INVESTIGATION OF THE BIOLOGICAL BACKGROUND OF DROUGHT STRESS IN MALTING BARLEY VARIETIES AND LINES

THESES OF DOCTORAL (PhD) DISSERTATION

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MOSONMAGYARÓVÁR
2021

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FACULTY OF AGRICULTURE AND FOOD SCIENCES
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Wittmann Antal Plant, Animal and Food Science Multidisciplinary Doctoral School

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1. INTRODUCTION

The most important cereal crop in Hungary, after wheat and maize, is barley. Booth spring and autumn barley are of outstanding importance in the food industry. Their cultivation is a great challenge for Hungarian farmers every year, as permanent or temporary water shortages are a common phenomenon in the continental climate of Hungary (PÁLFAI 2011). One of the requirements of profitable farming is the breeding of barley varieties that meet the current user requirements, have good nutritional content, can be grown economically, have a high-yield, and are also resistant to biotic and abiotic stresses. In order to expand the possibilities of breeding for drought tolerance, the author, in cooperation with the Plant Breeding Research Station of Cereal Research Non-Profit Ltd. at Táplánszentkereszt, Hungary, carried out laboratory tests based on field data of 22 spring barley varieties and breeding lines already in public cultivation. The drought tolerance of the cultivars was determined in a 5-repeat small-plot field experiment at 5 Great Plain and 4 Transdanubian experimental sites by Tomcsányi (2012) using the so-called bioassay method.

The following goals were set during the work:

- Can plant responses associated with drought tolerance (shoot and root growth, soluble sugar and proline content, photosynthetic activity, evaporation, etc.) be used in seedling testing? Can the effect of dehydration treatment be demonstrated by seedling tests, and do barley varieties and lines differ in this respect?

- How does the expression of the Hsdr4 gene, responsible for drought tolerance, change in response to drought, and does the rate of gene expression change when examined in a European variety range?
- Is it possible to deduce the drought tolerance of varieties / lines in the field by examining seedlings?
- Is it possible to develop a cost-effective, fast, reliable laboratory selection methodology for drought tolerance in the field with the help of the examined parameters?
- Can natural biostimulants reduce the effect of drought, can there be a difference in the barley varieties in this regard?

2. MATERIALS AND METHODS

2.1. Experimental plants, variety selection

A total of 23 cultivated spring barley (*Hordeum vulgare* L.) varieties were used for laboratory tests. The effect of osmotic stress on seedlings was studied on 22 spring cultivars and breeding lines included in the region-variety multi-plot experiments of the Plant Breeding Research Station of Cereal Research Non-Profit Ltd. at Táplánszentkereszt, Hungary. The field drought tolerance of the varieties has already been known; it was determined on the basis of the field yield results in 2011 (TOMCSÁNYI 2012). For laboratory experiments to reduce drought stress by algae treatment, 9 different spring barley cultivars and one drought-tolerant genotype were selected. *Hordeum vulgare* L. cv. Rihane, (ROMDHANE et al. 2020) were provided to us by the Plant Diversity Center in Tápiószele.

2.2. Cultivation of experimental plants

30-30 grains per barley variety were germinated at room temperature (25 °C) in a humid environment and then placed on a germination net on day 2. Seedlings were grown in liquid culture for the first six days in tap water at room temperature under laboratory light conditions. The plants were subjected to stress treatment on the 7th day after germination (with 20% PEG6000 solution or with 16 hours drying technique), then 10-10 plants were sampled in a representative manner (along a X-shaped line) on the ninth day for laboratory measurements. In the experiments aiming to reduce drought stress by algae treatment the photosynthesizing prokaryotic *Nostoc entophytum* (MACC-612) and the eukaryotic *Tetracystis sp.* (MACC-430) algae were provided in a lyophilized state by the Mosonmagyaróvár Algae Collection. Algae suspensions (ÖRDÖG 2015) were used in the liquid culture at a concentration of 2 g L^{-1} from the 2nd day after germination. Algae treatment was also used together with the dehydration treatment on the 7th day.

2.3. Laboratory experiments

In our laboratory studies on the effects of osmotic stress and algae suspensions on seedlings, we measured the root and shoot length of the seedlings of the control and treated cultivars, and the total wet weight of the shoots and roots. The condition of the plants was characterized by the water saturation deficit (WSD%) parameter according to STOCKER (1929). Stoma conductance, proportional to stoma openness, was measured in four replicates per cultivar, on three plants with a LI-6400 instrument, whereas changes in photosynthetic activity was recorded with a pulsed amplitude modulation (PAM) portable MINI-PAM chlorophyll-a fluorimeter. From the measured parameters (Fo, Fm, F'm) was calculated the light utilization of the plants (Fv/Fm = (Fm-Fo)/Fm) (BILGER and SCHREIBER 1986), the effective fluorescence yield of PSII (Y = (F'm – F)/F'm) and non-photochemical quenching (NPQ = (Fm – F'm)/F'm) (BILGER and BJÖRKMAN 1990).

The detection of proline in seedlings was performed according to the work of BATES (1968) and the soluble sugar content was measured according to the work of DUBOIS (1956).

The expression of the Hsdr4 gene involved in drought tolerance was examined based on the work of SUPRUNOVA et al. (2007). TRIzol® reagent was used to extract total RNA from plant samples. After synthesis of the cDNA, the reaction was run on a Mx3000P qPCR System (Agilent Technologies) Real-Time PCR in normal mode. Hsdr4 expression levels, resulting from the treatments, was calculated based on the work of LIVAK and SCMITTGEN (2001).

2.4. Statistical methods

Growth, physiological and molecular biological parameters were measured in n=4 replicates during the different treatments. Student's t-test for equal and unequal variances (based on F-test) was used in Microsoft Excel for pairwise comparison of group means. For data showing non-normal distribution, comparisons were made using the Mann-Whitney test. The difference in the mean of two groups was considered significant if the probability of the null hypothesis was less than 5% ($p < 0.05$). The relationship between some parameters of barley cultivars and field drought tolerance was analyzed by regression analysis in Microsoft Excel.

One-way ANOVA (BM SPSS Statistics 25.0 program) was used to analyze the effect of multiple treatments. To confirm the effect of each treatment, the Bonferroni test was used as a post-hoc test, which was considered significant at $p < 0.05$.

3. DISCUSSION OF RESULTS AND CONCLUSIONS

3.1. Effect of dehydration treatment on seedlings

There were several changes observed in the growth parameters of the 9-day-old seedlings of the studied spring barley cultivars and lines as the effect of stress. The root length and shoot length of the 22 spring barley cultivars decreased significantly ($p < 0.05$) in the dehydrating treatment group compared to the control.

Proline content increased significantly ($p < 0.05$) in the drought tolerant (based on field drought tolerance data) GK Foam cultivar as the effect of osmotic stress. The soluble sugar content of the treated varieties increased significantly ($p < 0.05$) compared to the untreated group.

The maximum quantum efficiency (Fv/Fm) of the photochemical system II was significantly ($p < 0.05$) decreased in seedlings exposed to 16 hours of desiccation treatment compared to control, in contrast to seedlings subjected to milder stress (2 days 20% PEG treatment). Although PEG treatment did not significantly reduce the total Fv/Fm values of spring barley plants in overall, but PEG and desiccation treatment resulted in significantly ($p < 0.05$) lower Fv/Fm values in most field drought tolerant spring barley cultivars compared to control plants.

In contrast to the literature data, PEG and desiccation treatment significantly ($p < 0.05$) decreased the value of non-photochemical quenching (NPQ) of spring barley cultivars in overall and also individually compared to the control. We hypothesize that the development of defense mechanisms in seedlings is not yet complete. Stomatal conductance was reduced by 84% with treatments (PEG and desiccation). This might indicate that stomatal closure could be triggered by a relatively weaker stress-inducing treatment (PEG), which causes disturbances in the repair mechanisms of the photosynthetic apparatus.

As a result of dehydration treatment, the overall Hsdr4 transcript level of spring barley cultivars increased (Hsdr4 $\Delta\Delta C_T$: 0.474). The increase was smaller in the medium and poor field drought tolerant cultivars such as GKS 901, Explorer, Chill and KH Lédi, whereas it was higher in GK Habzó, Marthe and GKS9413 which were shown to be tolerant according to field drought tests.

3.2. Correlation of field drought tolerance and laboratory parameters of spring barley varieties

Growth, physiological, and gene expression parameters were correlated with field drought tolerance calculated from the region-variety small-plot field experiments. There was a strong ($p < 0.01$) correlation between field drought tolerance and the difference in root weight of control and PEG-treated plants as well as Hsdr4 transcript levels. There was medium ($p < 0.05$) correlation between shoot length, shoot weight and root weight of control plants, proline content of PEG-treated plants, difference between proline content of control and PEG-treated plants, light utilization of control plants, and stoma conductance of control plants. There was a weak ($p < 0.1$) correlation between the soluble sugar content of the control plants, non-photochemical quenching, and the difference in NPQ of the control and PEG-treated plants.

3.3. Estimation of drought tolerance of spring barley cultivars using tolerance index (TI) and extended tolerance index (KTI)

In selection for field drought tolerance, the developed tolerance index may be suitable for pre-selection of breeding lines, so that extremely drought sensitive lines can be screened at the seedling age (SCHMIDTHOFFER et al. 2018). First, cost-effective and easy-to-test parameters related to field drought tolerance (shoot weight of control plants, root weight of control plants, and proline content of PEG-treated plants) were pooled and correlated with field drought tolerance of cultivars ($r = 0.62$, $p < 0.001$). 1/3 of the varieties can be safely distinguished in terms of drought tolerance using this index. For more efficient selection, another extended tolerance index (KTI) consisting of several added parameters (difference in root weight of control plants, light utilization of control plants, and expression of Hsdr4 gene) was generated. The extended tolerance index correlated even more closely with the field drought tolerance ($r = 0.69$) and was significant at $p = 0.001$ probability level. On the basis of the extended index, varieties that are tolerant to drought can be distinguished even more precisely from varieties which are drought sensitives, thus, more than 50% of the lines could be selected with high certainty for drought tolerance in the early stages (just after genotyping) of the breeding process.

3.4. Changes in osmotic stress tolerance of spring barley cultivars as a result of algae treatments

Algae treatments (0.2%) after osmotic stress had a positive effect on root growth mainly in weakly or moderately water-intensive cultivars. Shoot length was significantly ($p < 0.05$) increased in Tatum cultivar after both osmotic stress treatments. Shoot weight was also significantly ($p \leq 0.05$) higher due to both algal suspension treatments (*Nostoc entophytum* treatment alone and after post-osmotic stress).

Significantly ($p < 0.05$) higher Fv/Fm values, compared to PEG treatment alone, were measured for both algae treatments, mainly in drought-tolerant cultivars. It is hypothesized that photosynthetic pigments in these cultivars suffer less osmotic damage because of the beneficial effect of algae suspension treatments. Non-photochemical quenching (NPQ) increased significantly ($p < 0.05$) in the Grace variety in both algae suspension treated groups compared to PEG treatment alone.

The change in Hsdr4 gene expression ($\Delta\Delta C_T$) relative to PEG treatment decreased the most in the drought sensitive KH Lilla variety as the effect of *Tetracystis sp.* algal suspension treatment (70.8%) whereas it decreased by 33.5% with *Nostoc entophytum* treatment after osmotic stress in the case of the extremely drought-tolerant Rihane variety. The decrease in gene expression levels is a sign of drought tolerance, which is confirmed by the fact that a strong $p < 0.01$ correlation was found between field drought tolerance and changes in Hsdr4 gene expression. Both microalgal strains can be used to mitigate the drought effect.

Our new scientific results can be applied in the practice of crop production. In order to increase crop safety, we consider important to use natural biostimulants and to extend the methodology of the tolerance index proposed in the dissertation when selecting other plant species for drought tolerance.

4. NEW SCIENTIFIC RESULTS

1. The response of spring barley cultivars / genotypes to dehydration treatment (20% PEG 6000 and drying) can be studied at seedling age.

2. Some parameters (root length and fluorescence yield) showed no correlation with field drought tolerance of the cultivars.

3. Many of the parameters studied may be potential selection markers during plant breeding as they correlate with field drought tolerance. These properties are:

- Control shoot length - medium correlation r = 0,41
- Control root weight - medium correlation r = 0,48
- Control shoot weight - medium correlation r = 0,51
- Shoot weight difference (control - PEG) - medium correlation r = 0,49
- PEG proline content - medium correlation r = 0,45
- Difference in proline content (control - PEG) - medium correlation r =-0,46
- Soluble sugar content - weak correlation r = -0,38
- Control Fv / Fm - medium correlation r = 0,41
- Control NPQ - weak correlation r = 0,39
- NPQ difference (control - PEG) – weak correlation r = 0,35
- Control stoma conductance - medium correlation r = -0,43
- Root weight change (Control-PEG) – strong correlation r = 057
- Hsdr4 transcript level - strong correlation r = 0,55

4. The efficiency of selection for drought tolerance can be increased by creating a cost-effective tolerance index (r= 0.62) and an extended tolerance index (r= 0.69) from the parameters. The closer correlation (r = 0.69) resulted in more efficient selection.

5. The methodology described in the dissertation is suitable for the early selection for drought tolerance.

6. Both *Nostoc entophytum* and *Tetracystis sp.* microalgae can be used as biostimulants to mitigate the effects of drought.

5. PUBLICATIONS RELATED TO THE TOPIC OF THE DISSERTATION

Peer-reviewed journal article in a foreign language

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Peer-reviewed journal article in Hungarian

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