Thesis of Doctoral (PhD) Dissertation

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Martonvásár

2025

HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

PHYSIOLOGICAL AND PRODUCTION-BIOLOGICAL INVESTIGATION OF DROUGHT STRESS TOLERANCE IN BARLEY (*HORDEUM VULGARE* L.)

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Gödöllő

2025

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1. Background and Objectives

In Hungary, the appearance of extreme weather elements in the future will be more frequent due to the changing climate. Forecasts suggest not only a rise in average temperatures but also an increasing frequency of extreme meteorological events. This trend may manifest in irregular precipitation distribution, leading to longer and more frequent drought periods. Such conditions have already been observed in Hungary: since 2015, the Ministry of Agriculture has declared prolonged drought periods each year. In drought years, the number of damage reports related to drought in the Hungarian agricultural compensation system has been particularly high (KELEMEN, 2024).

Among the abiotic stress factors affecting crops, drought is among the most critical ones, as it influences plant processes at physiological, biochemical, and molecular-genetic levels. These changes may, in turn, impact phenological events and morphological traits. For arable crops, particularly cereals, the emergence of extended drought periods during the growing season can critically hinder the realization of genetically determined grain yields and adversely affect crop quality.

Since regulating and modifying environmental conditions (for instance through irrigation) in arable crop production can be economically demanding, it is crucial to examine the drought tolerance of various cereals. It is also essential to enhance the adaptability of cultivated plants through breeding. Thanks to modern technologies, an increasing amount of information is available on the physiological and biochemical impacts of water deficiency, genes associated with drought stress, and the regulation and interaction of these genes. However, many unanswered questions remain, especially concerning cereals. It is vital for breeders to develop cultivars capable of maintaining stable yields even under drought stress, which is one of the most frequently occurring limiting environmental factors. In addition to field trials, climate chamber (phytotron) studies may provide even more reliable insights into varietal tolerance.

Globally, cereals are of great significance due to their vital role in human nutrition and as animal feed. Cereal-based products constitute the most widely consumed food items, forming a cornerstone of a balanced diet. In Hungary, barley (*Hordeum vulgare* L.) is the fourth most significant arable crop, cultivated across the entire country. According to the Hungarian Central Statistical Office (2024), in 2023, barley was grown on approximately 413,000 hectares, yielding 2.2 million tons with an average yield of 5.4 t/ha. It is utilized in feed, industry, and human consumption. Among cereals, barley is recognized as being drought-tolerant and, owing to its relatively small and simple genome, serves as an excellent model species for researching drought tolerance in field crops.

In the making of this dissertation, a comparative analysis of the drought stress tolerance of a genetically diverse group of barley cultivars was conducted under controlled climate chamber conditions, based on physiological and production-biological parameters.

The detailed objectives of the dissertation are as follows:

Drought stress may occur unpredictably during the growing season under field conditions, with the possibility of repeated or concurrent stresses. Controlled environments allow for the isolated study of individual abiotic stress factors under uniform environmental conditions. This setup ensures that stress affects each genotype at the same developmental phase, unlike in the field. For the experiments, 28 barley cultivars were selected from a genetically diverse population of 190 genotypes. These cultivars were subjected to standardized drought treatments of equal duration under phytotron conditions. The primary goal was to identify which developmental stages exhibit the greatest drought sensitivity among the tested genotypes. Additionally, the study aimed to observe physiological and morphological responses when water scarcity occured not just once but repeatedly across multiple stages of development. A further goal was to gain a comprehensive understanding of how persistent or recurring droughts, which are increasingly common under Hungary's changing climate, impact yield production across genetically distinct barley cultivars. The study also sought to characterize and compare varietal stress responses, with special focus on various drought tolerance strategies and their effectiveness. Ultimately, the aim was to obtain information that could support plant breeders in developing drought-tolerant barley cultivars by conducting complex drought stress experiments on the selected genotypes.

The main objectives of the dissertation can be summarized as follows:

1. To detect changes in morphological and yield components due to single and repeated drought stress under controlled conditions, and to analyze correlations among these traits in 28 barley cultivars.
2. To assess the following physiological parameters of the 28 cultivars under single and repeated drought stress in a controlled environment:
    - Chlorophyll content changes in the flag leaf (based on SPAD values)
    - Photosynthetic activity
    - Polyamine content
    - Relative water content (RWC) of the flag leaf
3. To identify drought-tolerant cultivars capable of maintaining relatively stable grain yields under suboptimal water availability, thereby serving as potential breeding material.

2. Materials and Methods

This dissertation examines the drought tolerance of barley genotypes included in the TKP2021-NKTA-06 project using production-biological and physiological methods.

2.1 Conditions of the Drought Stress Experiment

190 barley cultivars were selected forming the panel of BARGEN for examining barley ecological adaptation, yield formation and abiotic stress tolerance in the Agricultural Institute of the Centre for Agricultural Research (HUN-REN ATK MGI), Martonvásár, Hungary. Additional information on the 190 barley genotypes can be found in MUÑOZ-AMATRIAÍN et al. (2020) and HORVÁTH et al. (2024). From the BARGEN panel a subset of 28 barley cultivars was selected for the further testing of drought stress tolerance under controlled environmental conditions. The selection was performed to cover the genetic and phenotypic diversity present in BARGEN.

The experiment was carried out in the Phytotron facilities of HUN-REN ATK MGI, Martonvásár, using CONVIRON PGB-96 growth chambers (Conviron, Winnipeg, MB, Canada). The environmental conditions were constant throughout the experiment: a 16-h photoperiod, a PAR light intensity of 240 μmol/m2/s provided by metal halide lamps, and a constant ambient temperature of 18°C day and night. The experiment consisted of three treatments: control (C), single drought stress applied at the booting stage (Z49, ZADOKS et al. (1974), referred to as Ds), and combined drought stress (double) applied at first node appearance and again at booting stage, after a recovery period (Z31+Z49; referred to as Dd). For all three treatments, the germinated seedlings were vernalized for 60 days in peat blocks at 4˚C, with low light intensity and short days. The vernalized plantlets of one-two leaf stages were then transferred to individual pots (16 cm × 16 cm × 16 cm) containing a 3:2:1 mixture of about 2.5 kg of garden soil, compost and sand. For each barley genotype and each treatment, 24-24 plants were used as replicates and 4-4 plants were grown in individual pots. During the stress the watering of the variant was stopped until the soil moisture content dropped to 15 vol%, which level was kept for one week, then re-watering was applied. Control treatment was carried out where the constant soil moisture content was maintained on 27 vol%. Three sensors (5TE- Decagon Devices, USA) were placed in every treatment. Soil moisture (tf%), soil temperature (°C) and electrical conductivity (mS cm^{-1}) were recorded in every two hours from planting until maturity.

The 24 replicates (plants) of each treatment and each genotype were harvested at full maturity and the following morphological and yield-related parameters were measured: length of the last internode (LIN), length of the main ear (EaL), number of spikelets in the main ear (SPS), main ear density (DENS), number of reproductive tillers (RT), aboveground dry weight of plant without the ears (BIOM), seed number per spikelet of the main ear (MSSN), main ear weight (MEaW), weight of grains in the main ear (MSW), number of grains in the main ear (MSN), total side ears weight (SEaW), total grain weight in the side ears (SSW), total grain number in side ears (SSN) and grain yield per plant (GY). Thousand kernel weight in the main ear (MTKW), average thousand kernel weight (ATKW), average number of grains per spikes harvested (ASN) and average weight of grains per spikes harvested (ASW) were also calculated from these data.

Chlorophyll content of leaves was measured in four phenological stages—first node detectable (Z31), booting (Z49), late milk (Z77) and early dough (Z83)—using a Konica-Minolta SPAD-502 portable chlorophyll meter (Minolta Camera Inc. Tokyo, Japan). This instrument measures relative chlorophyll content (SPAD index) based on the ratio of red and infrared light transmitted through the leaf. SPAD values are linearly correlated with chlorophyll concentration.

Photosynthetic activity was measured using the Cyras 2-Portable Photosynthesis System (Tutorial version 2.03; Amesbury, MA, USA) halfway through the water withdrawal period, at the Z49 stage. The following parameters were evaluated: intercellular CO_2 concentration (CI), net photosynthetic rate (PN), transpiration rate (EVAP) and stomatal conductance (GS).

Samples for polyamine and relative water content (RWC) analysis were collected on the last day of Z49 developmental stage from control, from single, as well as from repeated water withdrawal treatments. Sample preparation and HPLC analysis were carried out according to the protocol of PÁL et al. (2013), allowing determination of both free and conjugated polyamines (putrescine, spermidine, and spermine).

For RWC% measurements, samples were collected from the flag leaf of three plants per treatment group at booting stage (Z49), on the last day of the drought stress.

Data organization, processing, and statistical analysis were carried out using Microsoft Excel (2016). Detailed general statistical analyses were performed using R version 4.1.1 (R Core Team, 2021) and GeneStat (VSN International Ltd., 18th ed.). Various RStudio packages were also employed.

3. Results and Discussion

3.1 Production-Biological Results of the Controlled Climate Chamber Drought Stress Experiment

Two-way ANOVA analysis revealed that drought stress had a strong and statistically significant effect ($p \leq 0.001\%$) in the case of both treatments. Among the 14 two-row barley cultivars, morphological and yield component parameters were influenced the most by the type of treatment (control, single, or repeated drought). Similar trends were observed in the average values of the 14 six-row cultivars. Generally, the treatment type exerted the most considerable effect on yield components. However, traits such as spikelet density (Dens), number of reproductive tillers (RT), and total grain number in side ears (SSN) showed the highest phenotypic variance attributable to genotype.

Drought stress induced numerous negative effects in the tested cultivars, showing varying intensities across the evaluated traits. Comparing single versus repeated drought treatments, the two regimes produced similar results for most parameters, with negligible or non-significant differences. Grain yield (GY) was particularly sensitive to drought in both two-row and six-row cultivars. Similar patterns were observed for average grain number (ASN), average grain weight (ASW), and average thousand kernel weight (ATKW). The ASN value dropped by more than one-third following drought treatment in both spike types. This reduction led to an even greater decrease in ASW, although ATKW was reduced by only 24.9–32.7%. A similar level of decline was recorded in side ears.

Morphological traits displayed analogous patterns. Aboveground biomass (BIOM) was reduced by approximately 25%, and the length of the last internode (LIN) was halved under drought conditions.

The relationship networks between morphological and yield components varied considerably depending on drought treatment. Repeated water withdrawal generally resulted in a decrease in the number of significant correlations, observed across both spike-type groups. In two-row cultivars, strong positive correlations were identified between grain yield (GY) and main spike parameters, such as MSN and MSW. Positive correlations were also observed between spike length (EaL), spikelet number (SPS), and MSN. These interrelationships remained under both drought treatments.

After single drought stress, main spike traits were positively correlated with RT, ASN, and ASW. These correlations weakened or disappeared under repeated stress, except for the relationships among grain numbers and grain weights. Among six-row cultivars, more interrelationships were found than those present in the two-row group. Positive associations between EaL and SPS, and between MSW and GY, were consistent across all treatments. Regardless of spike type and treatment, ASW consistently correlated positively with grain yield.

3.2 Discussion of Morphological and Yield Component Results

Our controlled climate chamber experiment confirmed that drought exerts a clearly negative impact on barley yield, regardless of spike type. The traits examined were influenced the most by the drought treatment in both spike-type groups. Similar results were reported by EL-SHAWY et al. (2017) and POUR ABOUGHADAREH et al. (2013), who also observed significant effects of treatment, genotype, and their interaction on trait variances. EL-HASHASH et al. (2019) similarly found that the treatment had the strongest influence during their drought stress experiment, followed by genotype and the interaction term.

Single and repeated water withdrawal treatments significantly reduced the average grain number per spike (ASN) and the average grain weight per spike (ASW). Our findings are in line with previous studies (MORGAN & RIGGS, 1981; JAMIESON et al., 1995; SAMARAH, 2005; AJALLI & SALEHI, 2012; EL-SHAWY et al., 2017). The results also support the conclusion that under drought conditions, the yield loss in barley is not significantly different from that observed in related species, such as wheat (MEHRABAN et al., 2019; YASHAVANTHAKUMAR et al., 2021; JAVED et al., 2022).

Water deficiency also altered the relationship networks among the examined traits. Strong positive correlations were observed between main spike yield parameters (MSN and MSW), as well as between average grain number (ASN) and average grain weight (ASW), independently of treatment and spike type. Moreover, both drought treatments and spike types exhibited a strong negative correlation between aboveground biomass (BIOM) and the main spike grain number (MSN) and grain weight (MSW).

The significant impact of drought stress on main spike parameters can be attributed to the fact that the stress occurred during critical periods of yield formation. The absence of significant differences between the two treatments for these traits suggests that prior stress exposure did not induce an adaptive response in the plants to mitigate the impact of subsequent stress. NOSALEWICZ et al. (2016) found that priming effects from drought stress only manifested in the next generation of plants, and no somatic memory was detected within the same generation in their experiment.

ARSHADI et al. (2018) recommend the use of secondary traits—such as spike weight, duration of grain filling, grain weight of the main spike, and awn length—in breeding programs targeting improved drought tolerance in order to increase selection efficiency. These traits are relatively easy and cost-effective to measure, and due to their genetic variability, they are suitable for identifying drought-tolerant genotypes.

3.3 Genotypic Differences in Response to Drought Treatments

To facilitate a detailed analysis of genotype-specific drought responses and account for the variability in the magnitude of changes observed among traits, values for each cultivar were expressed as a percentage relative to the control treatment. This dataset was used to construct a heatmap, enabling simultaneous grouping of traits and genotypes based on their drought stress responses via UPGMA cluster analysis.

Based on the distribution of positive and negative values across morphological and yield components, the 28 barley genotypes were divided into three distinct groups under both drought treatments. Each group contained both two-row and six-row cultivars. The most drought-sensitive cultivars clustered in Group 3, whereas genotypes in Group 2 showed greater tolerance to water withdrawal.

There was substantial overlap in genotype composition between the similarly responding clusters identified in both drought treatments (Group 1 and Group 2). The moderately drought-tolerant GroupDd-1 (under repeated drought stress) comprised six cultivars that also belonged to the stress-tolerant GroupDs-2 under single drought stress: ‘Dahlia’, ‘Elan’, ‘Faraday’, ‘Parasol’, ‘Robur’, and ‘Sombrero’. Additionally, among the 14 cultivars in GroupDs-2 (single stress), eight retained their tolerance under repeated drought conditions (GroupDd-2): ‘Aldebaran’, ‘Balda’, ‘Bereke 54’, ‘Cinnamon’, ‘Coriolis’, ‘Full Pint’, ‘Maja’, and ‘Mascara’.

The least drought-tolerant cultivars—‘Carola’, ‘Dolphin’, and ‘Surtees’—consistently appeared in the most sensitive groups (GroupDs-3 and GroupDd-3). Interestingly, four cultivars (‘Lambada’, ‘Spinner’, ‘Ketos’, and ‘Lonni’) shifted from the most sensitive group under single drought (GroupDs-3) to the moderately tolerant group under repeated drought stress (GroupDd-1), indicating some degree of acquired resilience.

3.4 Discussion of Drought Stress Response Patterns

Both drought treatments (single and repeated) and both spike-type groups of barely exhibited a strong negative correlation between biomass and grain number and weight. This trend was particularly evident in Group 3. Under both water withdrawal regimes, Group 3 cultivars had above-average biomass values, while Groups 1 and 2 displayed below-average biomass production. Conversely, cultivars in Groups 1 and 2 retained higher grain number (MSN) and grain weight (MSW, MTKW) values compared to control under stress conditions. Despite having a comparable number of reproductive tillers, Group 2 genotypes produced higher grain numbers and grain weight under both drought treatments. In contrast, Group 3 cultivars showed significantly increased vegetative biomass, indicating a preferential allocation of resources to vegetative rather than reproductive structures.

According to SELEIMAN et al. (2021), plant responses to drought can be categorized into three survival strategies: avoidance, tolerance, and recovery. Avoidance involves accelerated development, rapid flowering, and grain filling. In our study, this mechanism was excluded due to the controlled application of drought stress at specific developmental stages, which prevented stress avoidance.

Tolerance, the ability to maintain near-normal physiological function under stress through effective structural, physiological, and genetic regulation, was variably expressed among the barley genotypes in both treatments. Tolerant plants maintain higher water potential by reducing transpiration losses through stomatal regulation (DOBRA et al., 2010; BOULARD et al., 2017; SELEIMAN et al., 2021). This often correlates with reduced productivity and plant size, which indirectly affects yield components depending on the severity of change (ARAUS et al., 2023).

In our barley experiments, two types of drought tolerance were identified. The first group focused on retaining grain number and weight in the secondary spikes (moderately tolerant). The second group maintained grain number and weight in the main spike while preserving reproductive tillers (more tolerant). Both tolerance types appeared under both drought regimes but with different intensities. Preservation of main spike traits proved more evident and measurable under controlled conditions, in which case stress coincided precisely with developmental stages and recovery occurred under optimal circumstances (HORVÁTH et al., 2024).

The repeated drought treatment also aimed to assess the priming effect. For most traits, mean values under repeated stress were similar to or lower than those under single stress, with the exception of spikelet density (Dens) and number of reproductive tillers (RT). Half of the cultivars produced more yield after repeated stress, though not reaching control levels, regardless of spike type. The priming effect under drought stress seems highly genotype-dependent, and may also be stress-specific (ABID et al., 2018; BALLA et al., 2021; THABET et al., 2024). The duration of somatic memory may vary by genotype and stress type. The underlying physiological and genetic regulatory mechanisms of these processes remain insufficiently explored.

3.5 Physiological Parameters

Visible wilting of the plants was observed following water withdrawal, attributable to reduced turgor pressure. Measurements indicated a marked decrease in relative water content (RWC): 38% under single drought stress and 33% under repeated stress, on average.

Photosynthetic activity was assessed by examining the flag leaves. Drought stress significantly reduced photosynthetic performance, as stomatal closure limited both transpiration and CO_2 uptake necessary for photosynthesis. Compared to the control, all four parameters—intercellular CO_2 concentration (CI), evaporation (EVAP), stomatal conductance (GS), and net photosynthetic rate (PN)—were significantly reduced in both drought treatments. While EVAP, GS, and PN showed strong significance ($p \leq 0.01$), CI was significantly affected at $p \leq 0.05$.

In addition to photosynthetic measurements, chlorophyll content in flag leaves (SPAD values) was assessed at four developmental stages (Z31, Z49, Z77, and Z83). Prior to drought treatment, SPAD values in Z31 and Z49 stages did not differ significantly from controls. However, repeated drought stress caused a substantial decrease in SPAD values at both stages, particularly at Z31. A similar decline was observed under single drought stress at Z49. Following rewatering and recovery, SPAD values nearly returned to control levels by the Z77 (milk ripening) stage. No significant differences were found in the maximum SPAD values across treatments, indicating successful chlorophyll recovery after stress cessation. Notably, cultivars with higher maximum SPAD values (e.g., 'Balda', 'Bereke 54', 'Mavlono', 'Lorena', 'Ketos', and 'Maja') also maintained higher grain yields under both treatments.

All three measured polyamines—putrescine (PUT), spermidine (SPD), and spermine (SPM)—increased in response to drought. While most polyamine levels showed no significant differences between treatments, spermidine content was highest under repeated drought and significantly exceeded values in both single-stress and control plants. Total polyamine content (TPA, the sum of PUT, SPD, and SPM) more than doubled in response to drought. Interestingly, cultivars with the highest TPA in flag leaves ('Calcutta', 'Dolphin', 'Lorena', 'Dahlia', 'Lambada', and 'Surtees') consistently exhibited lower grain yields in both treatments.

3.6 Summary of the Results from the Physiological Parameters Measured in a Controlled Climate Chamber Drought Stress Experiment.

Results of our controlled climate chamber experiment confirmed that both single and repeated drought stress caused significant changes in the physiological processes of barley. Measurements of parameters related to the functioning of the photosynthetic system showed a significant decrease compared to the control. Based on this, stomatal limitation of photosynthesis was a key factor in the examined varieties, which is one of the indicators of the restrictive effects caused by drought stress (IZANLOO et al., 2008). In addition to stomatal conductance (GS) and evaporation (EVAP), the relative water content of flag leaves also showed a clear decline, indicating that the plants were unable to prevent water loss from the leaves. This phenomenon is supported by previous research findings as well (ROBREDO et al., 2007, 2010; BELLO et al., 2022; FERIOUN et al., 2022). Our analyses revealed a positive correlation between relative water content and photosynthetic parameters. No significant differences were found between the effects of single and repeated drought stress regarding the photosynthetic parameters studied.

The chlorophyll content of flag leaves significantly decreased due to water withdrawal, which was well correlated with changes in photosynthetic parameters. SPAD values measured on the final day of drought stress were significantly lower compared to the control. POUR ABOUGHADAREH et al. (2013) and FERIOUN et al. (2022) also reported a clear decrease in SPAD values under drought stress. In the experiment by ALGHABARI and IHSAN (2018), chlorophyll content in the leaves was reduced by 29% and 41% as a result of water withdrawal during flowering. Following our applied single and repeated water withdrawal treatments, chlorophyll content recovered to levels nearly equivalent to the control, and there were no differences in the maximum SPAD values between control and treated plants. Previous studies have already described that SPAD values can serve as indicators of drought stress severity (LI et al., 2006; KALAJI and GUO, 2008; ALAEI, 2011; ISLAM et al., 2014; MONOSTORI et al., 2016; EL-SHAWY et al., 2017). Our findings support this observation.

The levels of polyamines, as protective compounds, increase under stress conditions (GROPPA and BENAVIDES, 2008; FAROOQ et al., 2009; ZHOU and YU, 2010). In our phytotron experiment, the levels of the three studied polyamines—especially putrescine, spermidine, and spermine—also increased as a result of water withdrawal. We observed that spermidine levels were highest during repeated stress, while spermine was consistently present at significant levels in both treatments. Furthermore, we found that in control plants, relatively higher spermidine content was accompanied by lower putrescine levels. In contrast, under both single and repeated water withdrawal, putrescine levels increased significantly. Based on our results, barley varieties with the highest total polyamine content (‘Calcutta’, ‘Dolphin’, ‘Lorena’, ‘Dahlia’, ‘Lambada’, ‘Surtees’) had lower grain yield (GY) in both treatments. According to the literature, there is limited information available regarding changes in polyamine levels under drought stress in barley. In wheat, it has been observed that spermidine and spermine significantly increase the concentrations of zeatin, zeatin-riboside, and abscisic acid, while simultaneously reducing endogenous ethylene synthesis in the grains, which promotes grain filling under drought conditions. In contrast, putrescine enhances ethylene synthesis, leading to excessive abscisic acid accumulation in the grains, which subsequently inhibits grain filling under stress (YANG et al., 2016). Due to the limited literature, we have not yet found confirmation of this mechanism in barley. In our experiment, the drought-sensitive variety ‘Dolphin’ showed higher levels of putrescine after both single and repeated drought stress compared to the more tolerant varieties (‘Aldebaran’, ‘Coriolis’, ‘Maja’, ‘Cinnamon’, ‘Full Pint’, ‘Mascara’, ‘Balda’, and ‘Beeke 54’). The other two drought-sensitive varieties (‘Carola’ and ‘Surtees’) also showed greater increases in putrescine content under both stress treatments than the tolerant ‘Coriolis’ and ‘Balda’.

3.7 Relationships Between Physiological Parameters and Yield Components: Results and Discussion

The following conclusions can be drawn regarding the relationship between physiological parameters and yield components. Parameters related to polyamine content (PUT, SPM, SPD, TPA) formed a distinct group that showed a negative correlation with another group comprising photosynthetic activity parameters (EVAP, PN, GS, CI, SPAD_max), relative water content of leaves (RWC), grain number and grain weight of secondary spikes (SSN and SSW), total weight of secondary spikes (SEaW), and grain yield (GY). In contrast, FAROOQ et al. (2009b) found that exogenous application of polyamines in rice increased leaf water content and photosynthetic activity, thereby improving drought tolerance. Similarly, YANG et al. (2008), also working with rice, reported a significant positive correlation between drought-induced increases in polyamine content (spermidine and spermine) and grain weight.

It was also observed that while intercellular CO_2 concentration (CI) under control conditions correlated strongly with the grain number and grain weight of secondary spikes (SSN and SSW), in the single drought stress treatment, CI clustered with polyamines, whereas in the repeated drought treatment it was grouped with biomass (BIOM).

The evaluation of physiological parameters and their correlation with yield components revealed significant interrelationships. Under both single and repeated drought stress conditions, genotypes exhibiting higher relative water content (RWC) and chlorophyll content (SPAD values) generally achieved better yield outcomes. These physiological traits showed consistent positive correlations with grain number (MSN, ASN) and grain weight (MSW, ASW), particularly in the main spike.

In contrast, high polyamine levels—especially total polyamine content (TPA)—were negatively correlated with yield parameters in most cases. Cultivars that accumulated large amounts of spermidine and spermine during stress exhibited a clear tendency toward lower productivity. This suggests that although polyamines play a role in drought stress signaling and cellular protection, excessive accumulation may indicate high stress sensitivity rather than tolerance.

Photosynthetic activity parameters (PN, GS, EVAP) also showed positive associations with yield-related traits. Genotypes capable of maintaining higher net photosynthetic rates and stomatal conductance under drought conditions had better grain filling and yield stability. These relationships were especially pronounced in cultivars classified in Group 2 of the cluster analysis, which displayed the most drought-resilient profiles.

Taken together, the correlations identified between physiological parameters and yield traits support the use of integrative physiological profiling in drought tolerance studies. The findings reinforce that physiological indicators such as SPAD, RWC, and photosynthetic activity may serve as reliable predictors of genotypic performance under water-limited conditions, while high polyamine levels may indicate physiological stress rather than adaptation.

4. Conclusions and Recommendations

We investigated the impact of drought stress—an abiotic stress factor—on various barley cultivars under controlled conditions. Due to climate change, drought has already become one of the most common stress factors in crop production, and its importance is expected to increase further under future environmental conditions. Therefore, it is crucial to study the physiological, biochemical, and molecular genetic effects of drought stress on field crops. Our results confirm that data obtained from controlled climate chamber experiments contribute to a more comprehensive understanding of the complex effects of water withdrawal.

Our experiment, conducted on a population of 28 genetically diverse winter barley cultivars, enabled us to better understand the different survival strategies of winter barley based on key morphological and yield-determining traits. In analyzing yield components and morphological traits, we evaluated two-rowed and six-rowed genotypes separately. However, no notable differences were observed in their stress responses, suggesting that spike type does not play a role in yield variation caused by drought stress.

The physiological investigations revealed significant reductions in photosynthetic activity parameters, indicating that the inhibition of photosynthesis via stomatal closure was a major effect of drought stress in barley. We demonstrated that while control plants exhibited significantly lower levels of putrescine compared to spermidine and spermine, both single and repeated drought treatments led to a substantial increase in putrescine content. Elevated putrescine levels negatively affected grain filling under drought stress, while spermidine and spermine promoted this process. Consequently, hormonal interactions play a key role in the physiological regulation of grain filling during drought, and studying these interactions may be of high importance.

Overall, the results of the two drought treatments showed no or only minimal differences in most examined traits. This suggests that the cultivars did not develop a priming (conditioning) effect, and that a single, severe stress event is sufficient to cause a significant reduction in genetically encoded yield potential. Additional water withdrawal did not result in further significant yield loss. As priming effects in barley under drought stress are still poorly understood, future studies should explore this phenomenon in more depth. Investigating the potential transgenerational effects of priming and conducting various molecular genetic analyses could also yield important findings.

From an agronomic perspective, cultivars that are able to combine drought tolerance with recovery ability will be the most useful under changing environmental conditions. In our study, this criterion was best met by the cultivars ‘Aldebaran’, ‘Balda’, ‘Bereke 54’, ‘Cinnamon’, ‘Coriolis’, ‘Full Pint’, ‘Maja’, and ‘Mascara’. We therefore recommend these as parent lines for breeding drought-tolerant barley. Tolerance is also supported by secondary traits (e.g. polyamine content), which play an important role in yield formation and are therefore associated with adaptability.

To further enhance barley drought stress tolerance, we recommend conducting detailed phenotypic analyses on an even broader set of cultivars to identify optimal crossing partners. We also consider it important to examine the expression patterns of genes responsible for polyamine biosynthesis in the drought-tolerant cultivars identified in this study, and to study their regulatory networks in a comprehensive drought stress screening experiment. We believe that new breeding programs based on crossing genetically distant but drought-tolerant genotypes may offer the most promising avenue for improving drought resistance in barley.

5. New Scientific Results

1. We established that drought tolerance was statistically identical between two-rowed and six-rowed winter barley cultivars within the studied variety set.

2. Single and repeated drought stress affected yield-related parameters to a similar extent within the limits of statistical significance. Therefore, no somatic priming effect was observed in the cultivars tested under repeated drought stress.

3. We found that under water-deficit conditions, excessive aboveground biomass production had a negative interaction with grain yield formation under controlled conditions.

4. Based on drought stress responses, the 28 barley cultivars studied could be classified into three groups: one group showed strong drought sensitivity, while two groups exhibited relative drought tolerance.

5. The examined barley cultivars employed different strategies to mitigate the effects of drought stress. The first group attempted to retain grain number and weight in the secondary spikes (moderately tolerant). The second group included genotypes capable of maintaining grain number and weight in the main spike while also preserving the number of reproductive tillers (more tolerant). Both tolerance types were present in both treatments (single and repeated drought stress), but with varying intensities.

6. In the analysis of polyamine content in the 28 barley cultivars, we demonstrated that, similar to other cereals (e.g. wheat), elevated putrescine levels induced by stress had a negative effect on grain filling.

6. Author's Publications Related to the Dissertation Topic

Peer-reviewed Scientific Articles (English, with Impact Factor):

BERKI, Z., KISS, T., BÁNYAI, J., CSEH, A,, BALLA, K., KARSAI, I. (2025): Effect of drought stress during critical developmental stages on morphological and grain yield-related traits in winter barley (*Hordeum vulgare* L.). *PLoS One*, 20 (7) e0329391.

KISS, T., HORVÁTH, Á. D., CSEH, A., **BERKI, Z.,** BALLA, K., MAYER, M., TÓTH, V., KARSAI, I. (2025): Ambient temperature influenced co-expression network of major developmental, circadian, and photoreceptor genes in bread wheat. *Scientific Reports* 15, 27751.

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