



IS ESTONIAN BARLEY READY TO TACKLE CLIMATE CHANGE-INDUCED WATER REGIMES?

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ABSTRACT. The objective was to examine the effect of drought and flood on barley plants' biomass and growth rate in early vegetative development while comparing the stress adaption of different varieties. A greenhouse trial was conducted in the Estonian Crop Research Institute (ECRI) in 2021, where five Estonian grown spring barley varieties were grown in optimal, drought and flood treatments for six weeks to measure plants' projected leaf area (PA) and relative growth rate (RGR) through phenotyping. Both drought and flooding stress have a strong negative impact on plant biomass in early vegetative growth phases, causing PA at the end of the trial to decrease 26% and 49% respectively. Meanwhile, RGR throughout the trial decreased 6% in drought treatment and 16% in flood treatment. This indicates the greater impact of flood stress on plant's growth compared to drought stress. Genetic variation related to adaption to extreme water regimes in varieties is rather low, especially in drought stress conditions. In drought treatment, the variation coefficient (CV) was 14%, and in flood treatment 25%. Even as most varieties' PA and RGR varied between treatments, the difference between varieties in specific stress treatments was minimal. Estonian grown spring barley varieties are susceptible to extreme water regime related stress caused by potential climate change. This indicates the importance of assessing water-related stress tolerance in breeding material, adapting more accurate innovative evaluation approaches, and integrating climate-resilient genetic material into breeding programs, to hedge the risk caused by unfavourable growth environments in Estonian barley production.

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Introduction

Even though global population growth is projected to slow down by the end of the 21st century (Vollset *et al.*, 2020), the persistence and irreversibility of anthropogenic negative influence on the global ecosystem must be acknowledged. In the meantime, forecasted climate change and increasing demand for food will put even more pressure on plant breeders to develop cultivars with higher yields, quality, and climate resilience. In addition to changes in temperature and atmospheric gas composition, precipitation patterns will also be altered, affecting global and local hydrological cycles (Konapala *et al.*, 2020). This altogether increases the likelihood of extreme weather conditions with excessive or lacking precipitation, resulting in drought or flood induced abiotic stress in plants. Exposure of crops to abiotic stress thereby limits the biomass and yield of crops, which is something we can't afford.

At present, a great amount of crop plants' genetic diversity to adapt to the environment has been lost due to the long-term yield-oriented selection bottleneck (Dawson *et al.*, 2015). To improve adaption to abiotic stress, the suitable genetic material must be screened for and transferred to new varieties. With the help of non-destructive phenotyping, adaption of plants' phenotypes to abiotic stress can be observed in time on a larger scale, making it possible to evaluate breeding material and to connect its phenotype with a responsible QTL (quantitative trait locus) or a gene.

In this experiment, a cost-effective greenhouse phenotyping platform was used to measure the relative growth rate (RGR) and projected leaf area (PA) of Estonian grown barley varieties in extreme water regimes. Evaluating varieties' adaption to extreme water regimes gives an overview of their climate



resilience at present, making it possible to prepare better for future challenges.

The objective was to examine the effect of drought and flood on barley plants' biomass and relative growth rate in early vegetative development while comparing the stress adaptations of different varieties.

Material and Methods

A six-week trial (04.01.–16.02.2021) was conducted in controlled greenhouse conditions at the Estonian Crop Research Institute (ECRI) in Jõgeva, Estonia (58.759097° N, 26.406711° E). Five common Estonian grown spring barley varieties of various origins were used: 'Maali' (ECRI), 'Tuuli' (ECRI), 'Katniss' (Nordic Seed A/S), 'Feedway' (Nordic Seed A/S) and 'Bente' (Nordsaat Saatucht GmbH).

An experiment was carried out with five replicates per genotype in each of the three treatments: control, drought and flood. Single plants were grown next to each other in two-litre plastic pots with 1.7 kg of the growth substrate, in a randomized design. For growth substrate, a mix of soil, peat and sand was used in a volume ratio of 3:2:1.

Three seeds were sown into each pot and trimmed to a single plant two weeks later. For light conditions, 16:8 h light regime was secured with plant growth lamps and temperature between 15–25 °C. At the end of the experiment, the shoots were cut from basal conjunction to determine wet and dry biomass.

Induced stress lasted for two weeks in drought treatment and a week in flood treatment. For the first 14 days after sowing (DAS), all treatments were kept at a water level of 20% gravimetric water content (GWC). In the control treatment, 20% GWC was sustained throughout the experiment. To induce drought, watering was reduced until 10% GWC was achieved, starting from 14 DAS and kept until 28 DAS. For flood treatment, a water level of 1 cm above soil level was sustained from 14 DAS to 21 DAS. Both stress treatment's water level of 20% GWC was restored post-stress until the end of the experiment at 42 DAS. This method is based on the trial conducted by Honsdorf *et al.* (2014), and modified to add flood treatment conditions.

Phenotyping was done weekly from 14 DAS to 42 DAS. Every week, three pictures of each plant were captured (front, side 90° and top). Captured photos were analysed in the program EasyLeafArea, where green pixels were separated from the background and summed. To calculate RGR, the formula:

$$RGRPA = \frac{\ln\left(\frac{PA_2}{PA_1}\right)}{t_2 - t_1} \quad \text{was used, (1)}$$

where PA is projected area (pix) at time t (Armoniené *et al.*, 2018).

For descriptive statistics of PA and RGR, average and standard error were calculated. Tukey HSD was used to calculate the significant difference between varieties and treatments. One-way ANOVA and variation

coefficient (CV) were used to determine variation in treatments and varieties. All data analysis and statistical tests were done in R (R Core Team 2021).

Results and Discussion

Relative growth rate (RGR) and projected area (PA)(pix) of five Estonian grown barley varieties were measured through phenotyping in control and extreme water regime conditions.

Effect of stress

In both drought and flood treatment, PA was significantly lower than control treatment from the end of stress until the end of the experiment ($P < 0.001$). When the decrease in the first post-stress week of drought plants was only 7%, it slumped for the second and third post-stress week to 31% and 42% (Fig. 1). By the end of the experiment, PA in drought treatment was 26% lower in the control treatment. In flood treatment, PA decreased 28% by the first post-stress week, decreasing even more in the following weeks to 52% and 55% accordingly. At 42 DAS flood treatment, PA was 49% lower compared to the control treatment. Variation between both stress treatments and control treatment at 42 DAS was 97%, while variation within groups was 3% ($P < 0.001$).

In the meantime, RGR decreased 6% overall in drought treatment and 16% in flood treatment ($P < 0.001$). Although the decrease of RGR was greater in flood than drought treatment, in both treatments the significant effect of stress appeared only during the stress and the following week. By the last week of the experiment, RGR in both flood and drought exceeded control treatment by 48% and 21% ($P < 0.001$), compensating the former stress with faster growth. Here we can conclude that flood had a more severe effect to plant biomass growth than drought, as a greater decrease in PA and RGR indicate.

Different physiological processes targeted by stress cause the difference. As known, growth reduction in drought treatment can be explained by dehydration of cells due to the plant's limited access to water, harming basic growth-related physiological processes like cell/leaf expansion and metabolic activities. Meanwhile, excess water in flood treatment leaves plants' roots in anoxic conditions, inhibiting their respiration and energy availability, which is necessary to provide water and nutrients for the growth and metabolism of above-ground parts.

The effect of abiotic stress on the biomass of barley varieties from different backgrounds has been measured before by Honsdorf *et al.* (2014) and Zhao *et al.* (2010) with drought and Yordanova and Popova (2001), Bertholdsson (2013) and Luan *et al.* (2018) with the flood, where uneven severity depends strongly on the origin of varieties, developmental stage of exposure and other methodical approaches. Overall, that points to the presence of genetic variation and even resistant varieties in-between different gene pools

tested, which can be exploited for climate-resilient breeding in other regions.

Here we can conclude that there is the widespread vulnerability of juvenile barley to potential climate

change-induced flood and drought stress, which could inhibit achieving sustainable development goals if action is not taken in time.

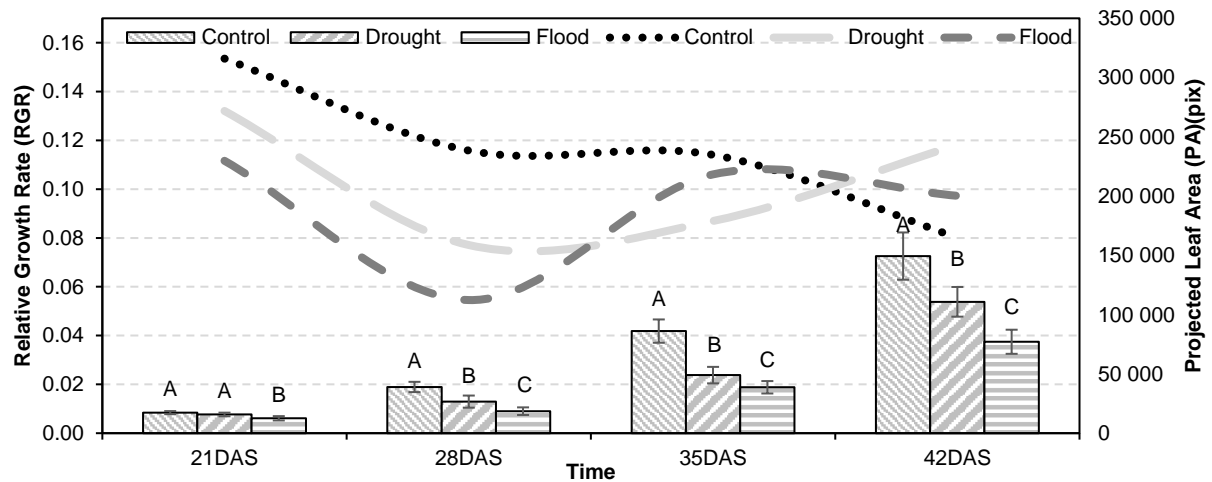


Figure 1. Average projected leaf area (PA-columns) and relative growth rate (RGR-lines) of control, drought, and flood treatments (all varieties). (I represent \pm SE (standard error); different capital letters represent statistical difference $P < 0.05$ between treatments; DAS – days after sowing)

Varieties in stress treatments

The effect of stress caused by extreme water regimes depends on the plant's genotype. Drought stress did not decrease PA of all varieties. The PA of varieties 'Tuuli' and 'Maali' did not differ significantly from the control treatment ($P > 0.05$), while 'Tuuli's average PA value exceeded the control treatment by 9%. A negative effect of drought was observed with varieties 'Katniss' (28%), 'Feedway' (40%) and 'Bente' (34%) ($P < 0.001$). The latter's PA was significantly decreased from the end of stress exposure to the end of the experiment (28–42DAS), in the situation where 'Tuuli' and 'Maali' were significantly lower than the control treatment only the week after the stress (35DAS). Flood stress decreased PA in all varieties from the second post-stress week until the end of the trial. PA decreased in varieties: 'Maali' (67%), 'Tuuli' (42%), 'Bente' (41%), 'Katniss' (45%) and 'Feedway' (46%) ($P < 0.001$).

Variation between varieties in flood treatment was 85% and in drought, treatment was 79% ($P < 0.05$). Meanwhile, the variation coefficient (CV) between varieties in drought treatment was 14% and 25% in flood treatment ($P < 0.05$). Wild barley introgression varieties tested for drought by Honsdorf *et al.* (2014) showed a variation coefficient of 72%. The higher variation in response to flood treatment in this experiment indicates greater genetic variation related in genotypes than in drought treatment, while still staying relatively low for both treatments compared to wild relatives. That points out the stronger negative effect of flood stress to plant growth together in combination with to some extent greater genetic variance in the phenotypic response.

A similar pattern to PA occurred with RGR, wherein drought treatment 'Tuuli' and 'Maali' did not differ significantly ($P > 0.05$), while other varieties had 28–44% lower RGR compared to the control treatment ($P < 0.05$). On the other hand, 'Tuuli' and 'Maali' did not show the highest PA in the control treatment of all varieties, pointing out their robustness in their biomass growth. In flood treatment, RGR decreased unevenly across all varieties between 14–35DAS in between 29–58% ($P < 0.05$), without a single variety indicating resistance.

For the most part, varieties in treatments did not differ from each other in stress treatments (Fig. 2). In drought treatment, PA of 'Tuuli' was 37% higher than 'Feedway' and in flood treatment, PA of 'Bente' was 36% higher than 'Tuuli' and 49% higher than 'Maali' ($P < 0.05$).

Varieties' low CV with the scarce significant difference in RGR and PA affirm relatively narrow genetic variation in their genotypes for these specific abiotic stress responses, common to modern top-yield varieties. Low genetic variation for early flood and drought tolerance was also pointed out with local varieties in neighbouring Finland by Hakala *et al.* (2012), where all other climate change risk-related traits had variation in local genotypes. That points out the demand and need for more climate-resilient breeding material for spring barley in the region.

A better overview of individual varieties' growth in control and stress treatment is seen while comparing the performance in both. As seen, 'Maali' had the second highest average PA in control and drought treatment compared to other varieties, despite great variation in-between replications (Fig. 3). At the same time, the variety 'Feedway' had one of the lowest PA in control and drought treatment.

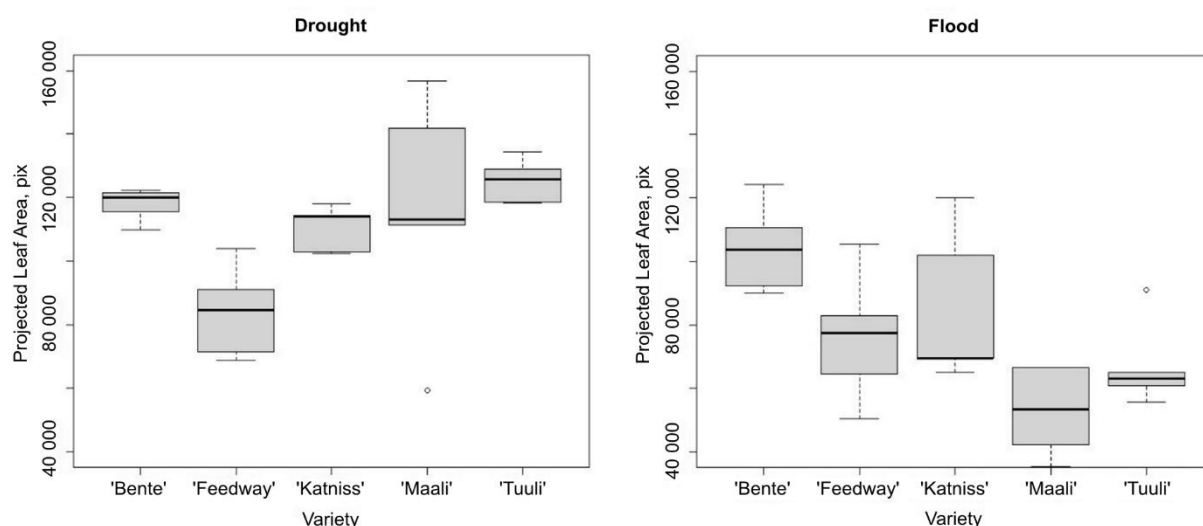


Figure 2. Projected leaf area (PA) of varieties at 42 DAS in drought and flood treatment. I represent 95% confidence interval, the bottom and top of the box are the 25th and 75th percentiles, the inner line as the 50th percentile (median), and outliers are shown as open circles.

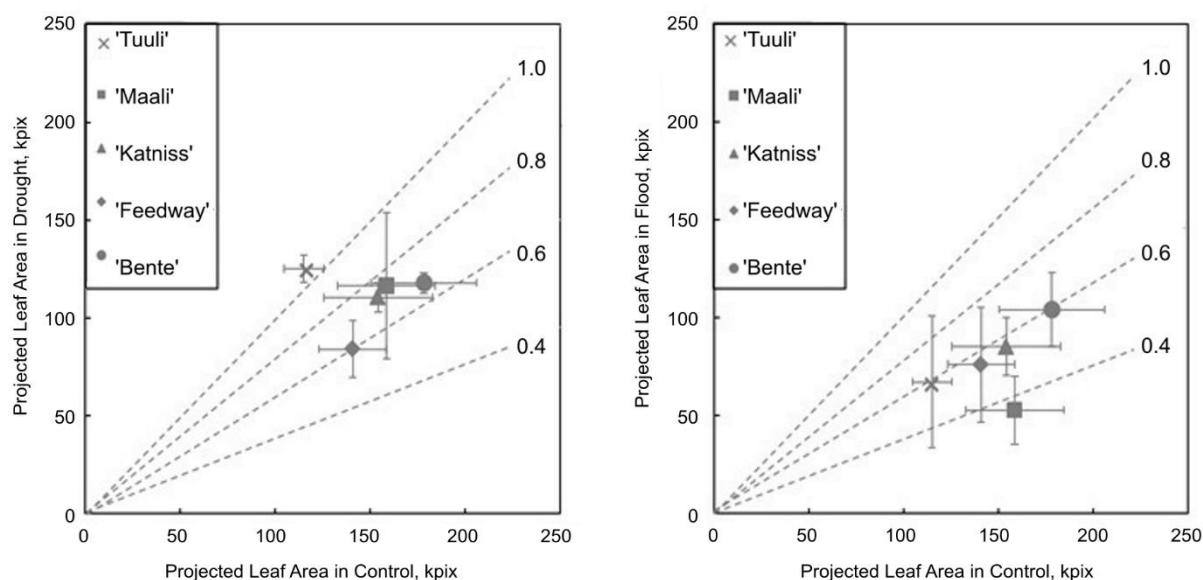


Figure 3. Projected leaf area (PA) of varieties in control and drought treatment and in control and flood treatment. I represent \pm SE (standard error).

In flood and control treatment, 'Bente' performed above varieties' average, meanwhile 'Tuuli' stayed below. Indeed, it also shows the complexity of breeding material evaluation for abiotic stress resistance, which could benefit from the use of stress indexes and yield data in future studies, to get output that is even more accurate.

Based on the results, we can state that Estonian grown spring barley varieties are overwhelmingly susceptible to extreme water regimes caused by water-related abiotic stress, an effect, which is likely caused by their narrow gene pool common to high-performing varieties. Even though breeding for extreme weather events still has a limited capacity (Olesen *et al.*, 2011), it will become more relevant with pessimistic climate change scenarios already becoming reality.

For more accurate evaluation in future studies, plants' grain yield data can also be collected, which makes it possible to better understand the effect of abiotic stress growth in time and its relation to grain yield formation (Ciancio *et al.*, 2021). In addition, adapting other phenotyping stress indexes and developing genetic markers combined with gene expression measurements will make it feasible to precisely determine the nature of yield-limiting bottlenecks in plant physiology. Thus, having a deeper insight into limitations of growth and yield-formation, more efficient selection of crossing parents can be done.

Conclusion

The spring barley varieties tested were vulnerable to potential climate change-induced water regime changes in juvenile growth. Genetic variation of abiotic stress

response-related genes is relatively low, drawing attention to the need for more climate-resilient breeding material. To achieve climate-smart barley production, better screening of abiotic resistance and integration of resistance-related traits must be adopted in plant breeding.

Conflict of interest

The authors declare that there are no conflicts of interest.

Author contributions

SSS, ÜT – study conception and design, analysis and interpretation of data.

SSS – acquisition of data, drafting of the manuscript.

SSS, ÜT, EL – critical revision and approval of the final manuscript.

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