.*

In addition to the overall reaction patterns for grain yield, treatment-specific differences were noted between the responses of the various clusters (Figure 2.), which could best be visualised as the average yield reduction expressed as a % of the average control values for each cluster. The results of Figure 2. showed the heat sensitivity of the individual clusters in the different developmental phases, independently of their productivity. Of the three best-yielding clusters (Cluster 1, 2, and 3), the sensitivity of Cluster 2 was always the greatest and was more pronounced in the two earlier developmental phases. At ZD49, Cluster 3 proved to be the most tolerant of heat stress, whereas Cluster 1 gave better results at ZD72. Of the three intermediate clusters (Cluster 6, 7, and 8), Cluster 8 was the best in all three developmental phases, whereas Cluster 6 showed the greatest sensitivity to heat in the two earlier developmental phases (ZD49 and ZD59). The heat sensitivity of the lowest-yielding Cluster 4 was intermediate for the early and late developmental phases but was among the best at ZD59. However, the heat sensitivity of Cluster 5 increased in later developmental phases, as a result of which this group became the most sensitive at ZD72.

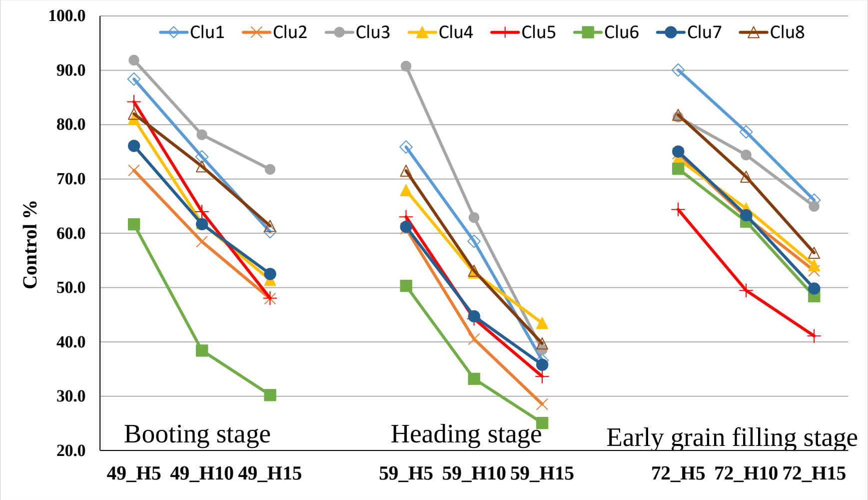


Figure 2. Treatment specific changes of various clusters. Changes in grain yield in % on the control – in different phenophases – under various heat stress treatments *(H5-H10-H15: Heat stress lasting for 5, 10 and 15 days).*

Topic 2. *Studying the effect of single and repeated heat stress on the yield related components on winter wheat varieties. Studying the possibility that the thermo-tolerance capacity of wheat plants can be improved by heat priming.*

Topic 2. of the project consisted of two independent experiments. A total of 51 winter wheat varieties with different geographic origins were included in a series of experiments under controlled conditions in the greenhouse and phytotron to study their responses under single and repeated heat stress conditions applied at different developmental stages (ZD-31: stem elongation, ZD-49: booting, ZD-59: full heading). The plant raising protocol was the same in the two experiments. In Experiment I., a single heat stress event was applied at stem elongation (**SE:** 28°C, 10 days of treatment) and booting (**B:** 36°C, 10 days of treatment), and the repeated heat stress was applied at both of these stages (**SE+B:** 28°C+36°C, 5+5 days of treatment). In Experiment II., the single heat stress was applied at stem elongation (**SE:** 28°C, 10 days of treatment) and full heading (**CH:** 36°C, 10 days of treatment), while the repeated heat stress was applied at both stages (**SE+CH:** 28°C+36°C, 5+5 days of treatment). Temperature was between 19-25°C in the greenhouse under control conditions. The plants were raised till the given phenophases in the greenhouse, the application of the heat stress were carried out in the growth chambers of the phytotron (Conviron PGV-36). After the heat stress treatment, the plants were returned to the greenhouse and raised with the control plants till maturity. The effects of single vs. repeated heat stresses were examined by measuring the changes in morphological and grain yield-related traits and photosynthetic parameters.

Results of the single versus repeated heat stress experiments in wheat

Physiological processes and agronomical traits of wheat plants were determined on the effect of single vs. repeated heat stresses at different developmental phases in two independent experiments. With a possible reference to heat priming and to characterize the extent and variation in the heat stress responses, differences could be identified among the examined 51 selected wheat varieties. This research provided the basis for the article which was published in PLOS ONE in 2021 (K. Balla, I. Karsai, T. Kiss, Á. Horváth, Z. Berki, A. Cseh, P. Bónis, T. Árendás, O. Veisz (2021): Single versus repeated heat stress in wheat: What are the consequences in different developmental phases? PLoS ONE 16 (5): e0252070. IF: 3.240 (2021) https://doi.org/10.1371/journal.pone.0252070).

The results showed that the genotypes were more important factors than the heat stress for determining the morphological and yield-related traits, while the photosynthetic parameters was mostly influenced by the treatment effect (with the exception of the chlorophyll content). The temperature stress of 28^{o}C caused the least damages to the plants at stem elongation stage. The heading stage was more sensitive to heat stress than the booting stage, which was primarily due to the larger decrease in the average seed number (AS). The importance of biomass in contributing to grain yield intensified with the heat stress treatments.

Averaged over the genotypes, the effects of repeated heat stress were closer to those of the control and the single heat stress at stem elongation than to the single stresses at the two later phenophases, but beyond this result, significant differences in the various magnitudes and directions could be detected across the different traits, phenophases and wheat genotypes.

Averaged over the wheat genotypes, the highest effect of repeated heat stress was detected mainly on average seed number (AS), the harvest index (HI), thousand kernel weight (TKW) and grain yield (GY), while this stress caused the smallest changes in morphological properties, such as plant height (PH) and biomass (FBIOM). In single stress treatments, the large reduction in AS was compensated by increased TKW, and this compensating mechanism was apparent in the repeated stresses as well.

The possible priming effect of heat applied at the stem elongation stage prevailed in both experiments and was clearly visible in the case of the double heat stress treatments (SE+B and SE+CH). If we compare B with SE+B (Exp. I.) and CH with SE+CH (Exp. II.), the differences achieved due to the heat priming can be clearly seen: Exp. I.: GY: +17.8%, AS: +22%, and HI: +13.3%; Exp. II.: GY: +9.5%, AS: +20.5%, and HI: +14.3%.

The results of the photosynthetic activity showed that net assimilation (PN) and transpiration (E) could be intensified in plants stressed at later developmental phases when they were also subjected to early heat stress at the stem elongation stage, which could be attributed to the greater capacities of photosynthesis maintenance under heat stress. A possible priming effect due to heat stress applied at the stem elongation stage could be detected for PN and E. In the case of PN, the extent of the increase in response to repeated heat stress was +13.6% at SE+B and +4.3% at SE+CH compared to the B and CH stages, respectively, while for E, these values were +16.2% at SE+B and +2.6% at SE+CH.

The correlation analysis showed that weak correlations were obtained between the physiological and production biological properties in response to different heat stress treatments.

There was a large variation between the wheat cultivars not only in yielding abilities under control conditions but also in sensitivities to the various heat stresses, based on which 7 distinct groups with specific response profiles could be identified at a highly significant level. The majority of these 7 clusters formed separate groups in the scatter plot (Figure 3.), especially Cluster 2, 4, 6 and 7. There was only a slight overlap between Cluster 1 and 3. The 7 wheat groups were also characterized by their reaction patterns of different magnitudes and directions in their responses to single vs. repeated heat stresses, which depended on the phenological phases during the second cycle of heat stress. The most characteristic members of each group are listed in Table 1.

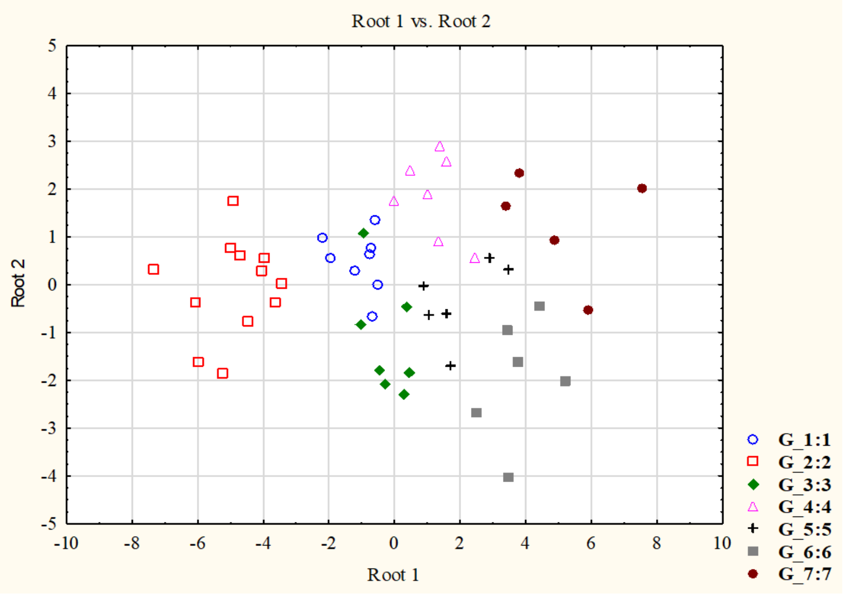


Figure 3. Discriminant analysis of the seven phenotypic clusters of 51 wheat genotypes. The clusters were established by K-means clustering of the grain yield data matrix of Exp. I and II. under heat stress treatments.

Table 1. The most characteristic members of the seven phenotypic wheat groups identified via K-means clustering and discriminant analyses based on their grain yield profiles.

Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**	**Group 7**
(n= 8)	(n= 12)	(n= 7)	(n= 7)	(n= 6)	(n= 6)	(n=5)
ave dist= 0.601	ave dist= 0.785	ave dist= 0.768	ave dist= 0.597	ave dist= 0.666	ave dist= 0.809	ave dist= 0.830
Divana (HR)	Feng-you-3 (CN)	Disponent (DE)	Briana (RO)	Bastide (FR)	GK-Göncöl (HU)	KWS-Scirocco (DE)
Turkmen (TR)	Mv-Amanda (HU)	Cutter (US)	Soissons (FR)	Hallam (US)	Balada (CZ)	GK-Hattyú (HU)
Ravenna (IT)	Blasco (IT)	Buratino (CZ)	Libellula (IT)	Altay-2000 (TR)	Agent (US)	Dumbrava (RO)
Buck-Panadero (AR)	Mv-Verbunos (HU)	Klein-Flecha (AR)			Lupus (AT)	Cadenza (GB)
	Chara (AU)					
	Mv-Palotás (HU)					

Only in Cluster 1 was the heat stress tolerance significantly enhanced in both later developmental phases as a result of the high temperature pretreatment (Figure 4.). Clusters 2, 5, and 6 responded positively to the high temperature pretreatment only in the case of heat stress applied at the booting stage (SE+B), but this stimulating effect was much smaller (Clusters 5 and 6) or not significant (Cluster 2) at full heading. In contrast, for Groups 4 and 7, the stimulating effect of the high temperature pretreatment was only significant when it occurred before full heading (SE+CH). The wheat genotypes in Cluster 4 showed the strongest enhanced heat stress tolerance among all the groups; plants pretreated with high temperatures at stem elongation showed a 90% increase in grain yield at full heading (SE+CH). Cluster 3 was the only cluster that did not respond positively to the pretreatment in either stage.

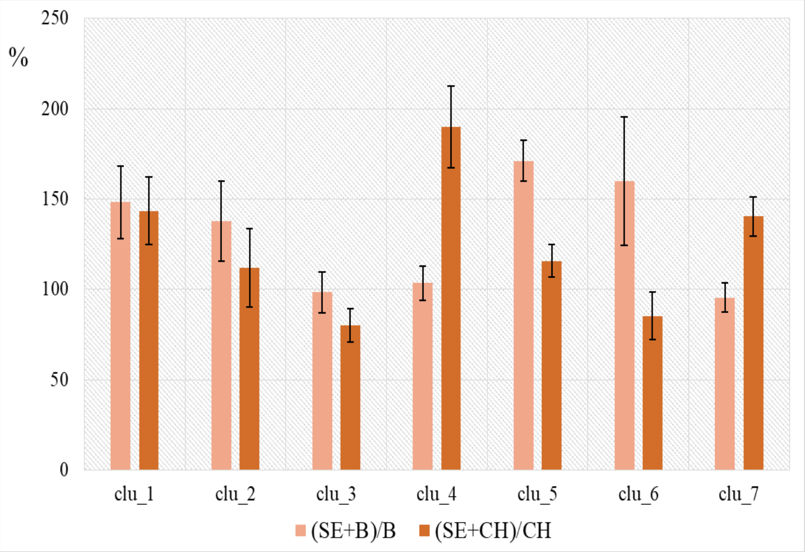


Figure 4. Grain yield profiles of the seven phenotypic clusters of 51 wheat genotypes measured by the effect of repeated heat stress to the single heat stress treatment in two developmental phases expressed as % of change.

B (single heat stress at the booting stage) and SE+B (repeated heat stress at the stem elongation and booting stages) from Exp. I.; and CH (single heat stress at full heading) and SE+CH (repeated heat stress at stem elongation and full heading) from Exp. II.

Our results achieved in a larger number of wheat genotypes with various geographical origins and ecological adaptation backgrounds may provide evidence that the phenomenon of heat priming exists; it is not a universal response of wheat but depends strongly on genotypes and developmental phases, in addition to the various parameters of the stress itself. Further studies are necessary to establish the conditions that may initiate heat priming and to identify their possible genetic backgrounds before practical applications in breeding can be considered realistic.

Topic 3.

Task 3.1. *Genetic analysis of heat stress response and association analysis between the morphological and physiologic properties.*

The results of the genetic examinations

The genome wide association (GWA) analyses were prepared between the LD map and the phenotypic data matrix. For the genetic analysis the LD map of the 100 wheat cultivars group was already available, consisting of 7853 SNP markers throughout the wheat genome. Together with controls, 25 morphological, yield related components and physiological properties were analysed in 12 different treatments, determining significant marker-trait associations (MTAs).

The number of markers with significant associations at least in one treatment and in one trait was 1074, which was the 13.7% of all SNP markers.

Significant SNP markers were clustered into 32 chromosome regions of the length of 10-30 cM, the roles of which were variable depending on the examined properties and the applied heat stress treatments (Figure 5.). We identified regions that played a role in defining a property independently of treatment. Thus, the 40-60 cM region of chromosome 2B contributed substantially to the determination of plant height, last internode length and number of spikelet per ear, but its effect on other traits was also enhanced in the case of heat stress in late phenophase (ZD-72).

The 50cM region of 4D, the 10-27 cm region of 2B and the 110-120 cM region of 6D played roles in the determination of biomass production. The significant effect of other chromosome regions manifested in the heat stress treatments, but not at all developmental phase. The 0-10 cM region of 7B influenced the grain yield in booting stage due to heat stress, primarily through the determination of grain yield of side ears. The 50-75cM region of 2D became significant under the heat stress in early grain filling stage (ZD-72), defining several yield related components and thus grain yield.

20 SNP markers were identified in the entire genome that had more than 10 marker-trait associations (Figure 5.). None of these was in complete linkage with each other (Figure 6.). The 5D160 (24), 2B48 (21), and 6A7 (20) SNP markers had the highest MTA numbers.

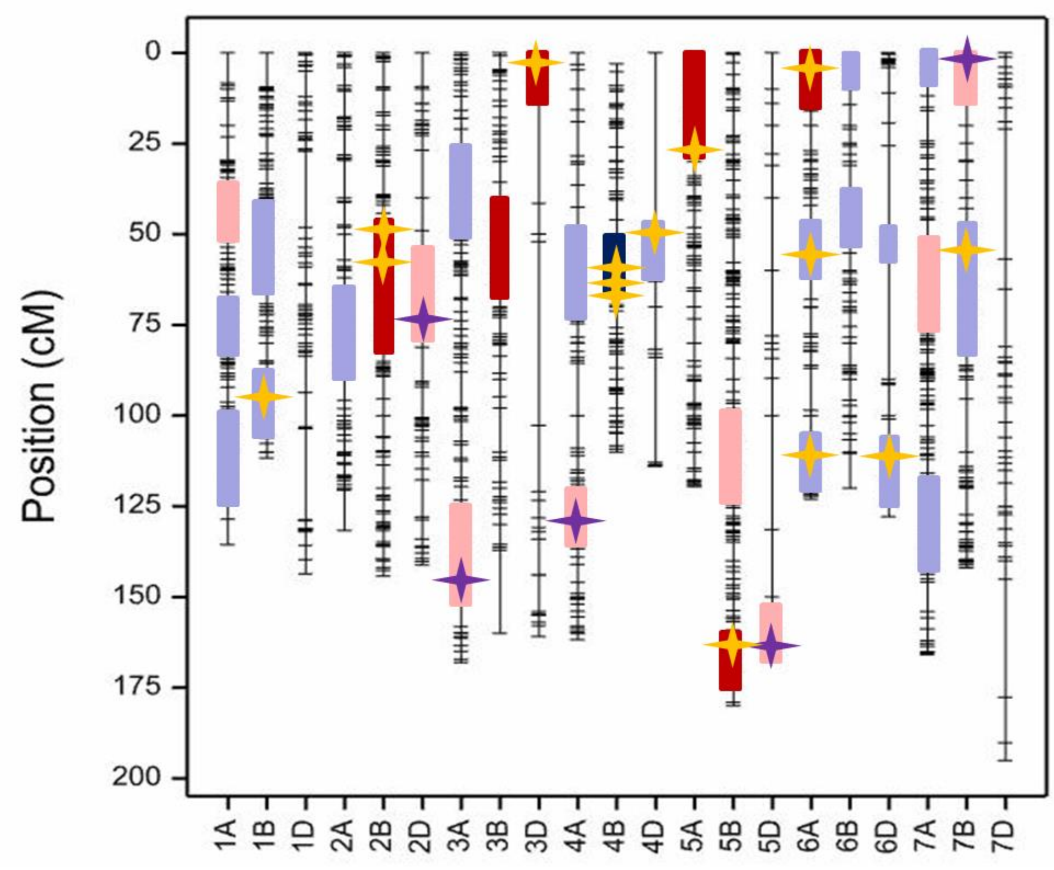
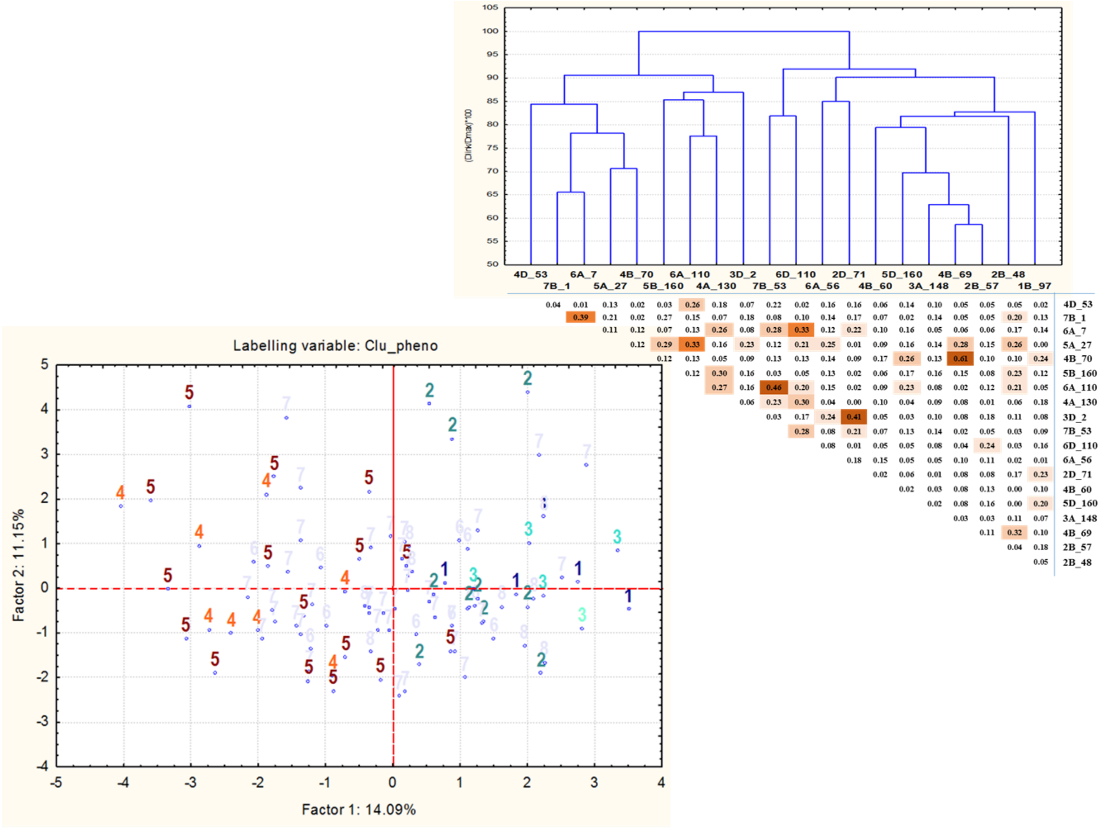


Figure 5. Significant chromosome region – trait associations identified in the genome wide analyses of 25 morphological, physiological and grain yield related traits in the control and heat stress treatments (legends: light rectangles less than 5 traits, dark rectangles more than 5 traits; pink – red included grain yield related traits as well. Asterisks: the 20 most significant SNPs showing associations with more than 10 traits)

We carried out Principal component analysis (PCoA) on the data matrix of the marker allele compositions of 100 wheat cultivars in the 20 most significant SNP markers, which resulted in a diffuse scattering of cultivars. When, however the cultivars were labelled by their phenotypic cluster positions established in Topic 1, the three best yielding and more heat stress tolerant clusters (Clusters 1, 2 and 3) were distinctly differentiated from the lowest yielding and sensitive groups (Clusters 4 and 5) (Figure 6.).



7B_1	6A_7	5A_27	4B_70	5B_160	6A_110	4A_130	3D_2	7B_53	6D_110	6A_56	2D_71	4B_60	5D_160	3A_148	4B_69	2B_57	2B_48	1B_97	
0.04	0.01	0.13	0.02	0.03	0.26	0.18	0.07	0.22	0.02	0.16	0.16	0.06	0.14	0.10	0.05	0.05	0.05	0.02	4D_53
	0.39	0.21	0.02	0.27	0.15	0.07	0.18	0.08	0.10	0.14	0.17	0.07	0.02	0.14	0.05	0.05	0.20	0.13	7B_1
		0.11	0.12	0.07	0.13	0.26	0.08	0.28	0.33	0.12	0.22	0.10	0.16	0.05	0.06	0.06	0.17	0.14	6A_7
			0.12	0.29	0.33	0.16	0.23	0.12	0.21	0.25	0.01	0.09	0.16	0.14	0.28	0.15	0.26	0.00	5A_27
				0.12	0.13	0.05	0.09	0.13	0.13	0.14	0.09	0.17	0.26	0.13	0.61	0.10	0.10	0.24	4B_70
					0.12	0.30	0.16	0.03	0.05	0.13	0.02	0.06	0.17	0.16	0.15	0.08	0.23	0.12	5B_160
						0.27	0.16	0.46	0.20	0.15	0.02	0.09	0.23	0.08	0.02	0.12	0.21	0.05	6A_110
							0.06	0.23	0.30	0.04	0.00	0.10	0.04	0.09	0.08	0.01	0.06	0.18	4A_130
								0.03	0.17	0.24	0.41	0.05	0.03	0.10	0.08	0.18	0.11	0.08	3D_2
									0.28	0.08	0.21	0.07	0.13	0.14	0.02	0.05	0.03	0.09	7B_53
										0.08	0.01	0.05	0.05	0.08	0.04	0.24	0.03	0.16	6D_110
											0.18	0.15	0.05	0.05	0.10	0.11	0.02	0.01	6A_56
												0.02	0.06	0.01	0.08	0.08	0.17	0.23	2D_71
													0.02	0.03	0.08	0.13	0.00	0.10	4B_60
														0.02	0.08	0.16	0.00	0.20	5D_160
															0.03	0.03	0.11	0.07	3A_148
																0.11	0.32	0.10	4B_69
																	0.04	0.18	2B_57
																		0.05	2B_48

Figure 6. Linkage relationships between the 20 most significant SNP markers (above) and their grouping power via PCoA across 100 wheat cultivars (bellow) (Legends: In the PCoA matrix wheat genotypes are labelled by their phenotypic cluster numbers identified in Balla et al. (2019)).

Using multiple regression analysis, the probable weight of these 20 SNPs in determining grain yield under control and heat stress conditions was also established (Figure 7.). These analyses proved, that the allele compositions in the 20 SNP markers explained between 39 and 63% of the phenotypic variations in grain yield, depending on the treatment. The model was the strongest for ZD49, followed by ZD72, and the weakest for ZD59. While in the three control experiments the roles of 7B_1, 7B_53, 5B_160 and 1B_97 SNPs were the most pronounced, the significant markers in heat stress treatments depended on the phenological stages in which heat stress was applied and to a lesser extent on the length of the heat stress. Thus at booting stage, 7B_1 SNP was the most important determinant of GY followed by 6A_7, while at heading these were 6A_7 and 5B_160, showing relatively similar weights across the different lengths of heat stress. In the case of ZD72, however there were some changes in the contributing SNPs with the increase of heat stress treatment, in addition to the permanently significant 2D_71 SNP. At 5 and 10-day heat stresses the additional roles of 4A_130, while at 15-day heat 6A_7 became evident.

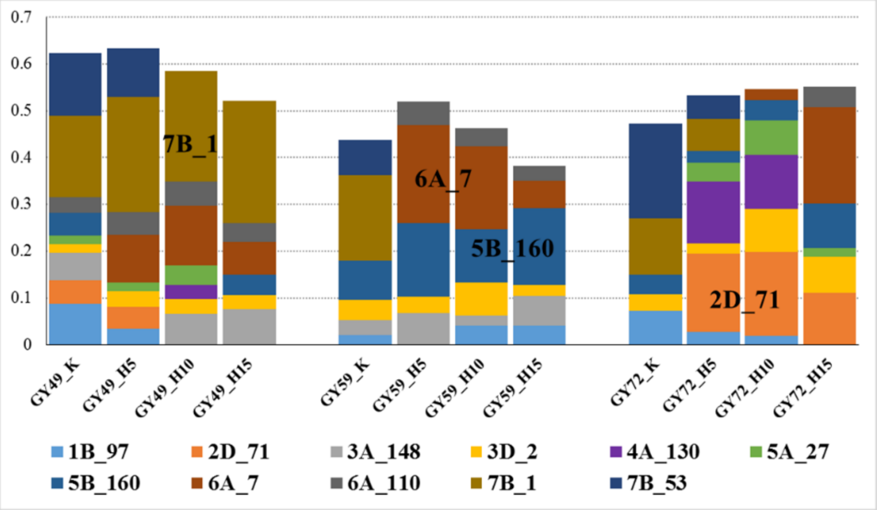


Figure 7. Stepwise multi-regression analyses of SNP markers in determining grain yield under the various control and heat stress treatments (y-axis is R^2 of each model explaining the phenotypic variance)

These results indicated that it is possible to use these 20 SNP markers for marker selections in order to identify heat stress tolerant, high yielding wheat genotypes with a greater probability. This hypothesis will be validated in the doubled haploid wheat population originated from F1 crosses between wheat cultivars of the various phenotypic clusters. A manuscript is under preparation on the GWA results of heat stress responses of the wheat cultivars.

Task 3.2. *Heat stress experiment with selected varieties for exploring the relationships between the morphological /physiological and heat tolerance performances.*

Eight winter wheat varieties (Mv Palotás: 'K6', Bayraktar: 'K55', Feng You-3: 'K57', Ellvis: 'K110', Tommi: 'K171', Mv Toborzó: 'K185', Mv Verbunkos: 'K188' and KWS Scirocco: 'K192') were selected based on the previous results under controlled environmental conditions and were subjected to more detailed physiological and analytical measurements. One group of the plants raised under control conditions throughout the development, the other group was subjected to a 10-day heat stress at the most sensitive phenophases, at complete heading (ZD-59) and then raised under control conditions till maturity in phytotron, in each case the daylength was 16 hour. In control chambers, the temperature was constant 18°C. In heat stress chambers, the temperature was 36°C/20°C (day/night). The heat stress kept for 8 hours.
The photosynthetic efficiency of the flag leaves were determined at 1st, 3rd, 6th and 9th day of heat stress treatment. The abscisic acid (ABA), proline and some carbohydrate compounds (e.g. trehalose-6-phosphate) were also measured under heat stress at the same sampling time intervals as for photosynthesis using complex analytical techniques (XEVO TQ XS UHPLC-MS/MS system, GC-MS equipment). The effect of the removed flag leaves was examined on yield components to clarify the role of flag leaf in yield formation under control and stress conditions.

The results of heat stress experiment with selected eight varieties

In Exp. 2019, the results of the most important selected morphological and yield related components showed different, mainly declining changes among the examined winter wheat varieties in response to heat stress treatment (Table 2.). During the heading stage, however, significant decreases were observed for most of the traits, the extent of which was the smallest for plant height and the last internode length and the largest for grain yield (GY) and average seed number (AS).

The plant height of the variety ‘K55’ decreased the most and ‘K57’, ‘K185’, ‘K192’ and ‘K110’ the least. The last internode length declined the most also in ‘K55’ which followed by ‘K192’, ‘K6’ and ‘K171’. Seven of the eight varieties had a significant decrease in biomass due to heat stress. The heat stress resulted in a reduction of between 59.28-86.64% in biomass. The harvest index decreased between 53.9-94.6% due to heat stress at heading. The harvest index of the varieties ‘K171’ and ‘K188’ dropped the greatest extent under heat stress. The HI of the other varieties was less affected by high temperature. The large reduction in AS was compensated by increased thousand-kernel weight (TKW) compared to the control, mainly in variety ‘K57’, ‘K110’ and ‘K188’. For the other varieties, the TKW did not increase, but the AS decreased, this was observed in varieties ‘K171’ (39.83%) and ‘K192’ (56.3%), where the grain yield (GY) reduced the greatest extent under heat stress. The large reduction in GY was also accompanied by a decrease in biomass for these varieties. The grain yield of ‘K185’ (81.83%) changed to the least extent, which was due to the fact that the AS remained high during heat stress.

Table 2. Changes of the morphological and yield related properties under control and heat stress conditions in Exp. 2019.

Traits		**K6**	**K55**	**K57**	**K110**	**K171**	**K185**	**K188**	**K192**	**LSD$_{5\%}$**
PH	C	36.25	62.20	33.50	28.80	35.50	51.25	41.40	57.95	1.48
	HS	31.10*	51.65*	34.35ns	28.35ns	33.90*	50.40ns	43.50*	56.95ns	
	Control%	*85.79*	*83.04*	*102.54*	*98.44*	*95.49*	*98.34*	*105.07*	*98.27*	
LIN	C	14.75	27.25	15.40	10.80	20.40	21.75	15.70	24.50	0.96
	HS	12.65*	21.15*	15.05ns	13.25*	17.95*	22.80*	15.60ns	20.00*	
	Control%	85.76	77.61	97.73	122.69	87.99	104.83	99.36	81.63	
FBIOM	C	9.04	13.28	10.19	18.59	15.32	10.90	11.69	15.37	0.74
	HS	9.18ns	10.37*	7.69*	14.29*	11.25*	9.44*	8.79*	9.11*	
	Control%	101.56	78.10	75.45	76.89	73.46	86.64	75.24	59.28	
GY	C	4.64	6.71	5.09	5.64	7.84	5.09	4.77	7.04	0.35
	HS	3.28*	4.81*	3.62*	4.12*	3.12*	4.17*	2.13*	3.96*	
	Control%	70.75	71.62	71.08	73.07	39.83	81.83	44.68	56.30	
HI	C	51.23	50.52	50.18	30.68	51.58	46.60	40.94	45.71	1.48
	HS	35.99*	46.36*	47.20*	29.03*	27.80*	44.36*	24.04*	43.07*	
	Control%	70.25	91.76	94.06	94.6	53.90	95.21	58.72	94.23	
TKW	C	43.01	49.65	43.06	26.43	35.52	53.30	36.60	47.83	1.79
	HS	36.91*	41.97*	51.33*	38.04*	33.36*	49.97*	39.20*	46.96ns	
	Control%	85.82	84.52	119.2	143.92	93.9	93.74	107.11	98.19	
SPS	C	2.55	2.12	2.41	2.36	2.57	1.41	1.80	1.87	0.15
	HS	2.14*	2.13ns	2.20*	1.84*	1.86*	1.32ns	1.40*	1.53*	
	Control%	83.89	100.38	90.99	78.07	72.25	93.62	77.79	81.89	
AS	C	34.25	33.00	32.11	47.15	39.41	16.72	26.43	29.24	2.13
	HS	26.33*	22.93*	21.21*	25.72*	13.23*	21.08*	11.30*	19.40*	
	Control%	76.89	69.47	66.07	54.55	33.58	126.13	42.74	66.35	

C - Control, HS - Heat stress; PH - Plant height, LIN - Last internode length, FBIOM - Total aboveground biomass (straw + all ears), GY - Grain yield, HI - Harvest index, TKW - Thousand *kernel weight, SPS - Grain number per spikelet, AS - Average seed number; * difference significant at 5% probability level, ns - not significant. Mv Palotás: 'K6', Bayraktar: 'K55', Feng You-3: 'K57', Ellvis: 'K110', Tommi: 'K171', Mv Toborzó: 'K185', Mv Verbunkos: 'K188' and KWS Scirocco: 'K192'*

The role of flag leaf in yield formation were studied under control and heat stress conditions (Figure 8. A-B), as the flag leaf was considered important as a source of carbohydrate for grain filling.

The results showed that the removed flag leaves affected the grain yield and grain number/ plant significantly compared to the control, but the flag leaves collected at different times (on the 1st, 3rd, 6th and 9th day of 10 days of heat stress treatment) did not show a tendentious (uniform) changes in yield formation. For most cultivars, removed leaves on the 9th day of heat stress treatment resulted in the largest grain yield and grain number reduction (28.4%-43%). Nevertheless, there were also differences between varieties due to flag leaves removed during heat stress. The greatest decline in the results was observed in the grain yield and grain number of the variety 'K171' and 'K188'. The varieties 'K55', 'K110' and 'K185' showed the lowest sensitivity to the remove of flag leaves, their yield formation remained the most stable.

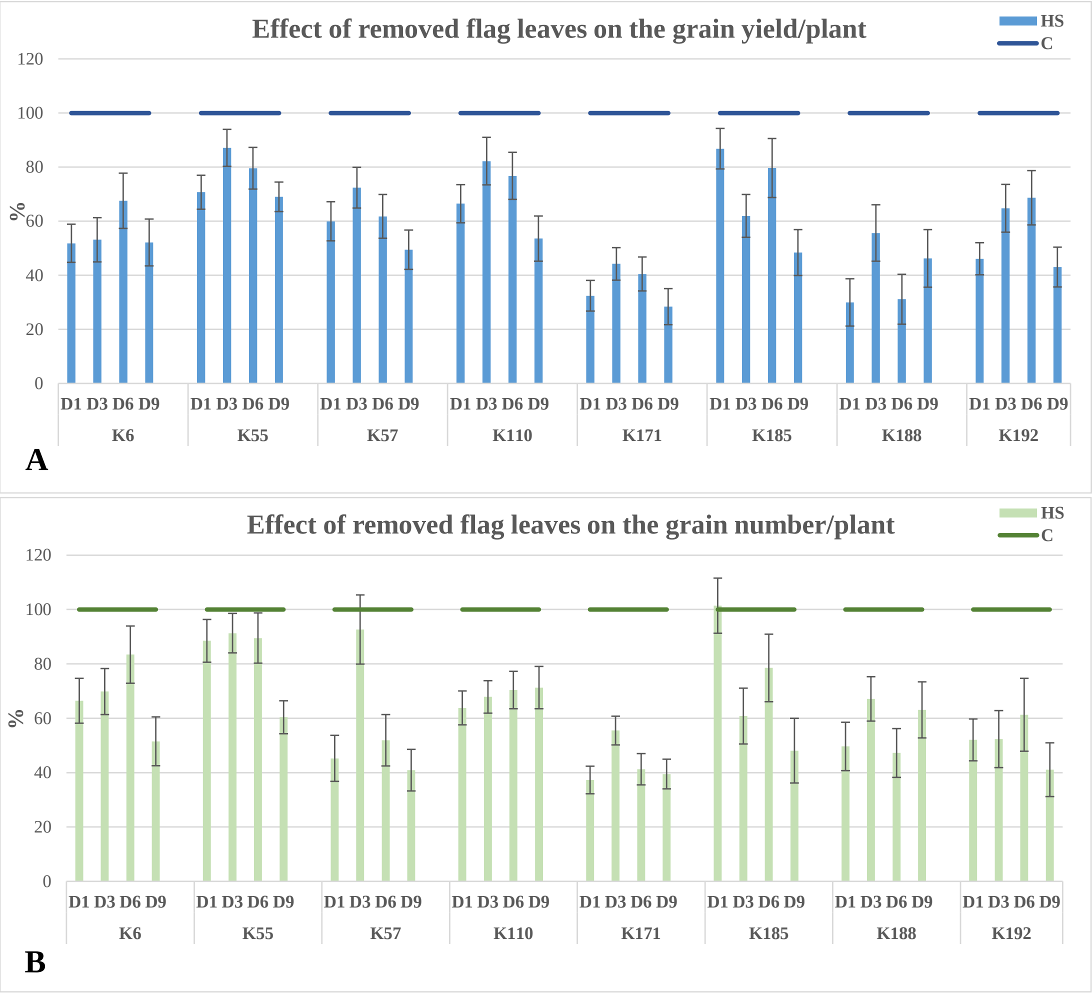


Figure 8. Effect of removed flag leaves on the grain yield (A) and grain number/plant (B) in control %.

Sampling of plant leaves on the 1st (D1), 3rd (D3), 6th (D6) and 9th (D9) day of the 10 days heat stress treatment; control (C); heat stress (HS)

The overall responses of the wheat varieties based on the photosynthetic properties such as net assimilation (PN), transpiration (EVAP), stomatal conductance (GS) and chlorophyll content (CLR) were similar across the experiment.

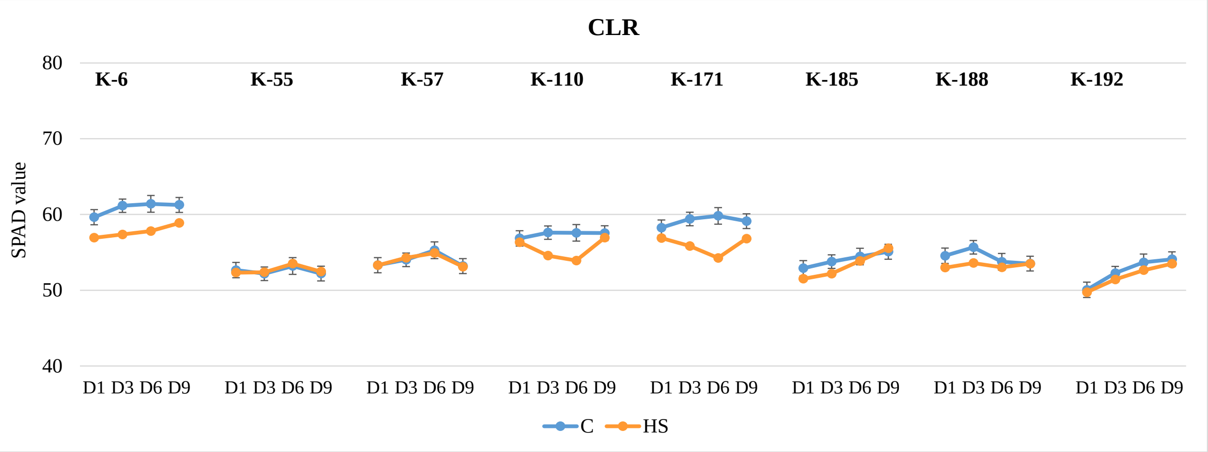
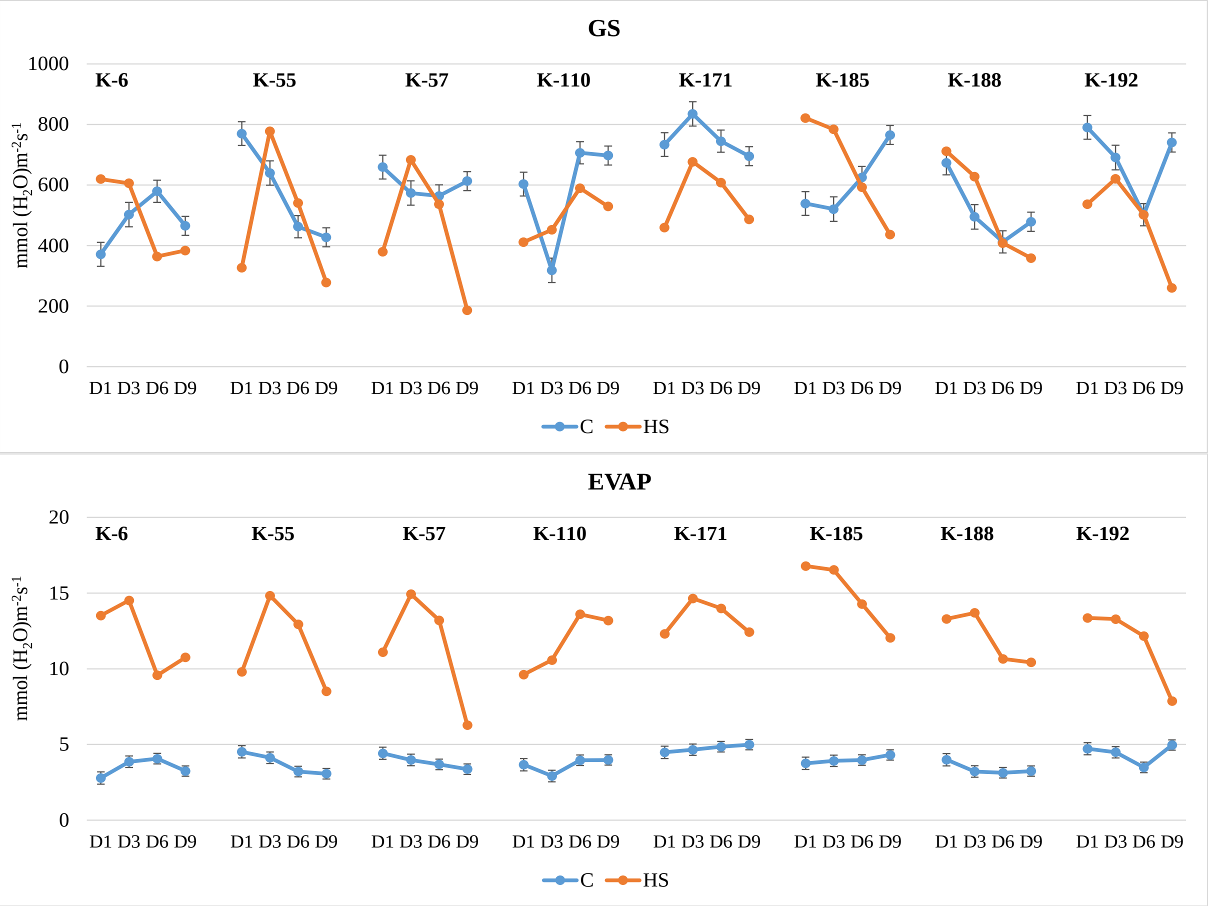


Figure 9. The changes of chlorophyll content (CLR) during heat stress treatment in eight varieties.

Measurements were performed on flag leaves on the 1st *(D1), 3*rd *(D3), 6*th *(D6) and 9*th *(D9) day of the 10 days heat stress treatment; control (C); heat stress (HS)*

The CLR was affected to the smallest extent by heat stress (Figure 9.). There were varieties (e.g. ‘K55’ and ‘K57’) whose chlorophyll content was almost unchanged under heat stress compared to the control. The heat stress resulted in the greatest significant reduction in CLR in the variety ‘K-6’, ‘K110’ and ‘K171’. In some varieties, changes of CLR showed an increasing trend due to heat stress applied during heading.



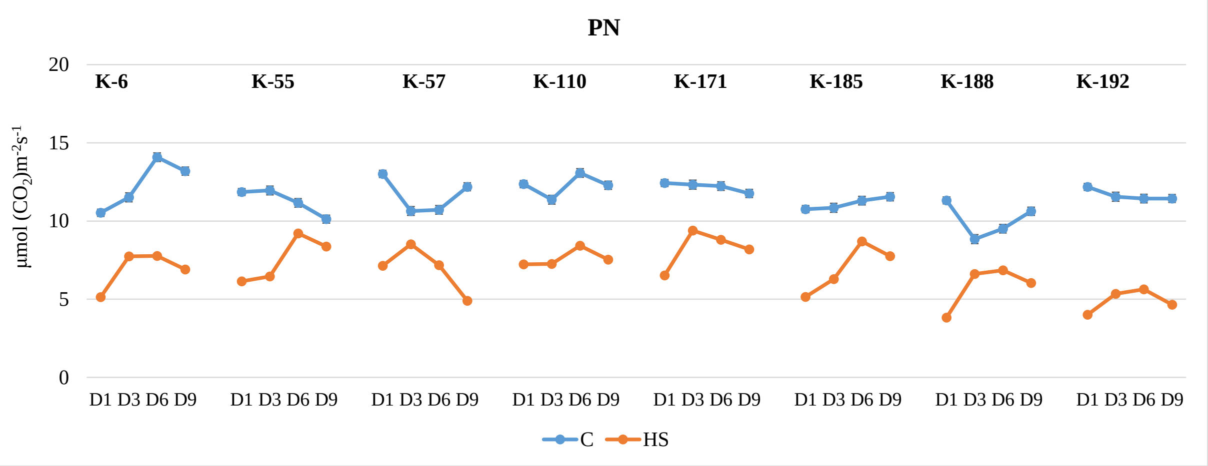


Figure 10. The changes of photosynthetic efficiency during heat stress treatment in eight varieties. *Measurements were performed on flag leaves on the 1st (D1), 3rd (D3), 6th (D6) and 9th (D9) day of the 10 days heat stress treatment; control (C); heat stress (HS), net assimilation (PN), transpiration (EVAP), stomatal conductance (GS)*

In general, PN and GS decreased significantly the most on the effect of heat stress treatment, while EVAP increased strongly (Figure 10.). For EVAP, the responses to heat stress increasingly intensified from the 1st to the 3rd day of heat stress treatment and then gradually declined until 9th day of measurement. The lowest EVAP value was measured at the end of the heat stress treatment (9th). The high temperature on 9th day was least tolerated by the varieties ‘K57’ and ‘K192’.

The GS values of these varieties also showed a strong decrease. Showing the highest EVAP value, the variety ‘K185’ tolerated the high temperature the best. The EVAP value did not decrease as much as for the other varieties. It remained the most stable for the first three days and then began to decline, followed by a steep decline in GS. The openness of the stomas also fluctuated strongly due to heat stress. In most varieties, the stoma was still less open on the first day of the heat stress, reached maximum openness (highest value) on the 3rd day of treatment, and then closed strongly on the 6th and 9th days (GS values decreased).

For PN, the responses of plants to heat stress were the highest on the 3rd or 6th day of treatment, then the values were slightly and significantly reduced to 9th day of treatment. The examined varieties tolerated similarly the high temperature stress, the PN values changed between 3.83 and 9.4 µmol (CO_2) $m^{-2}s^{-1}$. The PN value of variety ‘K110’ was the most stable, as the data values changed the least (7.23 and 7.53 µmol (CO_2) $m^{-2}s^{-1}$) between the first and last measurement of treatment. The heat stress caused the greatest drop in PN (7.14 and 4.9 µmol (CO_2) $m^{-2}s^{-1}$) for the variety ‘K57’.

Of the biochemical processes, changes in the contents of abscisic acid (ABA) and proline (PROL) proved to be associated with higher tolerance levels. Studies has shown that the proline increased faster than other amino acid in plants under drought stress and had a role in plant protection by acting as a cellular osmotic regulator between the cytoplasm and vacuole. The plant hormone ABA is known to positively regulate abiotic stress tolerance by affecting stomatal guard cells. Most of the experimental data on these protective compounds however originated from drought and osmotic stress experiments, much less information is known about their roles in heat stress.

The received results showed (Figure 11.), that the ABA and PROL compounds did not play important role as protection the plants against the effect of heat stress. Although the reactions of the varieties differed, overall, a decrease in ABA and PROL concentrations was observed during heat stress treatment. The ABA content of the varieties ‘K188’ and ‘K192’ alone was extremely high on the first day of heat stress treatment and then dropped sharply by the end of treatment. In the case of proline, its content became higher compared to the control in the varieties of ‘K171’ and ‘K188’ due to heat stress.

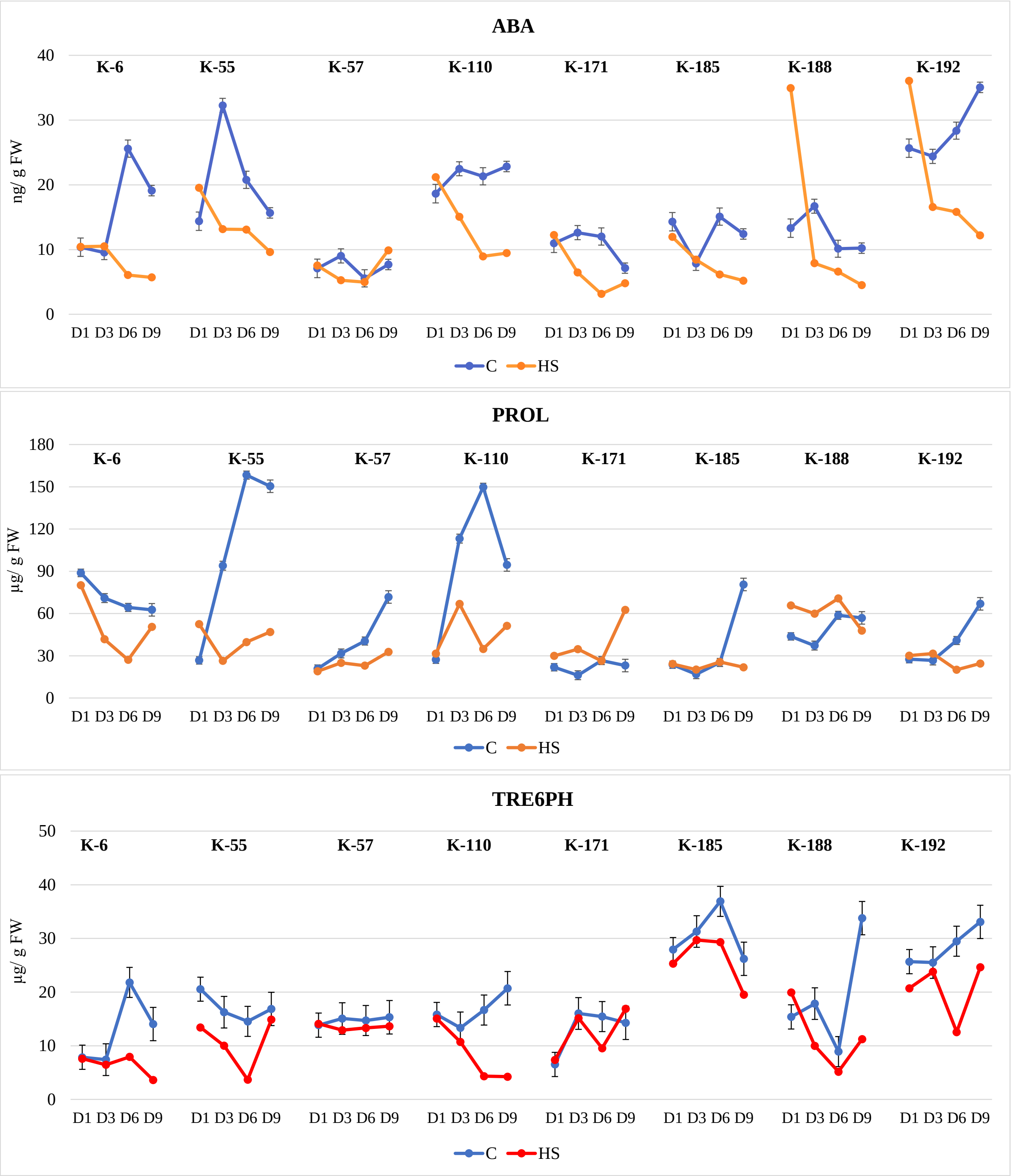


Figure 11. Changes of the concentration of the abscisic acid (ABA), proline (PROL) and trehalose-6-phosphate (TRE6PH) during heat stress treatment in eight wheat varieties. *Measurements were performed on the 1st (D1), 3rd (D3), 6th (D6) and 9th (D9) day of the 10 days heat stress treatment from collected flag leaves; control (C); heat stress (HS)*

The trehalose 6-phosphate (TRE6PH) is an essential signal metabolite in plants, with influence on growth and development. The TRE6PH is the phosphorylated intermediate of trehalose biosynthesis. It is a signal of sucrose status in plants and influences many metabolic process. Both TRE6PH and trehalose (as stress protectant) are implicated in regulation of stomatal conductance (Figueroa and Lunn, 2016). TRE6PH can be targeted to improve yield potential and resilience to diverse stresses by regulating whole-plant carbohydrate allocation and utilization (Paul et al., 2018). Increasing TRE6PH promotes biosynthetic pathways associated with grain yield, such as starch synthesis, while decreasing TRE6PH promotes resource mobilization and changes in sucrose allocation, enabling better performance under abiotic stress.

Most research has studied trehalose/trehalose 6-phosphate in the context of drought and osmotic stress. Less attention has been paid to this compound under heat stress conditions. Even its relationship to drought is not entirely clear (Pampurova et al., 2014). There also have been some reports (Garg et al., 2002) showing improvements in stress tolerance in plants with constitutively expressed trehalose pathway genes e.g. in rice. Attempts have also been made with trehalose pre-treatment to protect thylakoid membranes and photosynthetic capacity under heat stress conditions (Luo et al., 2010).

In our experiment, the concentration of TRE6PH were examined under 10 days of heat stress conditions. Overall, the level of TRE6PH in all eight selected wheat varieties changed negatively compared to the control. Significant changes were observed mainly on the 6th and 9th day of heat treatment. There were varieties ('K6', 'K110' and 'K185'), where the TRE6PH decreased significantly on the 9th day of heat stress, but in some varieties ('K55', 'K171', 'K188' and 'K192') an increase could be observed at the end of treatment. This positive change was not significant for 'K55' and 'K171', but was significant for 'K188' and 'K192', where the increase in TRE6PH remained below the elevated control values due to heat stress.

Correlation relationships among the examined morphological, yield related components, physiological parameters and analytical compounds were also evaluated during heat stress conditions (Table 3.).

The results showed, that some negative correlations were revealed between the photosynthetic parameters and grain number per spikelet (SPS) on the first day of heat stress treatment. The chlorophyll content showed strong significant correlation with plant height, last internode length and thousand-kernel weight on the 1st and with the exception of TKW on the 3rd day of heat stress treatment. The most correlations were found between the photosynthetic properties and yield related parameters on the 3rd day of heat stress and the least on the 6th day. The heat stress (on the 9th day) resulted in a significant relationship mainly between biomass and GS/EVAP and harvest index (HI) and GS/EVAP.

In cereals, the ABA content of the flag leaf was found to be strongly correlated with grain filling under stress conditions, such as during drought stress, but in our experiment under heat stress conditions, the ABA concentration did not show strong correlation with the yield related components. It was an exception, significant correlation was found between ABA concentration and plant height (PH) during on the 6th day of heat stress treatment.

The proline correlated significantly (positive) with biomass on the 3rd day of heat stress, and showed negative correlation with TKW on the 9th day of treatment.

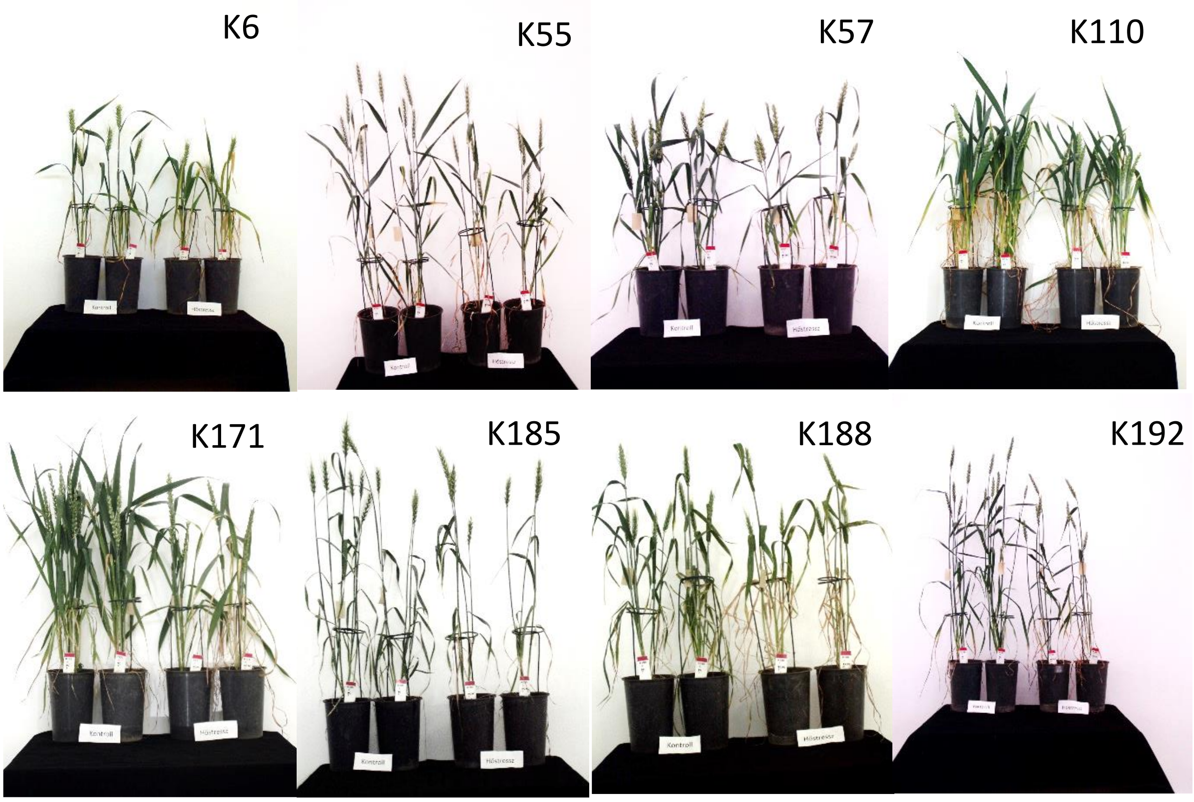
The TRE6PH showed the least correlation with morphological/yield related properties on the 6th day, in most cases on the 9th day of heat stress. Strong negative correlation was found between TRE6PH and SPS on the 1st and 3rd day of heat stress treatment. It showed the strongest correlation with morphological properties (PH and LIN) during heat stress conditions (on the 9th day).

Table 3. Correlation among studied morphological, photosynthetic properties, one amino acid, hormone and carbohydrate compounds under heat stress.

	PH_HS	*LIN_HS*	*FBIOM_HS*	*GY_HS*	*HI_HS*	*TKW_HS*	*SPS_HS*	*AS_HS*
EVAP-1_HS	0.36	0.39	-0.46	-0.26	0.07	0.29	**-0.68 ***	-0.24
PN-1_HS	-0.61	-0.26	0.48	0.40	0.11	-0.07	0.65	0.39
CI-1_HS	0.45	0.22	-0.51	-0.52	-0.19	0.00	**-0.66+**	-0.43
PS2-1_HS	0.65	0.61	0.04	0.59	0.50	0.49	-0.45	0.31
GS-1_HS	0.25	0.19	-0.32	-0.41	-0.19	0.13	**-0.76***	-0.26
CLR-1_HS	**-0.91*****	**-0.70***	0.49	-0.29	-0.58	**-0.76***	0.48	0.15
ABA-1_HS	0.52	0.12	0.03	-0.22	-0.30	-0.06	-0.56	-0.40
PROL-1_HS	-0.11	-0.38	-0.14	-0.39	-0.35	-0.53	0.16	0.06
TRE6PH-1_HS	**0.66+**	0.54	-0.21	0.13	0.24	**0.66+**	**-0.80****	-0.15
EVAP-3_HS	0.36	0.56	**-0.68***	0.03	0.50	0.40	-0.05	-0.17
PN-3_HS	**-0.79***	-0.47	0.11	-0.32	-0.30	-0.41	0.55	-0.13
CI-3_HS	**0.94*****	**0.75***	-0.50	0.25	0.50	0.53	-0.43	-0.17
PS2-3_HS	-0.19	0.08	0.62	**0.82****	0.34	0.05	0.47	**0.67***
GS-3_HS	0.58	**0.77***	-0.55	0.25	0.58	0.43	-0.06	-0.21
CLR-3_HS	**-0.88**	**-0.76***	0.16	-0.44	-0.47	-0.64	0.53	0.14
ABA-3_HS	0.34	0.09	0.45	0.52	0.10	-0.06	-0.08	0.46
PROL-3_HS	-0.53	-0.59	**0.79***	-0.06	-0.60	-0.43	-0.02	0.23
TRE6PH-3_HS	0.61	**0.75***	-0.16	0.30	0.39	0.59	**-0.67***	-0.14
EVAP-6_HS	0.09	0.51	0.38	0.52	0.25	0.29	-0.12	-0.03
PN-6_HS	-0.26	0.18	0.50	0.39	-0.02	-0.32	0.28	0.23
CI-6_HS	0.36	0.24	-0.10	0.02	0.13	0.48	-0.39	-0.35
PS2-6_HS	-0.20	-0.10	0.25	**0.77***	0.60	0.36	0.61	**0.86****
GS-6_HS	0.04	0.46	0.49	0.53	0.18	0.18	-0.08	-0.01
CLR-6_HS	-0.63	-0.57	-0.13	-0.13	0.02	-0.25	0.59	0.50
ABA-6_HS	**0.68***	0.37	0.07	0.54	0.39	0.24	-0.10	0.26
PROL-6_HS	0.03	-0.18	0.00	-0.54	-0.58	-0.33	-0.30	-0.49
TRE6PH-6_HS	0.25	0.56	-0.26	0.11	0.32	0.46	-0.45	-0.14
EVAP-9_HS	-0.38	-0.12	**0.71***	-0.14	**-0.66***	-0.61	-0.34	-0.03
PN-9_HS	-0.16	0.22	0.59	0.30	-0.21	-0.51	0.09	0.12
CI-9_HS	-0.24	-0.32	0.24	-0.51	-0.62	-0.17	-0.62	-0.27
PS2-9_HS	-0.09	0.04	0.39	**0.75***	0.50	0.39	0.37	0.58
GS-9_HS	-0.44	-0.16	**0.77***	-0.11	**-0.67***	-0.63	-0.29	-0.01
CLR-9_HS	-0.65	-0.48	0.42	-0.17	-0.44	-0.57	0.11	0.32
ABA-9_HS	0.29	0.08	0.06	0.61	0.55	0.44	0.28	0.43
PROL-9_HS	-0.47	-0.17	0.39	-0.33	-0.59	**-0.78***	0.22	-0.43
TRE6PH-9_HS	**0.90*****	**0.74****	-0.49	0.36	0.65	**0.79***	-0.46	-0.09

*1, 3, 6, 9: Sampling dates: on the 1st, 3rd, 6th and 9th day of the 10 days heat stress (HS); EVAP - Evaporation, PN - Net assimilation, CI - Intercellular CO_2 concentration, PS2 – Effective quantum yield, GS - Stomatal conductance, CLR - Chlorophyll content, PH - Plant height, LIN - Last internode length, FBIOM - Total aboveground biomass (straw + all ears), GY - Grain yield, HI - Harvest index, TKW - Thousand kernel weight, SPS - Grain number per spikelet, AS - Average seed number, ABA - Abcisic acid, PROL - Proline, TRE6PH - Trehalose-6-phosphate; $^{+}$, *, **, *** critical r values of the correlation coefficient at P=10%, 0.666 P= 5%, 0.798 P= 1%, 0.898 P= 0.1%; n= 8.*

Photographs of the selected eight varieties (Mv Palotás: **'K6'**, Bayraktar: **'K55'**, Feng You-3: **'K57'**, Ellvis: **'K110'**, Tommi: **'K171'**, Mv Toborzó: **'K185'**, Mv Verbunkos: **'K188'** and KWS Scirocco: **'K192'**) studied under control and heat stress conditions at the end of treatments (Picture 1.).



Picture 1. Two control (left) and two treated wheat plants after heat stress (right)

Experiments built on each other in recent years have also allowed a comparative analysis of eight selected varieties among three main experiments (Figure 12.).

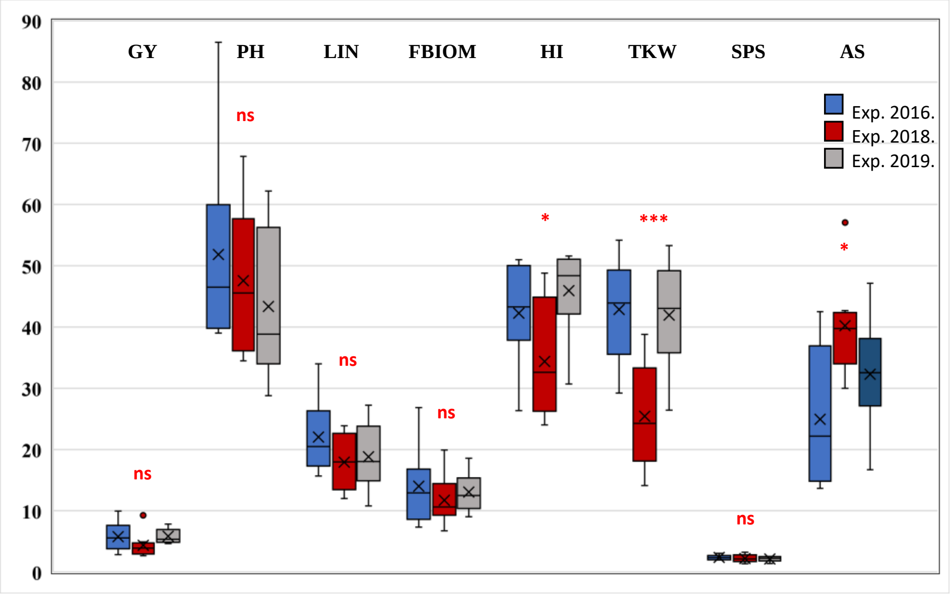


Figure 12. Comparison of control treatments for eight yield properties tested in three heat stress experiments (2016, 2018 and 2019).

GY - Grain yield, PH - Plant height, LIN - Last internode length, FBIOM - Total aboveground biomass (straw + all ears), HI - Harvest index, TKW - Thousand kernel weight, SPS - Grain *number per spikelet, AS - Average seed number; ns - not significant,* *** - *significant at the P≤ 0.05 level,* ***** - *significant at the P≤ 0.001 level*

During control treatments, the changes in yield components of the eight varieties were compared from 3-year series of experiments to prove the similarity of the results obtained in recent years (Figure 12.). The highlighted eight yield components showed in most cases that there was no significant differences among the control treatments of the three experiments (2016, 2018 and 2019) in grain yield, plant height, last internode length, total aboveground biomass and the grain number per spikelet. These results emphasised that the wheat plants grew equally in the three experiment and their databases were comparable. Of the results, the largest jump out was shown by the TKW (in 2018).

The results of GGE Biplots could identify the relationship between the environments and the examined varieties (Figure 13.). The test environments were the three year experiments (1: Exp.2016, 2: Exp.2018, 3: Exp. 2019). In this analysis, the two principal components, PC1 and PC2, accounted for 64.56% and 24.09% of GGE sum of squares, respectively, explaining a total of 88.66% of the variation (Figure 13.). The scatter pot concluded that the experiments in the three year were positively correlated among each other. Strongest correlation was between the Exp. 2018 and Exp.2019. Based on the length of the vectors, the Exp. 2016 was the most discriminating and informative environment.

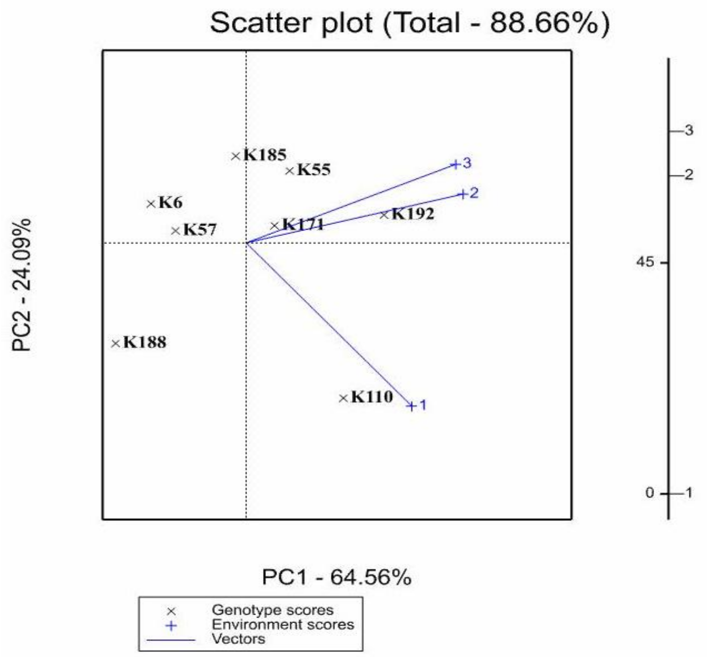


Figure 13. The relationship among the test environments with environment vector view of the GGE biplot based on the grain yield.
+1: Exp. 2016, +2: Exp. 2018, +3: Exp. 2019; Mv Palotás: 'K6', Bayraktar: 'K55', Feng You-3: 'K57', Ellvis: 'K110', Tommi: 'K171', Mv Toborzó: 'K185', Mv Verbunkos: 'K188' and KWS Scirocco: 'K192'

The "Average-Environment Axis" (AEA) appears on the ranking biplot, which has the average coordinates of all test environments. The AEA axis passes through the biplot origin and the Average Environments Coordinate (AEC). The result of ranking biplot (Figure 14. A) showed that the Exp. 2018 was the most representative of the test environments (based on the smallest angle with AEA). The ranking biplot was suitable for evaluating the studied genotypes based on their mean performance and stability across environments. The result of ranking biplot

(Figure 14. A) indicated that the variety of 'K192' had the highest mean yield, followed by 'K110' and 'K55' while 'K188' and 'K6' had the lowest mean yield. The varieties 'K185' and 'K171' had a mean yield similar to the grand mean. The variety 'K110' was highly unstable, while 'K57' and 'K171' were found to be stable.

When evaluated the mean performance and stability of the varieties in the different experimental years separately (Figs. 14. B, C and D), we found that in Exp. 2016 (Figure 14. B), the 'K110' had the highest mean yield and 'K6' the lowest. The variety 'K188' was highly unstable, while 'K110' was found to be stable. In Exp. 2018 and 2019 (Figs. 14. C-D), the 'K192' had the highest mean yield and 'K188' had the lowest. The varieties 'K171' and 'K192' was highly stable while 'K110' was mostly unstable.

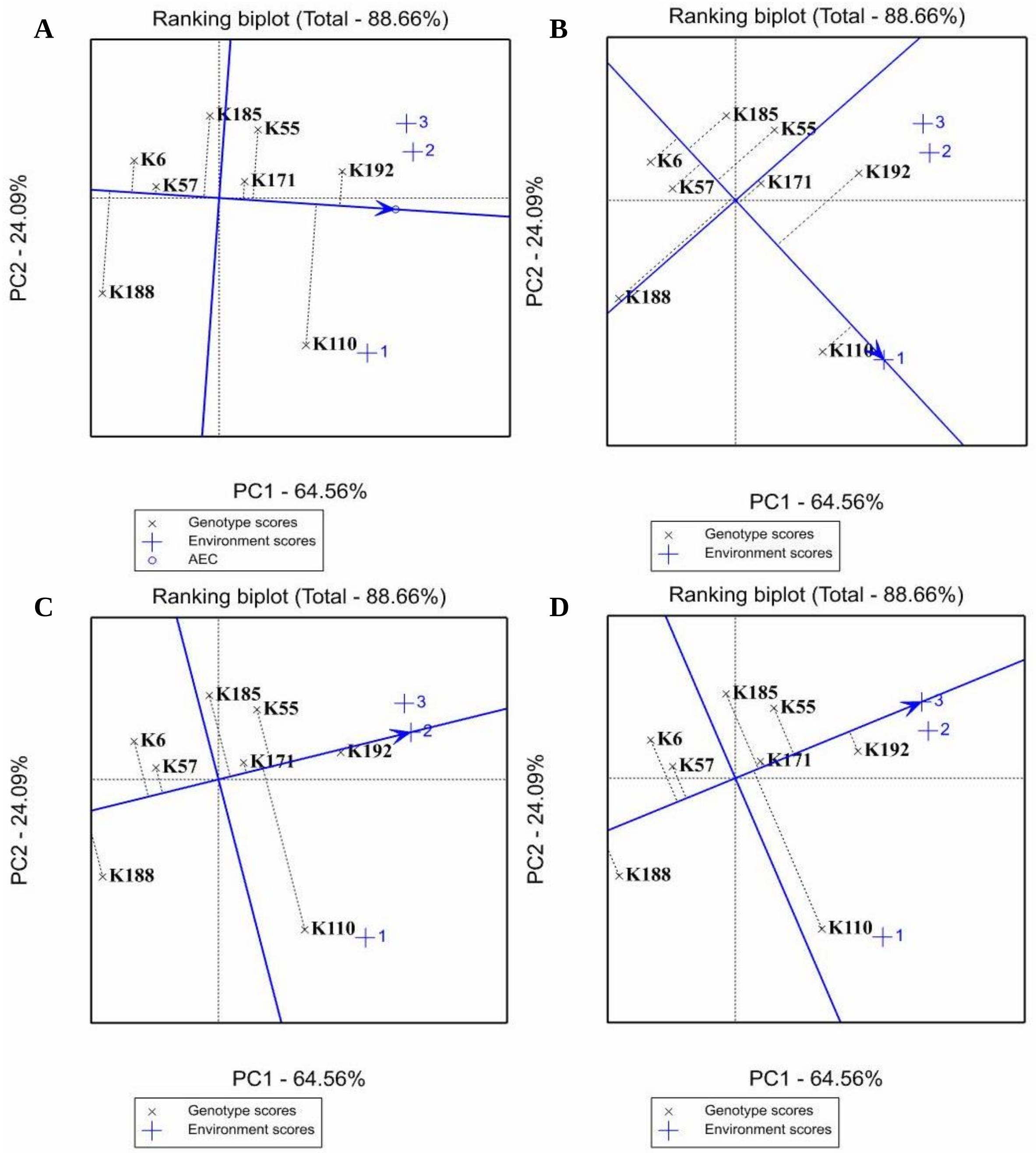


Figure 14. Ranking genotypes, based on mean yield performance (**A**) and based on grain yield of the three test environments (**B**: Exp. 2016 (+1), **C**: Exp. 2018 (+2), **D**: Exp. 2019 (+3)). *AEC: Average Environment Coordination view to show the mean performance and stability of the*

varieties. Mv Palotás: ‘K6’, Bayraktar: ‘K55’, Feng You-3: ‘K57’, Ellvis: ‘K110’, Tommi: ‘K171’, Mv Toborzó: ‘K185’, Mv Verbunkos: ‘K188’ and KWS Scirocco: ‘K192’

Overall, the results also provided answers to physiological and production biological questions that were still unclear in heat stress research. Detailed analyses of the tested varieties made it possible to select varieties of more flexible adaptation capacity, which could be used to breed new genotypes with better environmental plasticity across a wider developmental range more efficiently in breeding programs. As a result of the detailed analyzes and the favourable performances/ stability obtained under high temperature conditions, the varieties KWS Scirocco ('K192') and Bayraktar ('K55') were also included in the breeding program.

Topic 4. *Initiation of targeted crosses for the development of genetic population with high heat stress tolerance*

The aim was to create genetic mapping populations consisting of doubled haploid lines (DH) in which the various heat stress responses are combined. The anther culture technique was used to create these populations, as via this technique the homozygous state can be achieved within one generation and in addition, the genetic segregations are fixed in F2 stage. The crosses enabled us to transfer the genetic regions responsible for various heat tolerance responses and to develop genotypes, which have high heat tolerance properties across a wider developmental range.

Based on the previous results (published in Plos One, 2019), genetic populations were created from the selected varieties (GK Hattyú (HU), Mv Hombár (HU), Cutter (US), Valoris (FR), KWS-Scirocco (DE), Bayraktar (TR), Sunstar (AU)) showing contrasting heat tolerance at the three developmental phases.

After crossing the parental lines, anther cultures technique was applied in the F1 generation. The fertile DH_0 plants raised in the chamber of the phytotron under optimal conditions then harvested and their seeds were used in further heat stress experiments.

Results of the DH program

The results of the anther culture technique showed that the number cultured anthers was between 3252 and 5591 from the 6 F1 populations, with callus induction frequencies (CIF) ranging between 29.4% and 98.6% (Table 4.). The crossing of KWS-Scirocco x Bayraktar resulted in the best callus number (1801 pieces). The number of green plants ranged from 188 to 407 pieces and the number of albino plants ranged from 144 to 429 pieces. The obtained fertile DH_0 varied between 25 and 180 pieces of the six crosses.

The efficiency of the anther culture technique showed variations among the six crosses. The callus induction frequencies varied between 98.6% (KWS Scirocco x Bayraktar) and 29.4% (Valoris x Mv Hombár). The interval of total plant regeneration frequency was between 54.1% (Cutter x Mv Hombár) and 37.3% (KWS-Scirocco x Bayraktar), while the range of green plant regeneration frequencies was between 44.2% (Cutter x Mv Hombár) and 13.1% (KWS-Scirocco x Mv Hombár). The rediploidization rate was between 0.72 (KWS-Scirocco x Bayraktar) and 0.45 (KWS-Scirocco x Mv Hombár).

Table 4. Summary of the result of DH program

Pedigree	Cultured anthers	Callus	CIF	Green plant	RFGP	Albino plant	Fertile DH_0	DH_0PE
	No.	No.	%	No.	%	No.	No.	%
GK HATTYU(t)/MV HOMBAR(s)	3252	822	37.36	188	22.87	144	70	2.15
CUTTER(s)/MV HOMBAR(s)	4430	1251	41.2	407	32.53	270	180	4.06
VALORIS(t)/MV HOMBAR(s)	5071	694	29.36	184	26.51	162	50	0.99
KWS-SCIROCCO(t)/MV HOMBAR(s)	5271	1429	65.74	187	13.09	395	25	0.47
KWS-SCIROCCO(t)/BAYRAKTAR(s)	3369	1801	98.6	242	13.44	429	81	2.4
KWS-SCIROCCO(t)/SUNSTAR(s)	5591	1503	69.31	286	19.03	395	62	1.11

Callus induction frequency (**CIF**): % of embryogenic calli projected on the cultured anthers. Green plant regeneration frequency (**RFGP**): % of green regenerated plants projected on the embryogenic calli transferred to regeneration medium.
DH_0 production efficiency (**DH_0PE**): Number of fertile DH0 plants per 100-cultured anther.

The interval of the DH_0 production efficiency (DH_0PE) was between 0.5 (KWS-Scirocco x Mv Hombár) and 4.1% (Cutter x Mv Hombár). Altogether 391 DH_0 lines were created; the most – 148 DH_0 lines – from the cross of Cutter x Mv Hombár, while the lowest – 17 DH_0 lines – from the cross of KWS-Scirocco x Mv Hombár. As the crosses were created in two groups around two cultivars (Mv Hombár and KWS-Scirocco), Mv Hombár included in four, while KWS-Scirocco in three different crosses (with one overlapping cross between them). Thus, the DH_0 lines of each batch around one cultivar were analysed together. Thus, from the Mv Hombár crosses we had 269 DH_0 lines, while from the KWS-Scirocco crosses 139 DH_0 lines for further studies, 17 DH_0 lines overlapped between the two batches.

We started to carry out a heat stress experiment (in 2020/2021) on the DH0 lines of the Mv Hombár crosses, the results of which are still being processed.