

Research paper

Field versus controlled environmental experiments to evaluate the heat stress response of barley (*Hordeum vulgare* L.)

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ABSTRACT

The complexity of heat stress hinders both the exploration of the genetic basis of stress response and breeding of genotypes with increased stress tolerance. Our main goal was to analyze and compare the possibilities of evaluating heat stress responses of barley cultivars in field sowing and controlled environmental experiments. For this purpose, a four-year field-sown experiment was carried out at one location in a panel of 190 winter and facultative barleys. In parallel, a subset of 28 cultivars were included into controlled environmental tests, where their reactions were determined to single heat stress treatment applied at heading and to combined heat stresses applied at first node appearance and then at heading. Based on the grain-yield related parameters, seven distinct clusters of the cultivars could be established with specific reaction patterns across the years. There was one year with close to optimal weather conditions and one year, when heat stress occurred during flowering and grain setting, making it possible to evaluate the heat stress responses of the 190 barley genotypes. In the heat stress prone 2022 year, the general trends were a strong reduction in the reproductive tiller number and a slight reduction in the fertility. In several groups, these negative effects were compensated with significant increases in grain number per ears and with strong increases in the average grain weight. Under controlled conditions, heat stress significantly reduced most of the grain-yield related traits. Among the more tolerant genotypes, two basic response types could be distinguished. One group was able to better preserve the grain number and weight in the main ear under heat stress, while the other was more able to allocate resources into the side tillers during the recovery period. In the combined heat stress, the average trait values were similar to those in the single stress or even lower, and there was no general priming effect clearly detectable. In the case of the 28 genotypes, there were significant correlations between the stress-induced changes in grain-yield related traits measured under field and under controlled conditions, underlining the possibility of combining the information originating from the two different environments.

1. Introduction

The adverse effects of heat on plants strongly depend on the timing, duration and the intensity of the heat period, which can trigger different stress responses depending on the severity of the stress (Ugarte et al., 2007; Barnabás et al., 2008; Cossani and Reynolds, 2015; Dreccer et al., 2018; Kim et al., 2024). The plant responses to heat include

morphological, developmental, biochemical and genetic regulation changes that can lead to remarkable alterations on cell, tissue and whole plant levels (reviewed by (Bita and Gerats, 2013; Jacott and Boden, 2020)). These changes can cause serious losses in grain quality and yield. For many plant species, the effect of high temperature stress is more pronounced on reproductive development than on vegetative growth. Heat waves of a few days at any time during the reproductive

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development of the crop directly affect grain yield, irrespective to the type of environment (controlled or field). Days with temperature higher than 30°C during and after heading adversely affect inflorescence development, including male and female meiosis, pollen fertility, grain set and subsequent grain filling (Reynolds et al., 2000; Barnabás et al., 2008; Cantalapiedra et al., 2017; Végh et al., 2018; Jampoh et al., 2023; Fábíán et al., 2024; Kim et al., 2024). A single day with three hours of 35°C heat has already resulted in significant yield losses in wheat (Talukder et al., 2014). On the other hand, long-term, above-optimal temperature between 20°C and 28°C may also have indirect negative consequences on yield by shortening the plant cycle and/or disrupting optimal development patterns (Hemming et al., 2012; Cantalapiedra et al., 2017; Kim et al., 2024). This results in a dramatic reduction in dry matter accumulation (smaller plants, reduced plant canopy, less tillers), which ultimately leads to limited resources available for grain production (Cantalapiedra et al., 2017; Kiss et al., 2019; Kim et al., 2021; Slafer et al., 2022; Araus et al., 2023). The two categories of heat stress (short heat waves versus long term supra-optimal) may require different combinations of traits that are beneficial to enhance the tolerance of plants (Dreccer et al., 2018).

The effect of heat stress on grain yield also depends significantly on the genotype (Comadran et al., 2008; Rollins et al., 2013; Talukder et al., 2014; Barber et al., 2017; Balla et al., 2019; Fu et al., 2023; Kaseva et al., 2023). Plants use different adaptive mechanisms to achieve thermotolerance. Stress escape, stress tolerance and stress recovery are the most important coping strategies. Identifying and exploring the genetic components of the different regulatory cascades involved in the successful implementation of these strategies is of utmost importance (Cantalapiedra et al., 2017; Li et al., 2019; Fu et al., 2023; Mikołajczak et al., 2023; Abasi et al., 2024). In order to maintain cereal yields under changing environmental conditions, research and breeding should focus on identifying genotypes that are more tolerant to heat stress, key traits that contribute more to tolerance, and the development of effective screening methods for selection (Acuña-Galindo et al., 2014; Araus et al., 2023; Fu et al., 2023; Kumar et al., 2023). Of the temperate cereals, barley is grown in a wider range of environmental conditions and is the dominant crop in marginal areas, especially where drought and heat stress are more common (Ceccarelli et al., 2011).

Several methods have been used to investigate the responses of different genotypes to abiotic stresses, which can be grouped into two main approaches: field and controlled environment experiments, both hindered by different experimental bottlenecks. In field trials, the biggest problem is usually the complexity of the effects and interactions between plants, soil, geography, climate and weather conditions, not to mention other potential confounding factors such as different biotic stresses and crop production technologies. This complexity prevents a precise identification of the primary drivers behind the actual plant responses. Moreover, the seasonal occurrence of any stress, alone or in combination, is unpredictable, making it uncertain whether specific stress experiments can be conducted. To address the challenges posed by field conditions, experiments are performed either in multiple seasons and locations or under late-sown conditions to study different heat stress scenarios (Ogbonnaya et al., 2017; Wiegmann et al., 2019; Kaseva et al., 2023; Liu et al., 2019; Shirdelmoghanloo et al., 2022; Kumar et al., 2023; Abdelghany et al., 2024). Multi-location and multi-seasonal trials are of great importance in determining the environmental resilience and yield stability of different cereal genotypes. With additional meteorological information on the locations, the effect of high temperatures on the environmental adaptation of crops can also be modelled (Dreccer et al., 2018; Appiah et al., 2023). Caution is needed, however, when dealing with areas with very different climatic profiles in terms of day length, temperature and precipitation, as genetic determinants of developmental patterns can become excessively confounding factors (Comadran et al., 2008; Francia et al., 2011; Benaouda et al., 2022). Late planting experiments, supplemented by irrigation, are also used to mimic the effect of a general rise in temperature. However, the basic

criterion for this type of approach is that seasonal variations in day length should be small, which means that the experimental sites must be close to the equator and/or genotypic sensitivity to photoperiod is not a problem (Araus et al., 2023). Even in this situation, there may be other interfering factors, such as a shortened vegetative period, or exposure of the plant to a higher temperature profile (in the non-stressed range) during the growth cycle, which inevitably leads to lower biomass and, in itself, lower grain yield (Sukumaran et al., 2018; Li et al., 2019).

Controlled environmental experiments represent a completely different approach. These experiments are mostly carried out on individual plants and pots, so the results are considered by some to be irrelevant for field trials (Araus et al., 2023). An important advantage of controlled environmental studies, however, is precisely that complex plant-environment interactions can be unlocked by changing only one or a few selected factors in an otherwise continuously maintained environmental background. In studying abiotic stress responses, controlled environmental experiments can also be useful to rule out the confounding effects of differences in developmental stages between genotypes when the stress is applied. The various stress effects can be tested separately or in combination at specific developmental stages of each plant, using a defined intensity, duration and frequency of the stress treatment (Wang et al., 2012; Talukder et al., 2014; Barber et al., 2017; Balla et al., 2019; Fu et al., 2023; Jampoh et al., 2023; Fábíán et al., 2024). This kind of specificity provides a unique opportunity to explore in detail the physiological and genetic basis of plant stress responses.

In summary, the complexity of the response to heat stress requires a multidisciplinary, holistic approach that integrates physiological and genetic results from field and controlled environmental experiments to ensure that cereal production remains sustainable under the changing climatic conditions. However, not many results have been published so far directly comparing the results of these two basic experimental approaches (Talukder et al., 2014; Teklemariam et al., 2023). Based on this, our main objective was to analyse and compare the responses of barley cultivars to heat stress measured in field and controlled environmental experiments. To this end, a four-year field trial was carried out in a single location, under varying meteorological conditions, on a panel of 190 winter and facultative barley of different geographical origins. In parallel, 28 barley cultivars from the above panel were included into controlled environmental tests where their reactions were determined to a single and to a combined heat stress treatment. Single heat stress was applied at heading, while combined heat stress at first node appearance followed by an additional stress at heading.

2. Materials and Methods

2.1. Plant material

From the barley world collection of LTT panel set up for studying frost tolerance (Muñoz-Amatriaín et al., 2020), 190 barley cultivars were selected forming the panel of BARGEN for further examining barley ecological adaptation, yield formation and abiotic stress tolerance in the Centre for Agricultural Research (HUN-REN ATK), Martonvásár, Hungary (Suppl. Table 1). Additional information on the 190 barley genotypes can be found in Muñoz-Amatriaín et al. (Muñoz-Amatriaín et al., 2020). In the framework of this research, BARGEN was genotyped with the 45 K Infinium SNP chip of TraitGenetics (Suppl. Fig. 1) and phenotyped in a field-sown experiment in 2018 (Suppl. Fig. 2). From the BARGEN panel a subset of 28 barley cultivars were selected for the further testing of heat stress tolerance under controlled environmental conditions. The selection was performed to cover the genetic and phenotypic diversity present in BARGEN (Suppl. Figs. 1 and 2). Thus, 14 – 14, two and six-rowed barley cultivars were included in the controlled heat stress experiment (Suppl. Table 2).

2.2. Conditions in the multi seasonal field-sown experiment

The entire BARGEN panel of 190 varieties was field-tested for four consecutive years (2019–2022) at the same location, at the Centre for Agricultural Research, HUN-REN, Martonvásár, Hungary (Latitude: 47° 21' N, Longitude: 18° 49' E, Altitude: 150 m). [Suppl. Table 3](#) contains the meteorological data of the four years. Sowings were made around 10 October each year, following the same experimental design that was applied in wheat by Kiss et al. ([Kiss et al., 2019](#)) and Horváth et al. ([Horváth et al., 2023](#)); Five rows of each genotype with a plant density of approximately 150 per m² were sown in a 1.0 × 2.0 m plot, with a row spacing of 20 cm within each plot. 188 barley genotypes were sown without replications, while two (the early-heading 'Kompolti early' and the medium-late-heading 'Dicktoo') were sown as controls in seven replications, evenly distributed in the experimental area. This was done to assess the homogeneity of the experimental field, which was found to be adequate in all four years, ensuring comparative analyses (data not shown).

Of the developmental phases, the booting stage (ZD49, when the awns were visible just above the flag leaf sheath) was chosen to be recorded. It was then used as proxy for heading date in the field experiments ([Tottman and Makepeace, 1979](#)). This was done to minimize the uncertainty arising from the well-known phenomenon in barley that the elongation processes of peduncle can be abnormal under stressful conditions. The ear may remain partly or entirely within the flag leaf sheath, making it uncertain when later developmental stages occur. After full heading, the plant height was measured from soil surface to the bottom of the ears (PH), as well as the length of the last internode (LIN). Spikes from the 2 × 25 cm sections of two inner rows were harvested at full maturity as the two replicates of each genotype and various parameters related to grain yield were determined.

2.3. Conditions in the controlled environmental experiment

The heat stress experiment was conducted in CONVIRON growth chambers (PGV-36 and G-30 cabinets; Conviron, Winnipeg, MB, Canada), in the Phytotron facilities of HUN-REN ATK, Martonvásár. It consisted of three treatments: control (C), single heat stress applied at the booting stage (ZD49, ([Tottman and Makepeace, 1979](#)), referred to as Hs), and combined heat stress applied at first node appearance and again at booting stage, after a recovery period (ZD31+ZD49; referred to as Hd). For all three treatments, the germinated seedlings were vernalized for 60 days in peat blocks at 4°C, low light intensity and short days. The vernalized plantlets of one-two leaf stages were then transferred to individual pots containing a 3:2:1 mixture of about 1.5 kg of garden soil, compost and sand. All plants were grown under control conditions until a given developmental stage was reached, when the stressed batches were placed in the stress chamber for a given period of time and then returned to the control chamber for regeneration. All the plants were then kept under control conditions until maturity. The environmental conditions in the control treatment were constant throughout the experiment: a 16-h photoperiod, a PAR light intensity of 240 μmolm⁻²s⁻¹ provided by metal halide lamps, and a constant ambient temperature of 18°C day and night. The light conditions in the heat stress chambers were the same as in the control, but a specific daily temperature profile was applied. The nighttime temperature of 20°C was followed by a daytime temperature that gradually increased to 30°C for ZD31 and 35°C for ZD49, and was maintained for 8 hours of the 16 hour daytime period and then gradually reduced to 20°C at night. The length of the stress treatments was 5 days in stage ZD31 and 10 days in stage ZD49. Special care was taken to keep the plants well watered during the heat stress to exclude water deficit. For each barley genotype and each treatment, 7–7 plants in individual pots were used as replicates. After full heading, the plant height was again measured from soil surface to the bottom of the ears (PH), as well as the length of the last internode (LIN). The seven replicates (plants) of each treatment and each genotype

were harvested at full maturity and various parameters related to grain yield were determined.

2.4. Evaluations and transformation protocols of grain-yield related traits in the two experimental set-ups

After harvest, the same set of morphological and grain-yield related traits were evaluated in both the field and controlled experiments. The following morphological parameters included length of the last internode (LIN), length of the main spike (EaL), number of spikelets / main spike (SPIK), spike density (DENS) and in the case of the controlled experiment only, the weight of aboveground ripen plants without ears (BIOM). The grain yield related traits consisted of various parameters of the main spike such as number of grains / spikelet (SPS), number (MSN), weight (MSW) and thousand kernel weight of grains (MTKW). In addition, number of reproductive tillers/25 cm (for field experiment) or plant (for controlled experiment, referred to as RT in both cases) were counted. The total number (SSN) and weight of grains (SSW) in the reproductive tillers harvestes were measured. MSW plus SSW represented the 25-cm row (field) or the plant (controlled) grain yield (GY). With combining the data of main and side ears, the average grain number, weight and thousand kernel weight / spike (ASN, ASW, and ATKW) were also calculated.

In order to focus on year effects and genotype × year interactions, the dominant impact of headrow type on phenotypic variation ([Supplementary Figure 2](#)) had to be excluded for higher order analyses in the field-sown experiment. To this end, the annual ratio of each trait was calculated for each variety in relation to the four-year average, applying the following formula:

$$r_{p,y} = p_i / ((\sum_{i=1}^4 p_i) / 4), \quad (1)$$

where $r_{p,y}$ = yearly parameter (trait) ratio, p_i = original parameter value in the i^{th} year ($i = 1-4$). The matrix of the transformed data was then subjected to higher-level statistical analyses.

In the case of the controlled environmental test, a calculation method similar to the simple moving average technique ([Yudianto et al., 2021](#)) was used to normalize the data between the seven plants of each genotype in each treatment (batches). The two data of the lowest and highest replicates (plants) within one batch were replaced step by step by the respective mean value of the batch, until the number of replicates was reduced from the initial seven to four. The four replicates thus normalized were included in further analyses. In this case, the changes in the various parameters resulted by the heat stress treatments were expressed as percentage of the corresponding values in the control treatment.

In the case of both environments, we applied the methodology of Slafer et al. ([Slafer et al., 2022](#)) and Serrago et al. ([Serrago et al., 2023](#)) for evaluating the types of heat stress response across the individual barley genotypes. The only difference was that instead of the lowest value we used the treatment average acrc point, applying the following formula:

$$r_{p,t}^i = ((pHi / pCi * 100) / ((\sum_{i=1}^n (pHi / pCi * 100) / n)) * 100, \quad (2)$$

where $r_{p,t}^i$ = parameter change% of i^{th} genotype. "pHi" equals the original parameter value of the i^{th} genotype in the heat stress treatment (in field experiment – values measured in 2022; in controlled experiment – values measured either in single (Hs) or combined (Hd) heat stress). "pCi" equals the original parameter value of i^{th} genotype in the control treatment (in field experiment – values measured in 2019; in controlled experiment – values measured in the control treatment). "n" equals the total number of genotypes that was 190 in the field and 28 in the controlled experiment.

2.5. Statistical analyses

For analysis of the various morphological and yield component parameters, different commands of the statistical package R (R Core Team, 2016) was applied. The *tidyverse* package was used to generate general descriptive statistics (mean, standard deviation, standard error; (Wickham et al., 2019). Heatmaps and hierarchical cluster analyses were conducted using the *heatmaply* and *dendextend* packages, based on the percentage values of treatments compared to the control, to compare the effect of treatments on all species/genotypes relative to the control (Galili et al., 2017). The UPGMA statistical method (Unweighted Pair Group Method with Arithmetic means) was applied for dendrogram construction (Galili, 2015). Using the *qgraph* package, we visualized complex relationships between variables in large datasets for our analyses using Pearson's correlation statistics (Epskamp et al., 2012). The *qgraph* function has several options to generate specific types of graphical representations.

STATISTICA software package, version 13.5.0.17 (TIBCO Software Inc.) was used for carrying out further exploratory analyses including K-mean clustering, Discriminant analysis, and Principal Component Analyses for various grouping purposes of the genotypes.

3. Results

3.1. Multi-seasonal field-sown experiments

3.1.1. Yielding abilities of 190 barley genotypes

The four years of the experiment were highly variable in terms of temperature and precipitation profiles during the growing seasons (Suppl. Table 3), resulting in different degrees and combinations of abiotic stresses to which the plants were exposed. This meteorological variation was further exacerbated by the difference in the overall rate of barley development over the four years. Although the sowing date was similar each year (around 10 October), barley flowering was early and extended in 2019 (start date 24 April), normal in 2020 (1 May) and late in both 2021 and 2022 (8–12 May). This variation in heading dates meant that plants were at varying stages of development when different abiotic factors affected them in the respective years (Fig. 1). Thus, the spring of 2019 was warmer and drier than the 15-year average, except for May, which was the coldest and wettest of the 4 years, and even compared to the 15-year average. By the time of the first extended heatwave starting on 10 June, grain filling was completed under mostly favourable conditions, even for the later genotypes. Although May and June 2020 were the coldest, and the first heatwave did not occur until late June, this was coupled with the driest April and May, which negatively affected the intensive stem elongation and heading. 2021 was marked by a cool and wet May, especially during the flowering period, followed by the driest and warmest June. On the other hand, in spring 2022, rainfall was adequate, especially in April, but it was the hottest year, as there were often hot days from flowering to ripening (Tmax around or above 30.0°C). Thus, the year 2019 can be considered near-optimal for barley production, while drought was the dominant abiotic factor in 2020 and heat in 2022. In 2021, heat and drought occurred together, but only during the final stage of grain filling.

When the original data were analysed, both genotype and year and their interactions were highly significant components for all traits studied (Suppl. Fig. 3). With the exception of developmental stages, genotype contributed most of the variance in yield-related traits and grain yield, mainly due to fundamental phenotypic differences between the two and six-row type groups. The genotypic values ranged from 63.3 % (GY) to 91.5 % (MSN), expressed as a percentage of the sum of squared variances (SS%). The genotype $\times$ year interaction was highest for grain yield (36.1 %), indicating differential sensitivity of the genotypes to varying combinations of abiotic stresses over four years. The average grain yield over the four years reflected the meteorological conditions. It was highest in the optimum year 2019, followed by the

terminal stress year 2021, then the heat stress year 2022, and finally the drought year 2020 (grain yields were 56.5, 55.4, 54.4, 54.4 and 53.3 g/25 cm, respectively, with LSD_{0.05} = 0.2 between them). In all four years, grain yield showed the strongest positive correlation with average number of grains and weight per spike, followed by the correlation with number of grains and weight of the main ear (Suppl. Table 4). The determining role of these four parameters was strongest in 2021 and 2022. The number of reproductive tillers contributed significantly to GY in three years, but the level of correlation was mostly weak, between 0.26** and 0.38***. The correlation between other developmental, morphological and yield traits and GY was not significant in most years.

To exclude the effect of row type and to better investigate genotype $\times$ year interactions, the original trait data were converted to ratios relative to four-year averages using formula 1 described in subchapter 2.4. Using K-means clustering on the data matrix of 8 transformed traits (MSN, MSW, SPS, RT, ASN, ASW, ATKW and GY) $\times$ 4 years $\times$ 190 barley genotypes, the probability of seven phenotypic groups was the highest (Clu_{nd}1–7). The number of genotypes belonging to a given cluster varied between 19 (Clu_{nd}5) and 36 (Clu_{nd}3); the membership of each barley cultivar is listed in Suppl. Table 1. The correctness of the K-means clustering was verified by Discriminant Canonical Analysis, which proved that 100.0 % of genotypes were correctly assigned ($p < 0.0000$). Ten factors with Eigen values greater than 1 were identified in the Principal Component Analysis, explaining 87.9 % of the total variation. Of these, the first three factors were the most significant, with eigenvalues above or close to 5. Each of them alone explained more than 10 % of the total variance, which together accounted for 48.5 % (Suppl. Table 5). These three factors clearly separated the seven phenotypic groups from each other (Fig. 2a). Each of these factors had a primary effect in explaining GY and the different yield-related traits in a given year. In addition, however, each factor contributed to a lesser extent and with opposite sign to account for GY in a different year (Suppl. Table 5). Factor 1 showed the strongest negative correlation with the GY of the drought prone 2020 (-0.68****). Factor 2 stood in positive correlation with the GY of the optimal 2019 (+0.63****), while Factor 3 correlated negatively with GY of the terminal heat and drought stressed 2021 (-0.75***). It is interesting to note that GY of the heat prone year, 2022 had no primary PCO factor behind it; it stood in positive correlation with Factor 1 (0.58*** and to a lesser extent with Factor 3 (0.37***).

In the drought prone 2020 year, all seven clusters suffered varying degrees of reduction in main ear grain weight, and average thousand kernel weight (Fig. 2). In this setting, members of Clu_{nd}1, Clu_{nd}3 and Clu_{nd}4 performed better in terms of GY, due to the increased grain numbers both in the main and side tillers, which was also accompanied by the relative increase in average grain weight for Clu_{nd}3 and 4. This suggests that members of Clu_{nd}3 and Clu_{nd}4 are more drought tolerant. On the other hand, Clu_{nd}5 was the most sensitive to drought; the strong decrease in GY was mainly caused by a significant reduction in grain weight.

In 2021, the general trend in the seven clusters was a decrease in the number of main grains and an increase in the number of reproductive tillers. Only Clu_{nd}7 was more effective in maintaining GY, due to its ability to preserve average number of grains, weight and thus average thousand -kernel weight. Clu_{nd}1 was the most sensitive. In this cluster, the decrease in average number of grains and weight was so severe that neither the increased productive tiller number nor the slightly increased thousand-kernel weight could compensate for the loss, resulting in the most reduced GY.

In the heat stress-prone year of 2022, overall trends showed a sharp decline in the number of reproductive tillers and a slight decline in fertility (number of grains per spikelet). In several clusters, these negative effects were counterbalanced by a significant increase in the number of grains per spike and a particularly strong increase in average grain weight. This trend was strongest for Clu_{nd}5 and Clu_{nd}2, leading to an increase in GY. This year, Clu_{nd}4 was the most sensitive, since in this cluster, in addition to the general negative trends, a decrease in the

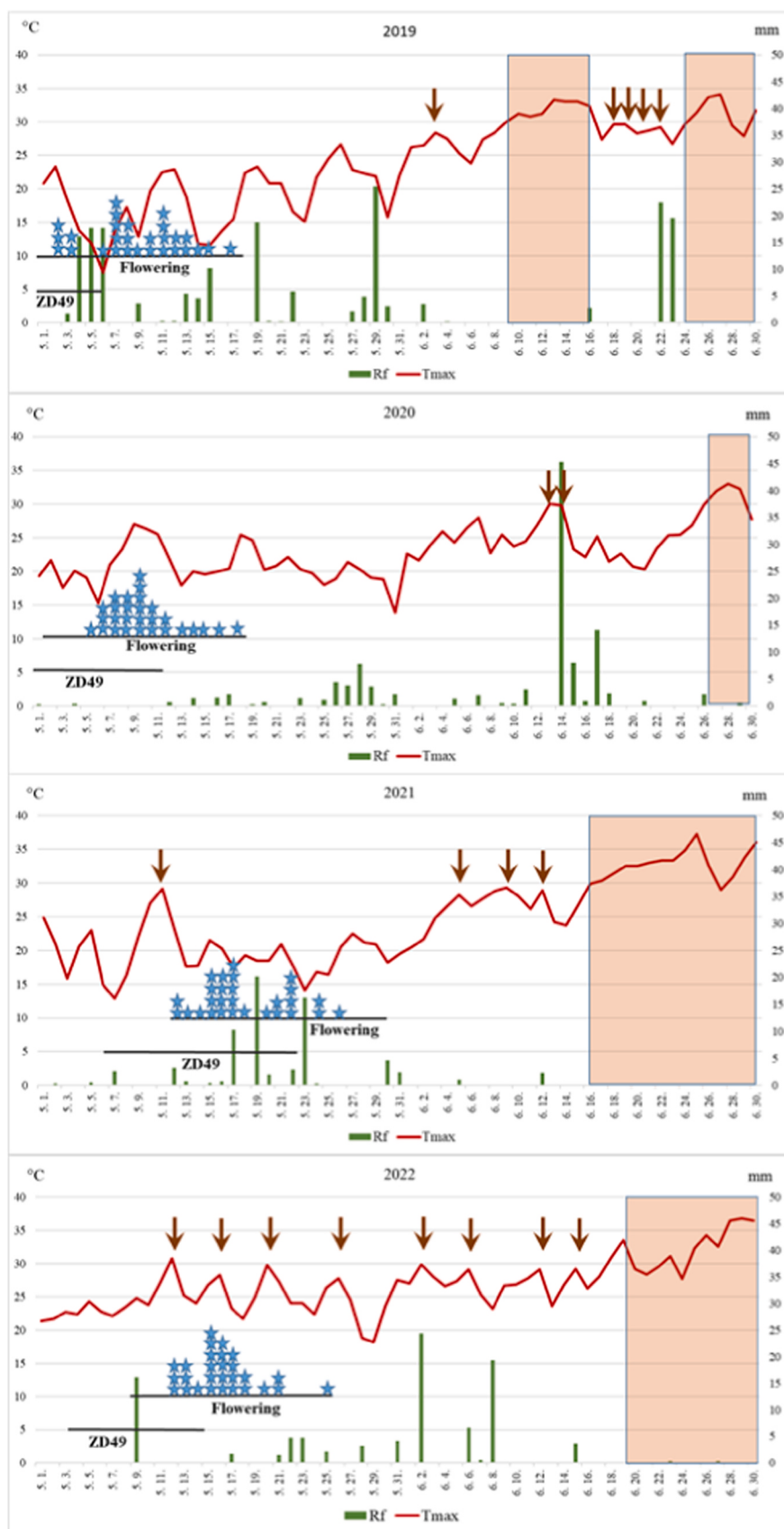


Fig. 1. Daily maximum temperatures (Tmax) and precipitation (Rf) in May and June of the four years and ranges of booting (ZD49) and flowering dates for 190 BARGEN barley varieties in the field trial, 2019–2022, Martonvásár. (blue asterisk: - flowering time distribution values for 28 barley cultivars included in the controlled environmental heat stress experiment, dark red arrows: - days with Tmax close to or higher than 30°C; pink rectangles: - heat waves with Tmax above 30°C for more than three consecutive days).

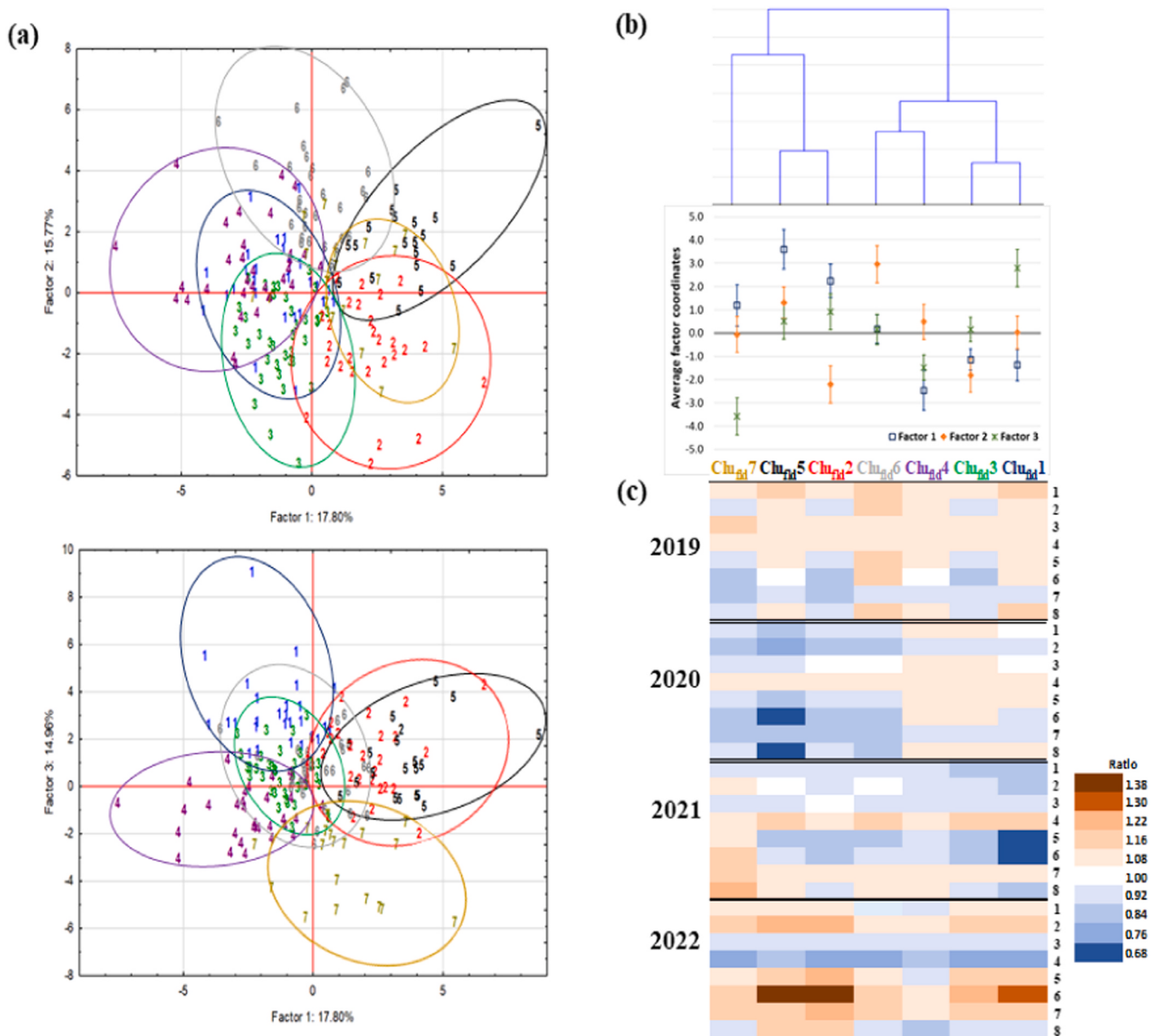


Fig. 2. Clustering of 190 barley genotypes based on their yearly ratio to the four-year averages in morphological, and grain yield related traits under the field-sown experiment, in 2019–2022, Martonvásár. (a) bi-plot graphs of the seven field clusters (Clu_d) including the 190 cultivars from Principal Component Analysis (PCA) (b) phenotypic relatedness of the seven field clusters based on the first three significant PCA factors, and (c) phenotypic heatmap of the seven field clusters (cluster averages of the yearly ratio to the four-year averages of the cultivars belonging to the given cluster). (where traits are the following: 1= main ear grain number, MSN; 2= main ear grain weight, MSW; 3= grain number per spikelet, SPS; 4= reproductive tiller number, RT; 5= average grain number, ASN; 6= average grain weight, ASW; 7= average thousand kernel weight, ATKW; 8= grain yield, GY).

number of grains was also observed.

3.1.2. Predicting the type of heat stress response under field conditions

To evaluate the responses of the 190 barley genotypes to heat stress under field conditions, the seven Clu_d change% in the various traits were compared to each other and to the average change% of the 190 barley genotypes using formula 2 (Subchapter 2.4). Based on the weather patterns of the four seasons, 2019 was considered a control year and 2022 a heat stress year. Clu_d2, followed by Clu_d5, produced the highest grain yield under heat stress conditions in 2022 (Fig. 3). In the case of Clu_d2, the higher productivity was maintained as a result of the higher number and weight of grains in the main and especially in the side ears, which together effectively compensated for the higher loss of the number of reproductive tillers. In Clu_d5, the higher proportion of

reproductive tillers was maintained, but this was associated with relatively lower grain numbers and grain weights in both main and side ears. On the other extreme, the heat stress tolerance of Clu_d1, Clu_d4 and Clu_d6 was found to be the lowest. However, the basis of sensitivity differed significantly between these clusters. For Clu_d1, grain number and grain weight were close to the average, but the reproductive tiller number was reduced the most. Clu_d4 showed the opposite trend. This cluster produced a remarkably high number of side tillers, but this was coupled with the largest reduction in grain number and weight out of all the clusters. In the case of Clu_d6, the grain weight, especially in the main ear suffered the greatest reduction, leading to the low grain yield of this cluster.

The 28 genotypes selected from the total set of 190 barley for the controlled environment study evenly covered the distribution curves of

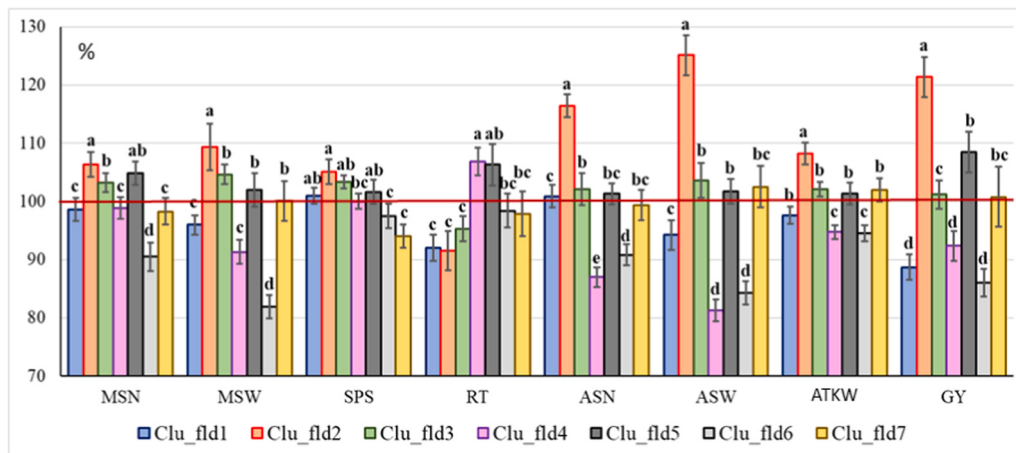


Fig. 3. Comparing the heat stress responses of the seven clusters (Clu_{fld}) of 190 barley genotypes identified under the four-year field sown experiment. (The cluster averages are based on the individual genotypic change % parameters expressed as a proportion of the main average change % of the 190 cultivars, where 2022 data were considered as heat stress, and 2019 data were considered as control. The bars represent standard deviation of the cluster members for each parameter, the pairwise differences were determined by Student T-test. Clusters with the same letter within a trait parameter are not significantly different from each other at $P=0.05$ level). (trait abbreviations: MSN – grain number in the main ear, MSW – grain weight in the main ear, SPS – grain number per spikelet, RT – reproductive tiller number, ASN – average grain number/ears, ASW – average grain weight, ATKW – average thousand kernel weight, GY – plant grain yield).

the change% values for the whole population. This is illustrated by the plots of total number of side grain (SSN) and grain yield (GY) in [Suppl. Fig. 4](#). There was slight underrepresentation only in the extreme positive range.

3.2. Heat stress experiment under controlled conditions

3.2.1. Effects of developmental stage specific heat stress on 28 barley cultivars

A heat stress experiment was conducted on 28 selected barley cultivars under controlled conditions to determine the sensitivity of barley to heat stress and the extent of variability in genotypic responses. Since the response to stress is significantly dependent on the developmental stage at which the plants are exposed to the stress, plants of each variety were stressed at the same growth stage. In the single heat stress treatment, this stage was the heading stage (ZD49), whereas in the combined heat stress treatment, the first stress treatment was applied at the first node appearance (ZD31), followed by a regeneration period and the second stress treatment at the heading stage (ZD49).

Two-way ANOVA confirmed a strong, significant effect of both heat stress and genotype, but their roles varied greatly depending on the individual traits ([Suppl. Fig. 5](#)). The morphological traits, particularly ear length (EAL), ear density (DENS), and total biomass (BIOM), were mainly determined by genotype. The same was true for spikelet number (SPIK) and number of grains per spikelet (SPS). In the latter two traits, the large genotype effect was attributed to the fundamental structural differences between two-row and six-row barley varieties. For yield components, the genotype exerted a greater effect on main ear parameters, while the treatment effect was stronger for the total spike and average spike parameters, especially for the total grain number and total grain weight per plant. As a result, the treatment effect primarily influenced grain yield per plant, while the role of genotype, although significant, was only minor.

High temperature treatment – both single and combined – caused numerous negative effects in the examined varieties, the ratio of which was strongly trait dependent. As the two groups of row-type differed in several yield related traits, the trait averages and intervals in the three treatments are presented separately ([Fig. 4](#)). In general, the morphological traits were less affected, while all the yield related traits showed strong depressions. Comparing the single vs combined heat stress, in most of the cases, the combined stress had similar effects than the single stress; it did not result in dramatically worse or better parameters. In

control treatment, the median of MSN in the six-rowed barley was 45 grains, which varied greatly due to the treatments and genotypes. After the single treatment, MSN decreased to 32, which was further reduced by 5 seeds in the combined treatment. These values for the two-rowed type were 24, 18, and 17, respectively. For the six-rowed barley varieties, the minimum and maximum values were quite wide, indicating a larger variation between the genotypes, especially after heat stress. Regardless of spike type, significant and similar reduction in MSW was caused by the single and combined heat stresses. MSW showed an overall 31–33 % decrease in response to the single treatment compared to the control, irrespective of the row-type. With the combined treatment, a further 12–14 % decrease occurred amounting to a 45 % loss in MSW. MTKW was generally lower in 6-row barley and was less affected by both heat stress treatments than in 2-row barley. The decrease in MTKW of 6-rowed barley were less than 10 % even in the combined stress. For the two-rowed type, this difference was larger underlining the stronger sensitivity of two-rowed barleys in context of thousand kernel weight to exposure to high temperatures during heading. Similar tendencies were apparent for the average grain number, weight, thousand kernel weight of the side ears, but to greater extents.

The grain yield of both two- and six-rowed ear types were heavily affected by exposure to high temperatures. In the case of two-rowed varieties, the average yield in the control treatment was close to 5 g, which decreased by 60 % due to the stress, resulting in a median value of 2 g in the single heat stress. This further decreased to 1.8 g in the combined treatment. For the six-row varieties, a 52 % reduction in yield was observed following the single treatment, while following the combined treatment the plants performed on average 60 % below the control.

3.2.2. Genotypic differences in responses to single heat stress

Heatmap dendrogram has been utilised for visualising the genotypic differences in stress response ([Fig. 5](#)). In general, grain yield after heat stress showed the strongest association with the total grain number, total ear and grain weight; together they formed a tight subcluster. This was followed by the next closest subcluster of the values measured in the main and in the average ears, with the exception of thousand kernel weights. The associations of GY were weak with the reproductive tiller number, ear morphology parameters, biomass and thousand kernel weight. These parameters formed two separate subclusters each distant from that including GY. While GY and its most important determinants of total grain characteristics showed various level of reductions

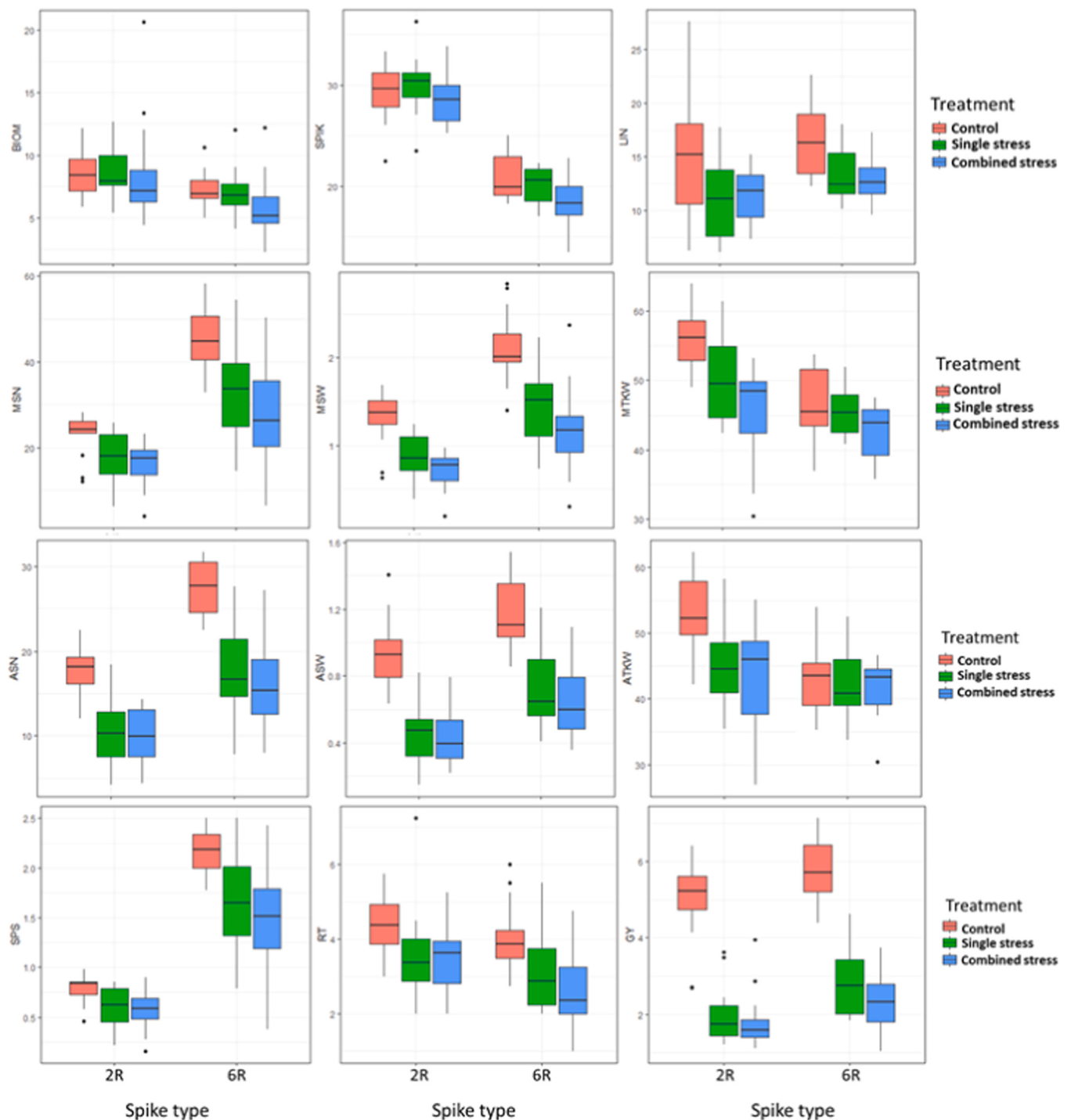


Fig. 4. Effects of single and combined heat stress on various morphological and yield-related traits as represented by the group averages of 14 two-rowed and 14 six-rowed barley cultivars. (2 R= two-rowed, 6 R= six-rowed). (trait abbreviations and units: BIOM – aboveground ripened biomass, SPIK – spikelet number in main ear, LIN – last internode length, MSN – grain number in the main ear, MSW – grain weight in the main ear in gram, MTKW – thousand kernel weight in the main ear in gram, ASN – average grain number per ears, ASW – average grain weight per ear in gram, ATKW – average thousand kernel weight in gram, SPS – grain number per spikelet in the main ear, RT – reproductive tiller number, and GY – plant grain yield in gram).

compared to the control values across the genotypes, biomass, ear morphology and thousand kernel weight demonstrated mostly increased values to various extents. The 28 barley genotypes however covered a wider range of negative to positive values in reproductive tiller numbers, main and average grain number and weight, which provided the basis of separating them into four distinct groups (the group position of each individual genotype is listed in [Suppl. Table 6](#)). None of these groups was uniform from the aspect of row-type, they included both two

and six-row barleys, in various ratios. From the heatmap it is clearly visible, that Groups 2 and 1 contained the most heat sensitive genotypes, while barleys in Groups 3 and 4 demonstrated a higher level of heat stress tolerance. For the purpose of better distinctions these groups are further referred with the abbreviation of Group_{HS}.

For further analysing the basis of the differences in heat stress responses between the four groups, their group average values of change in the various traits were compared to each other and to the average

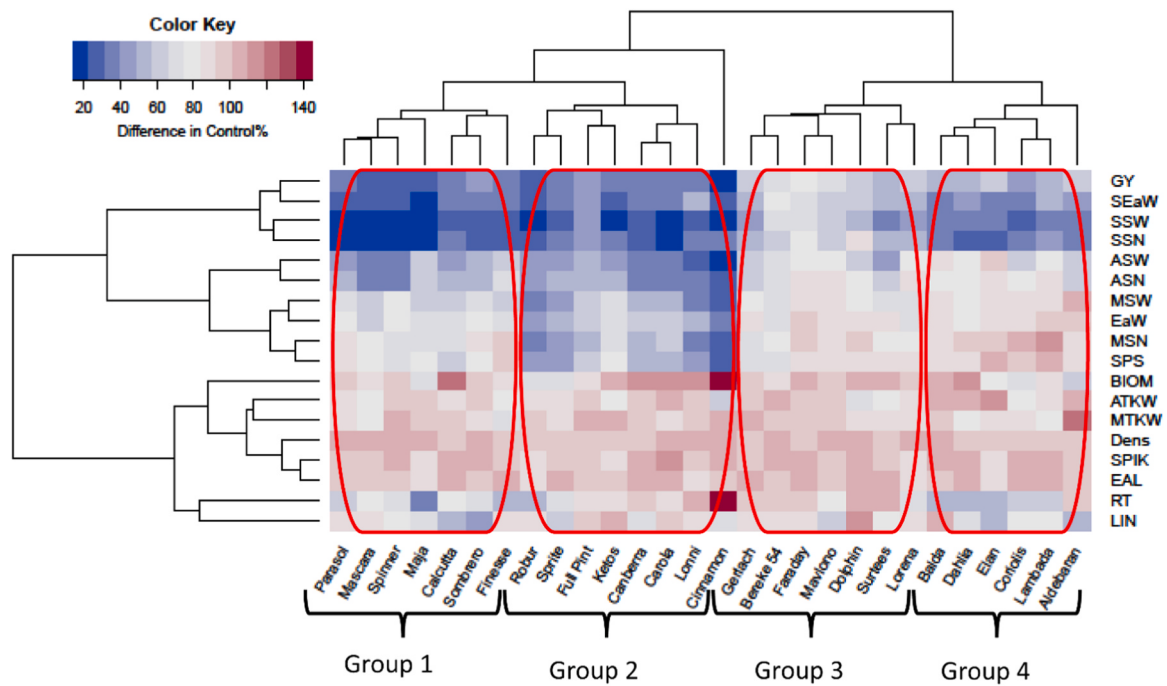


Fig. 5. Heat map dendrograms of 28 barley genotypes (columns) and 18 traits (rows) under a single heat stress applied at the booting stage (ZD49), expressed as % changes relative to control values. (trait abbreviations: Gy – grain yield/ plant, SEaW – total weight of the side ears, SSW – total grain weight in the side ears, SSN – total grain number in the side ears, ASW – average grain weight, ASN – average grain number/ears, MSW – grain weight in main ears, EaW – main ear weight, MSN – grain number in the main ears, SPS – grain number per spikelet, BIOM – aboveground ripened biomass, ATKW – average thousand kernel weight; MTKW – thousand kernel weight in the main ear, DENS – ear density, SPIK – spikelet number in main ear, Eal – ear length, RT – reproductive tiller number, LIN – last internode length).

change% of the 28 barley genotypes (Fig. 6). Under control treatment, there were no significant differences between the group averages for most of the traits, with only one exception, the reproductive tiller number. Under the single heat stress however, the differences between the four groups became significant in the cases of grain yield and its related traits. From the aspect of grain yield, Group_{HS}3 remarkably exceeded (141 %) and the next best group Group_{HS}4 (108 %) produced around the change% average of 28 barleys, while the grain yields of

Group_{HS}1 and 2 remained well below it (76 and 79 %, respectively). The phenotypic basis of better heat stress tolerance levels and thus stronger adaptive capacities detected in Group_{HS}3 and to a smaller extent in Group_{HS}4 remarkably differed from each other's. In addition to the almost similar number of reproductive tillers, genotypes in Group_{HS}3 were able to produce significantly larger total grain number and weights during the recovery period, while those in Group_{HS}4 were able to better retain the grain number and weight in the main ears

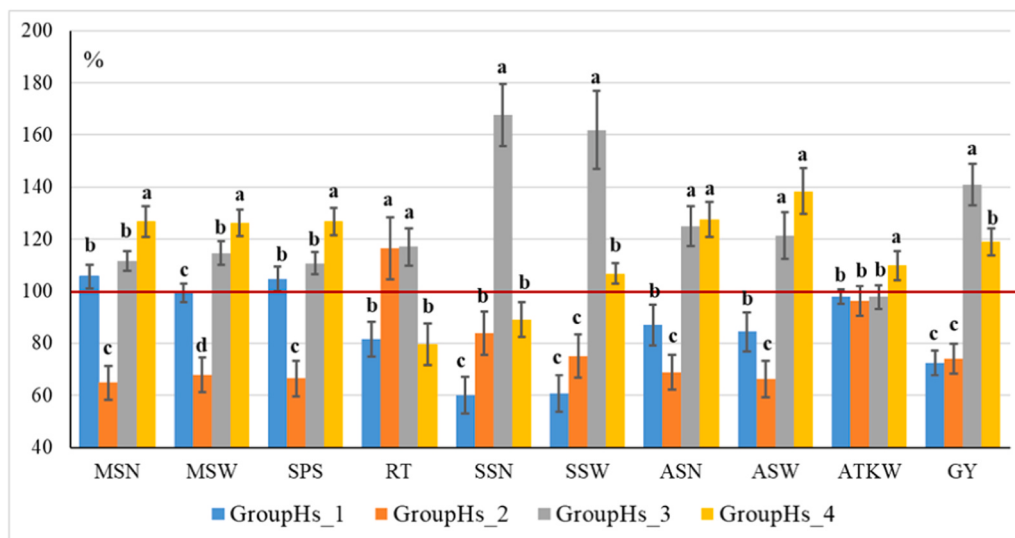


Fig. 6. Comparing the stress responses of the four barley groups (Group_{HS}), identified in the heatmap dendrogram under single heat stress treatment applied at booting stage (ZD49). (The group averages are based on the individual genotypic change % parameters expressed as a proportion of the main average change% of the 28 cultivars. The bars represent standard deviation of the group members for each parameter, the pair-wise differences were determined by Student T-test. Groups with the same letter within a trait parameter are not significantly different from each other at P = 0.05 level). (trait abbreviations: Gy – grain yield/ plant, SSW – total grain weight in the side ears, SSN – total grain number in the side ears, ASW – average grain weight, ASN – average grain number/ears, MSW – grain weight in main ears, MSN – grain number in the main ears, SPS – grain number per spikelet, ATKW – average thousand kernel weight, RT – reproductive tiller number).

during the heat stress period. Thus, they represented different strategies of heat stress responses; Group_{HS}_4 represented the true heat stress tolerant genotypes, while barleys in Group_{HS}_3 possessed better recovering and compensating capacities after the stress ceased.

The distribution of the 28 barley genotypes between these four groups were relatively even; seven cultivars belonged to Group_{HS}_1, eight to Group_{HS}_2, seven to Group_{HS}_3, and six cultivars belonged to Group_{HS}_4 (Suppl. Table 6).

3.2.3. Genotypic differences in response to combined heat stresses

In the case of the combined heat stress treatment, the ratio of positive changes in the morphological traits and thousand kernel weight, which was characteristic to the single heat stress, almost disappeared (Fig. 7). The associations between the various traits, however followed a pattern similar to that identified in the single heat stress, with one exception. While in single heat stress, biomass was closely clustered with thousand kernel weight, here it was the closest to the reproductive tiller number. Most varieties were severely damaged by the combined heat stress, and only a few tolerated it better. Based on their responses, the cultivars were divided into four groups (hereafter referred to as Group_{HD}), covering the range of sensitivity; four members of Group_{HD}_3 were more tolerant, followed by three varieties of Group_{HD}_2. Group_{HD}_4 contained the four most sensitive cultivars, while the remaining 16 cultivars formed Group_{HD}_1 with a sensitivity level close to that of Group_{HD}_4, but with a distinctly different trait pattern.

When examining the basis of the better tolerance, we again identified the two opposite types of response, similar to the one described for the single heat stress (Fig. 8). Cultivars belonging to Group_{HD}_2 were the best in preserving the grain number and weight in the main ear during the stress, and to a lesser extent in the side ears after the heat stress, thus they represented true tolerance. However, this group suffered the greatest loss in reproductive tiller number. The members of Group_{HD}_3 presented the opposite strategy. In their case, the ratio of number and weight of grains in the main ear was average, but they were better able

to maintain the number of reproductive tillers and the number and weight of grains in them. In short, they were better able to compensate for the adverse effects of heat, after the stress was removed. In terms of grain yield, however the two strategies proved to be similarly effective under the combined stress treatment.

There was strong genotypic overlap between the similar reaction type groups identified in the two treatments. Three cultivars of the stress tolerant Group_{HS}_4 from the single heat stress formed the stress tolerant Group_{HD}_2 of the combined heat stress. These three cultivars were Dahlia, Elan and Lambada, of which the first two genotypes produced good level of grain yield under control conditions (Suppl. Table 6). Balda, Coriolis and Aldebaran, on the other hand, have lost this ability due to repeated exposure to heat and have become members of the most frequent sensitive group (Group_{HD}_1). In the case of stress compensation, four of the seven barley varieties identified in the single heat stress treatment were able to retain this ability under combined heat stress conditions; these were Mavloro, Gerlach, Dolphin and Surtees. With the exception of Surtees, they produced good level of grain yield under the control conditions, as well (Suppl. Table 6). In this case too, the remaining three cultivars were transferred to the most frequent sensitive group. There was one cultivar, Sprite, however, which was grouped to the most sensitive cultivars in the single heat stress, but became member of the group with better compensation ability under the combined heat stress.

3.3. Comparison of the controlled and field data

In the case of the 28 barley genotypes that were included both into the controlled environmental test and into the field-sown experiment, comparisons could be made between their heat stress responses under the two environmental set-ups. For this purpose, we used the same transformed data of % change with the application of Formula 2 (Subchapter 2.4). This procedure removes the differences between experiments as source of variation in order to be focused on environmental/

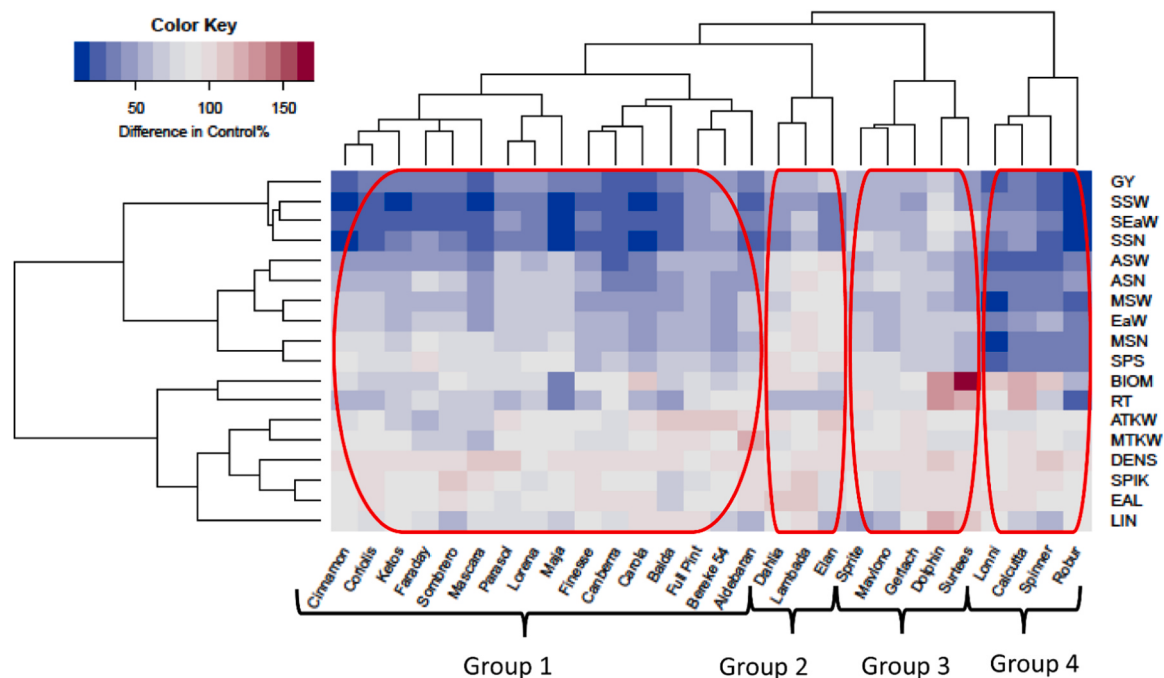


Fig. 7. Heat map dendrograms of 28 barley genotypes (columns) and 18 traits (rows) under a combined heat stress at first node appearance (ZD31) then at booting stage (ZD49), expressed as % changes relative to control values. (trait abbreviations: Gy – grain yield/ plant, SEaW – total weight of the side ears, SSW – total grain weight in the side ears, SSN – total grain number in the side ears, ASW – average grain weight, ASN – average grain number/ears, MSW – grain weight in main ears, EaW – main ear weight, MSN – grain number in the main ears, SPS – grain number per spikelet, BIOM – aboveground ripened biomass, ATKW – average thousand kernel weight; MTKW – thousand kernel weight in the main ear, DENS – ear density, SPIK – spikelet number in main ear, EAL – ear length, RT – reproductive tiller number, LIN – last internode length).

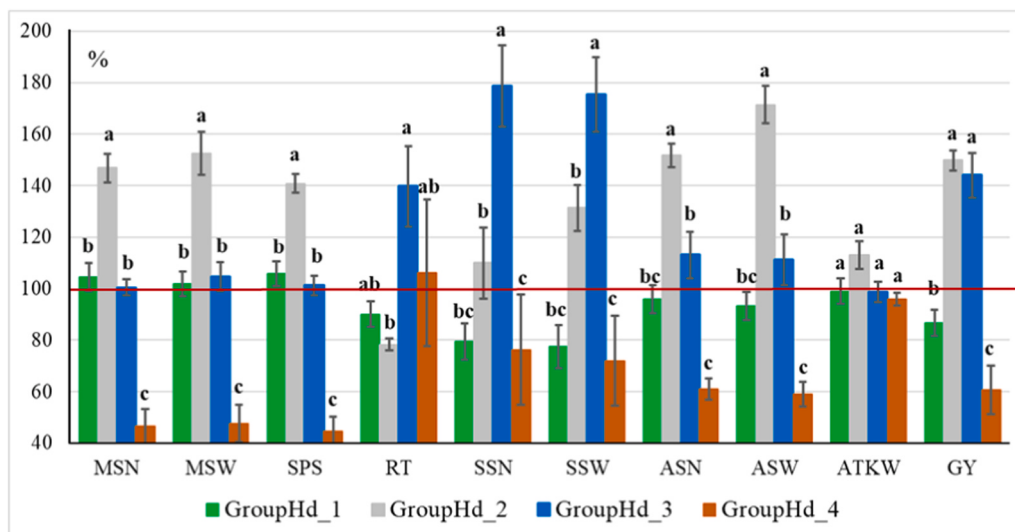


Fig. 8. Comparing the stress responses of the four barley groups (Group_{Hd}), identified in the heatmap dendrogram under combined heat stress treatment at first node appearance (ZD31) and booting stage (ZD49). (The group averages are based on the individual genotypic change% parameters expressed as a proportion of the main average change% of the 28 cultivars. The bars represent standard deviation of the group members for each parameter, the pair-wise differences were determined by Student T-test. Groups with the same letter within a trait parameter are not significantly different from each other at P = 0.05 level). (trait abbreviations: Gy – grain yield/ plant, SSW – total grain weight in the side ears, SSN – total grain number in the side ears, ASW – average grain weight, ASN – average grain number/ears, MSW – grain weight in main ears, MSN – grain number in the main ears, SPS – grain number per spikelet, ATKW – average thousand kernel weight, RT – reproductive tiller number).

genotypic effects across the datasets. There were significant associations between the parameters from the two environmental conditions confirmed both by Principal Component and by regression analyses. In the Principal Component Analysis, though the weights and orientations of the background grouping factors for both the field and the controlled data were similar in general, there were some differences, as well (Suppl. Fig. 6). Factor 1 (Eigenvalue 6.52, and explaining 54.4 % of the variance) showed the strongest correlations with most of the yield related traits in all treatments, with the exception of the main ear grain number, where its effect was less prominent in the case of the two controlled treatments. In addition, for the field data sets Factor 2 (Eigenvalue 1.64, explaining 13.7 % of variance) represented also significant grouping force, especially through total side grain number and weight and grain yield. The cultivars of the four Groups identified in the single heat stress treatment (Group_{Hs}) could be distinctly separated based on the data-matrix combining the results of field and controlled environmental tests.

The regression analyses carried out on the 28 barley cultivars identified significant positive associations between the change% of single

heat or combined heat stresses vs field heat stress, with the exception of the thousand kernel weight (Table 1). In the case of the single vs. field heat stress, the range of significant correlation coefficients of various grain yield related traits varied between 0.405* (main ear grain number) and 0.484** (total grain number in the side ears). While for the combined vs field heat stress it was between 0.339+ (main ear grain number) and 0.669*** (total grain number in the side ears). When the response profiles of the four Groups (Group_{Hs}) of single heat stress were compared with those under field, tendencies similar to the single heat stress could be identified between them, although within a much narrower interval (Fig. 9a). Group_{Hs}.3 with the best grain yield under both conditions, was the best in preserving reproductive tiller number, total grain number and weight. In the regression graphs, all or most of the cultivars of Group_{Hs}.3 were in the best quarter as was the case for both SSN, and GY (Fig. 9b and c). The main ability of Group_{Hs}.4 to preserve the grain number in the main ear was not that explicit under field conditions, but in spite of that, the grain yield change% of this group was the second best. On the other hand, Group_{Hs}.1 possessed the lowest

Table 1

Comparisons between the heat stress responses of 28 barley cultivars evaluated under controlled versus under field sown conditions based on linear regression models. (regression analyses were carried out on the transformed data of change% in the various traits of the 28 barleys using formula 2 (Subchapter 2.4) caused by the heat as compared to the appropriate controls).

Traits	Hs versus Hfield				Hd versus Hfield			
	r	R ²	P	Δ	r	R ²	P	Δ
Main ear grain number	0.405	0.164	*	1.42	0.339	0.115	+	1.39
Main ear grain weight	0.429	0.184	*	1.05	0.470	0.221	*	1.94
Main ear thousand kernel weight	0.180	0.032	ns		0.238	0.057	ns	
Reproductive tiller	0.457	0.209	*	1.05	0.375	0.141	*	1.00
Grain number in side ears	0.484	0.235	**	1.80	0.669	0.447	***	2.62
Grain weight in side ears	0.443	0.196	*	1.54	0.464	0.215	*	1.73
Average thousand kernel weight	0.239	0.058	ns		0.200	0.040	ns	
Grain yield	0.464	0.215	*	1.31	0.577	0.333	**	1.73

(In the field: data of 2022 as heat stress versus data of 2019 as control - Hfield; in the controlled environment: single or combined heat stress vs control treatment – Hs and Hd, respectively)

r – correlation coefficient

R² – determination coefficient

P – significance level: ns – not significant, +, *, **, *** - significant at P= 0.1, 0.05, 0.01 and 0.001 levels, respectively

Δ - steepness of the regression line

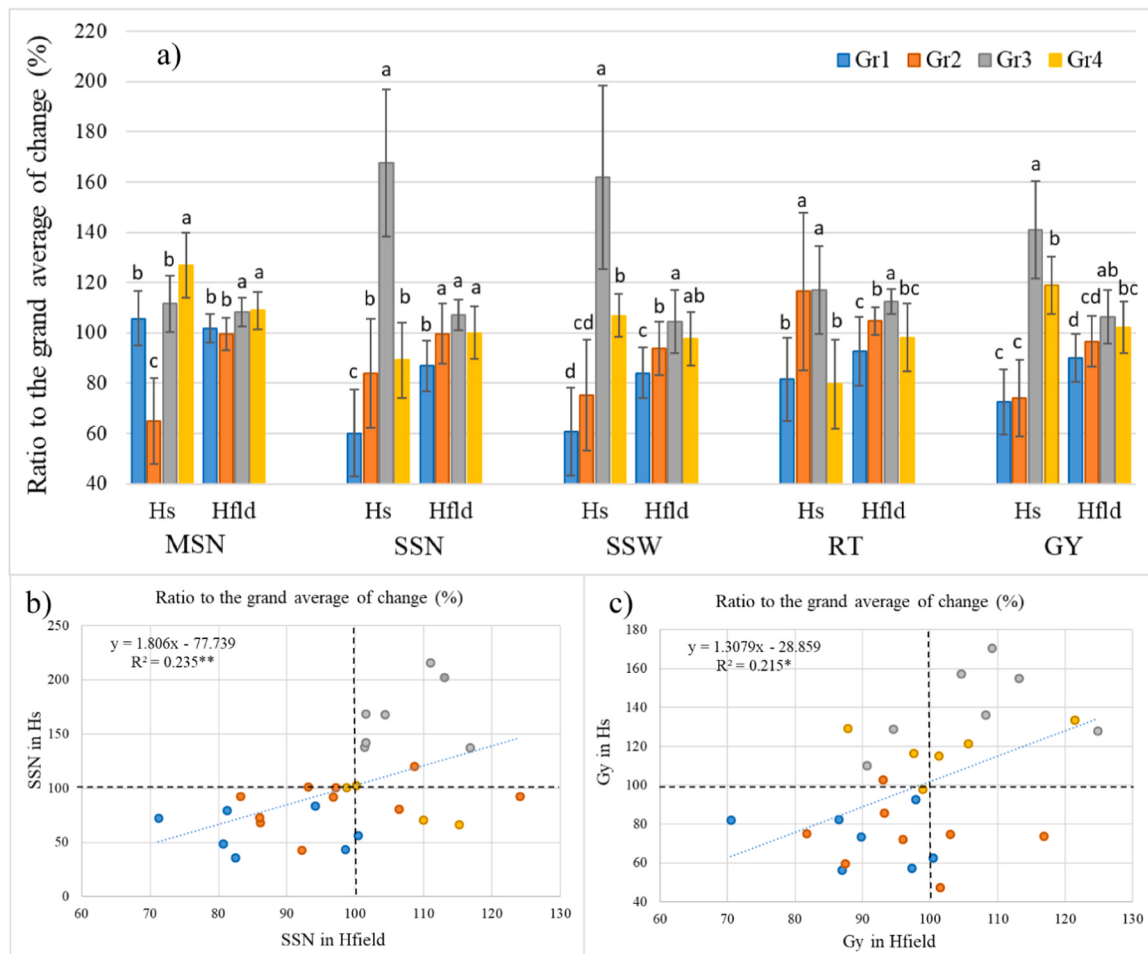


Fig. 9. Association between the heat stress responses of 28 barley cultivars measured under field-sown (Hfld) and under controlled environmental single heat stress conditions (Hs): (a) comparing the average change % values of the four single heat stress groups in some grain yield related traits under single heat versus 2022–2019 field conditions; and regressions (b) for total side grain number (SSN), and (c) for grain yield (GY). (trait abbreviations: MSN – grain number in the main ears, SSW – total grain weight in the side ears, RT – reproductive tiller number).

grain yield retention ability, irrespective to the environment. Its members were placed in the worst quarter for both SSN and GY. It is interesting to note, that the heat stress sensitivities of Group_{Hs}1 and 2 were accentuated by the control conditions. This was especially the case for main ear grain number of Group_{Hs}2 and for the total side grain number and weight of Group_{Hs}1. Similar tendencies and associations were detected in the case of the four Groups (Group_{Hd}) identified in the combined heat stress between their field and controlled stress responses (Suppl. Fig. 7).

4. Discussions

The main aim of the current research was to evaluate the heat stress responses of barley cultivars as measured under both field-sown and controlled environmental conditions, and to compare the results obtained.

4.1. Evaluating heat stress response under field conditions

The four consecutive years of our field-sown experiment provided wide ranges of various meteorological conditions, which also led to diverse barley developmental patterns among the years. Although the experiment was sown at a similar time, every year (mid-October), there was nevertheless an 18-day difference between the beginning of heading date during the four years. Moreover, the two years with later plant development had the most heat days ($T_{max} \geq 30^{\circ}\text{C}$) especially during

and after flowering. Shirdelmoghanloo et al. (Shirdelmoghanloo et al., 2022) when examining the heat stress tolerance of Ethiopian barley landraces under late sown conditions, found that on average very late sown barley was exposed to 15 days above 30°C after ZD49, compared to 10 days for late sown and 6 days for normally sown barley. In our case, there were 18–19 days with T_{max} being close to or above 30°C in the two late years. In spite of this however, heading date only weakly correlated with grain yield even in the two late years ($r = -0.23^*$). Therefore, the effects of developmental phase specific-genes on yield formation were minor at best. These facts made our multi-seasonal field experiment very similar to the use of late sowing for terminal heat stress simulation, with the added advantage of avoiding the confounding effects of shortened vegetative phase, and strong differences in plant developmental patterns.

Regarding the meteorological conditions, some variability between years could be expected, since the Hungarian climate is basically determined by the changing combinations of Mediterranean, oceanic and continental weather fronts. Together with the topographic situation of the Carpathian Basin, these could lead to variable climate from year to year (Bartholy and Pongrácz, 2007; Uzzoli et al., 2019). However, the magnitude of climatic variability experienced in these four consecutive years exceeded the usual level. This demonstrates one of the expected consequences of the intensifying climate change, namely the increased unpredictable fluctuations in the weather conditions of the local environments (Lobell et al., 2011; Mittler et al., 2012; Cammarano et al., 2019). Drought and heat stress occurred both individually and together

during this period, and in only one of the four years were conditions optimal for cereal production. As it follows, in the variance-analyses of the original data, the year main effect and the genotype $\times$ environment ($G \times E$) interaction were both significant factors in the trait variances, although the majority of the variances was covered by the genotype main effect. This was however primarily due to the fact that the BARGEN panel consisted of two and six-rowed genotypes, with distinct differences in grain yield related parameters including reproductive tiller number, grain number and thousand kernel weight (Serrago et al., 2023). In spite of this, the $G \times E$ effect covered more than 30 % of the variability even in the original dataset of grain yield underlining that wide variation existed between the barley genotypes for morpho-physiological characters conferring tolerance to various stresses (Francia et al., 2011). To exclude the row-type effect from further evaluating the bases of the strong $G \times E$ interaction in grain yield and to compare the stress responses of various genotypes, data standardisation was applied across the years for each barley cultivar (Slafer et al., 2022; Kaseva et al., 2023; Kim et al., 2024). This approach made it possible to identify seven groups of barley genotypes with diverse and relatively distinct grain yield response profiles across the four years. As the four years were greatly diverse in meteorological conditions, year with drought (2020) and with heat (2022) as primary stress sources could be identified and be compared with the relatively optimal year (2019). Here, we demonstrated the field data connected to heat stress response (2019 versus 2022).

4.2. Evaluating heat stress response under controlled environment

Under field, heat stress frequently occurs together with additional abiotic stresses such as drought stress, the impact of which has frequently been found to be intensified by heat (Talukder et al., 2014; Li et al., 2019). Thus, it is important to unravel the independent action and biological consequences of high temperature in order to ameliorate the effects of combined abiotic stresses. For this purpose, we selected a subset of 28 barley cultivars from the BARGEN panel that proximately covered the phenotypic and genotypic diversities present in the 190 barleys. This subset was further included in a heat stress experiment carried out in growth chambers. In the controlled experiment, we took advantage of the opportunity to apply the stress at the same specific developmental phase in each genotype. In addition, a recovery period with optimal conditions was provided for the plants after the stress treatment, which is rarely the case under field-sown experiments (Talukder et al., 2014; Balla et al., 2019). This experimental set-up contributed to a more accurate evaluation of the stress responses of various genotypes. Applying $T_{max} = 35^\circ\text{C}$ heat for 10 days (with a daily Tave of 27.5°C) from the pre-anthesis stage of ZD49 meant that flowering, fertilisation and early grain filling of the plants were all affected. Coinciding with other experimental results, this treatment hardly influenced the various morphological parameters such as biomass, plant height, spike length, spikelet number and last internode length, but it had a strong deteriorating effect on grain yield related traits. Grain number and weight suffered to the largest extent, a reduction of 61.9 and 56.6 % of the control treatment was observed in single and combined heat stress, respectively. Dreccer et al. (Dreccer et al., 2018) compared the effects of maximum temperature above 30°C during grain filling among several crop species and they found that heat stress had a more significant impact in wheat, followed by canola, chickpea and barley in decreasing order. In this research, the protocol of the single heat stress treatment was the same as in the case of the GY49_H10 treatment applied by Balla et al. (Balla et al., 2019) in wheat. When comparing the grain yield reduction caused by heat stress in wheat and barley, the stronger sensitivity of barley became apparent. While the average grain yield reduction was 38.1 % in 101 wheat cultivars, for the 28 barley of this research this value was 55.0 %. Although there was a significant and remarkable difference between the barley cultivars, none of them could preserve its yielding ability when subjected to heat. In

barley, the interval of grain yield loss due to single heat stress was between 22.4 % and 78.4 %. This result indicates that there may be a developmental phase specific crossover type relation in the heat stress tolerance of wheat and barley. Wheat generally exhibits greater tolerance in the pre-anthesis phases compared to barley, while the opposite is characteristic to the post-anthesis phases. The phenomenon of genotypic differences between the developmental phase specific sensitivities within plant species were reported for wheat (Bányai et al., 2014; Barber et al., 2017; Balla et al., 2019; Cseh et al., 2024).

4.3. Combined heat stress: priming or mimicking better the natural conditions?

Under field conditions, it is a more realistic scenario that plants are exposed to multiple stress events at different periods of their life cycle, as was the case during the four consecutive years of our field-sown experiment. It is hypothesised that exposure of plants to transient stresses often helps them to better tolerate subsequent more severe stresses through the process of priming effect that establishes a stress memory via phenotypic, molecular or metabolic adjustments. These changes may last from a few days to several months and enable the plants to give a stronger and faster response to subsequent stress stimuli (Wang et al., 2014; Menezes-Silva et al., 2017; Abid et al., 2018). There are results currently published that the priming effect can be even transgenerational as was suggested for drought tolerance in wheat (Thabet et al., 2024). The somatic stress memory, however, may depend on the duration of exposure, the nature and intensity of the priming stimulus, and those of the subsequent stresses (Hilker et al., 2016). The extent and mechanisms of stress priming are not well understood and the results are quite controversial, some demonstrating its positive effects (Wang et al., 2014; Fan et al., 2018; Thabet et al., 2024), while others show neutral or even negative effects (Wollenweber et al., 2003; Mendanha et al., 2018). Balla et al. (Balla et al., 2021), examining heat stress priming in a larger number of wheat genotypes, concluded that the phenomenon of heat priming may exist, but it is not a universal response of wheat. Its occurrence strongly depends on the genotypes and developmental phases, as well as various parameters of the stress itself. In the present controlled environmental experiment, heat priming in barley was examined with the application of the combined heat stress. The five-day 30°C heat stress at the beginning of stem elongation represented the priming treatment, which was followed by the main heat stress of 10-day 35°C at booting (ZD49 stage). In most of the traits, the average values achieved in the combined stress were similar to those in the single stress or even lower, with the only exception of the average thousand kernel weight, which showed slight (in the 2-rowed group) to remarkable (in the 6-rowed group) increase (Fig. 4). Averaged over the 28 barley genotypes, the grain yield in the combined heat stress was 86.4 % of that in the single heat stress with an interval of 42.7 and 160.3 % (Suppl Table 6). Ten cultivars of the 28 produced the same or higher grain yield as in the single stress, though none was close to the control yield, and that was independent of the row-type. The present results in barley therefore confirm the conclusions of Balla et al. (Balla et al., 2021) that stress priming seems to have a strong genotypic dependence as well. It is also possible that the priming effect is more stress specific, and other stresses such as drought may elicit stronger and more similar priming among the various genotypes (Abid et al., 2018; Thabet et al., 2024). In addition, the duration of the somatic memory may be also different between the various stresses and genotypes; it may be remarkably shorter for heat stress than for drought (Thabet et al., 2024). The physiology and genetics behind these processes however are not well understood yet. On the other hand, however, it is interesting to note, that in the case of the total grain number in side ears and grain yield, the association between the results originated from the field and from the controlled environment became more pronounced when the combined heat stress was compared with the field instead of the single stress (Table 1). This underlines that the combined stress produced a

closer dataset to real situations under field conditions, thus represented a stronger simulation power.

4.4. Heat stress response types in barley

Abiotic stress responses of the plants can be grouped into three basic categories, avoidance, tolerance and recovery (Abasi et al., 2024). Stress avoidance i.e. fastened plant development with earlier flowering and seed setting in order to finish the life cycle before the real stress hits, is mostly characteristic at locations, where the supra-optimal temperature conditions are prevailing throughout most of the life cycle. Late sowing in stress prone areas can simulate this situation better (Sukumaran et al., 2018; Liu et al., 2019; Shirdelmoghanloo et al., 2022; Kumar et al., 2023; Abdelghany et al., 2024). In this research, this type of plant stress mechanism was not possible to assess, either in the field or in the controlled tests. In the previous case, the occurrence of various abiotic stresses was apparent only from flowering and/or during the terminal developmental phases. In the controlled environment, the stress was applied in a developmental phase specific manner; therefore, stress avoidance was completely excluded. However, mechanisms of stress tolerance or stress recovery and specific barley genotypes demonstrating either of them could be detected in both sets of environments with varying clarity and to varying extent. Stress tolerance is the ability of the plants to maintain their close to normal functions during the stress as a result of effective structural, physiological, and genetic regulation adjustments, which can be indirectly detected in the level of changes in various grain yield related parameters (Araus et al., 2023; Abasi et al., 2024; Kim et al., 2024). In barley under these conditions, we identified two major types of heat stress tolerance: the ability of preserving grain number and weight in the main ears, and of maintaining the number of reproductive tillers. Both types appeared in either environments, but not with the same intensity. The preservation of the main ear grain number and weight was more evident and easily detectable under the controlled environmental experiments (both in the single and in the combined stresses), where the developmental phase specific application of a single definite heat stress event was followed by a recovery period under optimal conditions. Under field, however the heat stress occurred in several shorter spells following each other's at an uneven timing, and their coincidences with the exact plant developmental phases were also random. The ability to preserve the number of reproductive tillers as a stress tolerance mechanism was more characteristic under field conditions, where the soil-root aspect was not as limiting as in pot-based experiments. However, concerning stress recovery mechanisms, such as the compensating ability of lost resources after stress cessation, the same feature became apparent in both environments: the maintenance of grain number and weight in the side tillers. It is a well-known fact that the basis of grain yield is provided by the grain number and weight per unit area (Ugarte et al., 2007; Talukder et al., 2014; Serrago et al., 2023; Slafer et al., 2022; Kim et al., 2024). In our experiments, the association between the reproductive tiller number and the grain number, and weight in the side tillers was not or only weakly significant in either environments. In addition, the main ear grain number and weight represent only a small portion within the total values per unit area. Based on these facts, the ability of stress recovery proved to be more important than stress tolerance in itself in maintaining the yielding ability under heat stress both under field and under controlled conditions, when single heat stress was applied. In the case of combined heat stress however, the two strategies led to similar grain yield preservations indicating that efficiency of these two strategies depends on the actual features of heat stress. It would be worth further studying the genetics behind tolerance and recovery in order to be able to determine exactly the level of overlap or independence between them.

4.5. Comparisons between heat stress responses determined under field or controlled environments

The field grown and the controlled growth chamber experiments represent two distinct and distant environments differing from each other in many aspects (Araus et al., 2023). In comparing the stress responses of 28 barley cultivars that were included in both environmental conditions, we hypothesised that there is significant correlations between the heat stress induced changes in various yield-related parameters just as were found in some other research (Talukder et al., 2014; Teklemariam et al., 2023). Talukder et al. (Talukder et al., 2014) found that the impact of heat stress on grain yield was very similar for plants grown either in the field or controlled environment conditions and suggested that controlled environment studies could be an effective method for initial screening of wheat germplasm for tolerance to heat stress. Our results in barley confirm this suggestion. In addition, here we demonstrated that the stress response types identified in both experiments corroborate and complete each other. Thus, the heat stress sensitivity indices of the cultivars established in controlled conditions can later be included in field plant growth models as well.

5. Conclusions

We compared the heat stress responses of various winter barley genotypes as measured under field-sown and under controlled environmental experiments. Our results emphasised the fact that both set-ups of environments provided valuable, comparable, and more importantly, complementary information on the degree and type of heat stress responses detectable in barley cultivars. Though the field-sown experiment was carried out in one location, but its four consecutive years were characterised by very contrasting climatic conditions, the frequency and magnitude of which are expected to intensify in the near future due to the negative effects of climate change. Our results thus highlighted, that one-location, multi-seasonal experiments can complement the multi-location experiments, since the complexity of multivariable factors operating in the latter can be at least largely reduced to weather conditions in combination with plant development patterns. Under controlled conditions, the range of heat stress reactions was largely amplified, allowing for a more precise separation of different stress responses. In the combined heat stress experiment the phenomenon of heat priming could not be exactly clarified, but the results showed a stronger correlation with the field experiment indicating its closer simulation power. Heat stress was not constantly present in either of the environments, under such circumstances the ability of stress recovery proved to be more efficient in preserving the grain yield than the actual stress tolerance.

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CRediT authorship contribution statement

Tibor Kiss: Writing – original draft, Visualization, Conceptualization. **András Cseh:** Writing – review & editing, Supervision, Conceptualization. **Marianna Mayer:** Investigation. **Judit Bányai:** Investigation. **Krisztina Balla:** Writing – review & editing, Conceptualization. **Zita Berki:** Writing – original draft, Investigation. **Ádám Horváth:** Writing – original draft, Investigation. **Ildikó Karsai:** Writing – review & editing, Visualization, Supervision, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.envexpbot.2024.106038](https://doi.org/10.1016/j.envexpbot.2024.106038).

Data Availability

The data of this original contributions presented in the study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding authors.

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