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ASSESSMENT OF HETEROTIC ROOT AND SHOOT TRAITS OF BREAD WHEAT SEEDLINGS UNDER PEG-INDUCED DROUGHT

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Abstract

The phenotyping of desirable root and shoot traits is easier at early stages of development and can reveal drought-tolerance potential. A total of 11 genotypes from the collection of 101 bread wheat genotypes, with desirable traits in terms of increased tolerance to drought, were chosen for parents (P) and eight crosses were performed. The objective of this research was to determine heterotic root and shoot traits among nine traits evaluated in 19 bread wheat genotypes from P and F1 generations at the seedling stage, grown in hydroponic cultivation under PEG-induced drought stress and under control conditions. These two generations differed the most in mean values of distance to the first branch on the primary root and in angle of seminal roots, under control conditions, and in mean values of primary root length (PRL), number of seminal roots (NSR), stem length (SL), under induced drought stress. The F1 progeny was heterotic for PRL, NSR, SL, and these traits could have contributed to its greater tolerance to drought.

Key words: *Triticum aestivum* L., root system architecture, aboveground plant traits, crosses, drought tolerance

Introduction

The drought is a consequence of climate change manifested through global warming. According to computer projections, global surface temperature may warm from 1.3 °C to 8.0 °C by 2100, depending on the global climate model and the shared socioeconomic pathway scenario used for the simulations (Scafetta, 2024). Root system traits are increasingly becoming a key target for researchers and breeders to improve drought tolerance in wheat crops (Stangoulis and Nguyen, 2019). Root length at the seedling stage is a key genetic trait for increasing yield in arid environmental conditions (Ahmed et al., 2019).

The depth and branching of root systems are recognized as key components that enable plants to access water in the soil (Ober et al., 2021). Lopes and Reynolds (2010) showed that differences in root depth, that existed between closely related wheat isogenic lines were superior adaptations to drought. Root angle is considered an important adaptive trait that determines the vertical and horizontal distribution of roots in the soil (Slack et al., 2018). Root system architecture traits in wheat, such as the number and angle of growth of seminal roots, are genetically determined and associ-

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ated with drought resistance in a way that the narrow angle of the seminal roots and their greater number represent reference traits for selection in the early growth stages in breeding programs (Richard et al., 2015).

The objective of this research was to: a) compare mean values of the investigated root and shoot traits of wheat seedlings of selected parents and F1 offspring under induced drought stress and under control conditions; b) assess differences of investigated traits between induced drought stress conditions and control conditions in order to point to drought tolerance related traits.

Material and methods

A total of 101 genotypes of bread wheat (*Triticum aestivum* L.) from the collection of the Maize Research Institute "Zemun Polje", Serbia, originating from Serbia and 16 different countries of the world were analyzed in the seedling stage for root system architecture and shoot traits, in order to determine drought tolerant wheat varieties (Blažić et al. 2021; Blažić et al. 2023). A total of 11 genotypes of bread wheat were chosen for parents in this investigation and eight crosses were performed: 1. Euklid (FRA) x CHI 4 (CHN); 2. Dika (SRB) x Ingenio (FRA); 3. Pobeda (SRB) x Donska semi-dwarf (RUS); 4. Phoenix (USA) x NS 40S (SRB); 5. Pobeda (SRB) x Brigant (GBR); 6. Dika (SRB) x Donska semi-dwarf (RUS); 7. Zemunska rosa (SRB) x Ingenio (FRA); 8. WWBMC2 (USA) x Ingenio (FRA). Criteria for choosing parents in crosses were related traits to increased tolerance to drought: longer stem length, longer primary root, large number of seminal roots, large root dry mass and stem dry mass, beginning of root branching at the greatest possible distance from the beginning of the root. Contrasting genotypes that had the widest or narrowest angle between the outermost seminal roots were selected. Crosses were made with plants grown in pots under controlled greenhouse conditions. Each genotype was sown in three sowing dates with a period of three days between the dates, in order to neutralize the possible difference in the length of vegetation of the genotypes during crossing.

The F1 offspring and parents were simultaneously grown in hydroponic experiment (induced osmotic stress vs. control) in the laboratory of the Plant GeneBank of the Directorate for National Reference Laboratories of the Ministry of Agriculture, Forestry and Water Management of the Republic Serbia, in 2020. year. Seedlings of wheat genotypes were grown in a phytotron (KBW 720, Binder GmbH). Fifteen uniformly germinated seeds per genotype per treatment/control were placed on the perforated lid of the plastic box divided in the middle by a plastic partition, for the purpose of providing control conitions and conditions for applying stress treatment with two control genotypes-NS 40S and Zemunska Rosa. Wheat seedlings of different genotypes were grown in a completely randomized design with three replicates in both growth conditions. In one half of the box, there was an aqueous nutrient culture that represented control and in the second half of the box, the genotypes were first grown only in nutrient culture for three days, and then after those three days (on the seventh day from the start of germination), polyethylene glycol 6000 (PEG-6000, ACROS OrganicsTM) was added causing moderate desiccation stress which resulted in an osmotic potential of -0.4 MPa. The mode of operation of the phytotron was set as described in Branković et al. (2024). After 10 days of cultivation in the phytotron, 10 representative seedlings per genotype were selected, scanned, and the photographs were processed in the program ImageJ (Rasband, 2020).

The following traits were measured: primary root length (PRL), distance to the first branch on the primary root (DFBR), number of seminal roots (NSR), total seminal root length (TSRL), angle of seminal roots (ASR), stem length (SL), root dry mass (RDM), stem dry mass (SDM), and RDM/SDM. All statistical analyses were performed in the program MINITAB V. 16 (Minitab Inc., 2021).

Results and discussion

The differences in mean values for PRL of parents and F1 offspring under control conditions were not statistically significant. However, these differences under induced drought stress were statistically significant ($p < 0.05$). Under induced drought stress conditions, the mean value of PRL in the parents decreased by 11.6% on average, while in the F1 generation it decreased by 2.2% (Figure 1). Therefore, the PEG treatment had a much greater effect on the reduction of this trait in the parents than in the F1 generation, which showed greater resistance to the applied treatment. The results of this research confirm earlier reports that PRL in the seedling stage is an important trait that should be taken into account when choosing selection criteria for drought tolerance of wheat genotypes (Lu et al. 2022) as well as an indicator of drought tolerance in later stages of growth (Comas et al., 2013). Also, maximum root depth is potentially a good criterion for drought tolerance as root length has been shown to have a positive correlation with root-to-stem ratio (Kou et al., 2022).

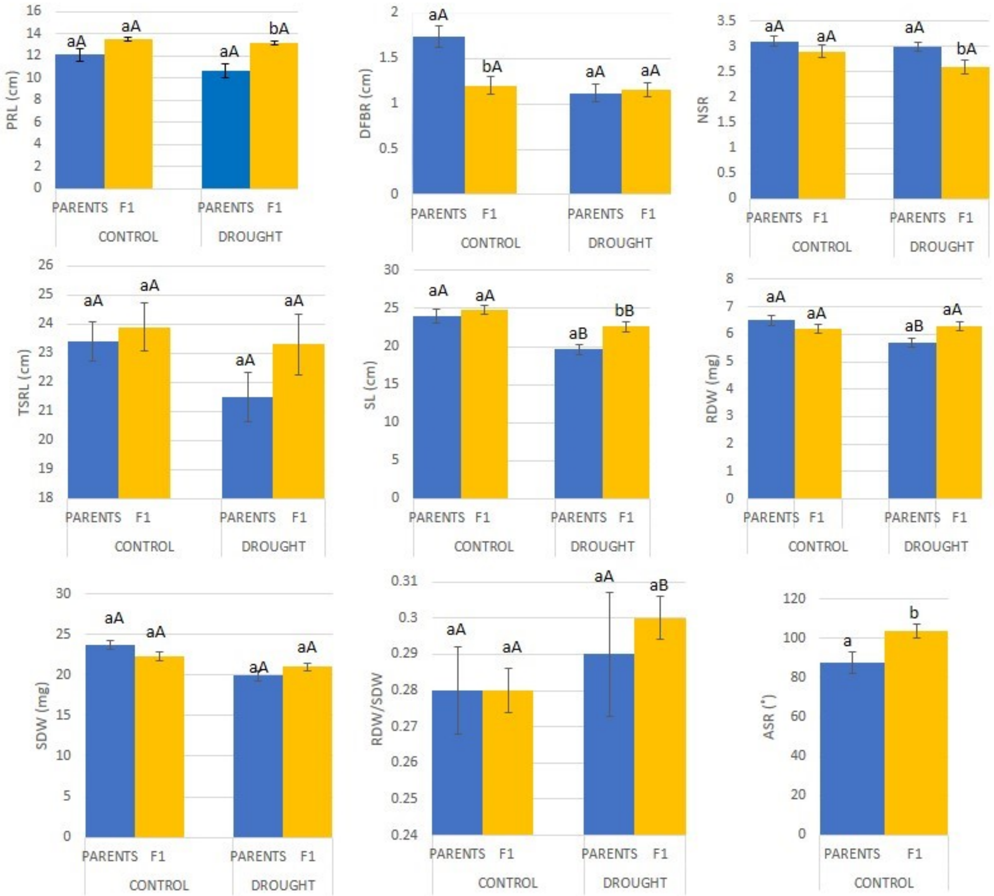

Figure 1. The mean values of root and stem traits for 11 parental genotypes and 8 F1 offspring of wheat seedlings under induced drought stress and under control conditions.

Grafikon 1. Srednja vrednost ispitivanih osobina korena i stabla 11 roditeljskih genotipova i 8 F1 potomstava klijanaca pšenice u uslovima indukovanog stresa suše i kontrole

PRL-primary root length, DFBR-distance to the first branch on the primary root, NSR-number of seminal roots, TSRL-total seminal root length, ASR-angle of seminal roots, SL-stem length, RDM-root dry mass, SDM-stem dry mass, RDM/SDM-the ratio root dry mass to stem dry mass.The combination of different lowercase letters (ab) indicates that there are statistically significant differences between the mean values of the examined traits of the parents and the F1 generation within the same treatment at the level of significance $p < 0.05$. The combination of different uppercase letters (AB) indicates that there are statistically significant differences between the mean values of the examined traits in the parental or F1 generation between different treatments (water-PEG) at the level of significance $p < 0.05$. ASR was measured only in conditions without induced drought stress.

It was shown that there was a statistically significant difference ($p < 0.05$) between the mean values for DFBR of the parents and the F1 offspring under control conditions. That value was higher in the parent generation (1.74 cm) than in the F1 generation (1.20 cm) (Figure 1). However, with PEG treatment, there was no statistically significant difference between parents and F1 offspring. Treatment with PEG resulted in a significant reduction of this trait in the parents (35.6%), while for F1 offspring the reduction was only 3.3%. As the reduction of this trait in the parents was significantly greater compared to the F1 offspring, it could be concluded that the F1 offspring were more resistant to the applied PEG treatment. Root systems branching at a shallower depth can take up surface water accumulated as a result of occasional rainfall, but not water stored in deeper soil layers.

The deep root trait and the dense root trait at greater depth are likely to be valuable where there is water scarcity in the late growing season, which is common worldwide. Selection for deeper, more efficient root systems has been shown to significantly improve water and nitrogen uptake (Odone et al., 2024). The difference in the mean values (0.2) of NSR between the parents and the F1 offspring under control conditions was not statistically significant. Also, the reduction of this trait under induced drought stress in parents was 3.2%, however, in the F1 offspring, that reduction amounted to 10.4%, which caused a statistically significant difference ($p < 0.05$) in the mean values of this trait between parents and F1 offspring under PEG treatment (Figure 1). This shows that the obtained F1 offspring were more sensitive to the applied PEG treatment compared to the parents regarding this trait. Thus, genotypes with higher NSR were more resistant to the applied treatment with PEG. A greater NSR results in more intensive root branching and root density at depth, which significantly increases the capacity of root systems for efficient absorption of water from deeper soil layers (Zhang et al., 2024). A greater NSR and their narrow growth angle are considered reference traits for selection at early growth stages in wheat breeding programs (Pais et al., 2022). The angle of seminal roots was not measured under PEG treatment. Under control conditions, the differences between the mean values of ASR of the parental and F1 generations were statistically significant ($p < 0.05$) (Figure 1).

The parents had smaller mean values of ASR (88°) compared to the F1 progeny (105°). Manschadi et al. (2008) suggested that selection for a narrow ASR results in deeper root growth and higher yields. The ARS of wheat seedlings can predict root system architecture and root depth (Kang et al., 2024). The total length of roots in the soil impacts the absorption of water and nutrients and the overall performance of the crop (Pais et al., 2022). It was shown that there was no statistically significant difference between the mean values for TSRL of the parents and the F1 offspring under control conditions, as well as under induced drought stress (Figure 1). Treatment with PEG caused a decrease in TSRL values for both the parental and F1 generations. That reduction was 8.1% in the parents, and 2.5% in the F1 offspring. F1 progeny had a greater TSRL and were more resistant to the applied PEG treatment in terms of this attribute. It has been shown that drought-resistant wheat genotypes have a greater TSRL compared to drought-sensitive genotypes (Tomar et al., 2016). The increased TSRL results from their greater number and greater length of each one individually, and when plants are grown in soils with insufficient water or nutrient content, extensive root systems are essential (Pais et al., 2022). SL mean values of parents and F1 offspring were not significantly different under control conditions (Figure 1).

The F1 progeny had an average of 0.8 cm longer stem. The difference in mean values of SL between parents and F1 offspring under induced drought treatment was 3 cm and was statistically very significant ($p < 0.05$). The results show that PEG treatment caused a significant decrease in the value of this trait in parents (18.3%) compared to the F1 generation (8.9%). Mean values for SL were significantly different ($p < 0.05$) between parental generation and beetween F1 offspring between different treatments (Figure 1). As the length of the first seedling leaf was recorded as the length of the stem, the F1 progeny had smaller length of the first leaf and was more resistant to the applied PEG treatment. The decrease in leaf length due to the effect of osmotic stress indirectly affects the decrease in leaf area. Limiting leaf area to control transpiration in drought conditions will also reduce potential yield by limiting the necessary assimilates for the grain filling phase (Zhao et al., 2020). For all three traits- RDW, SDW, RDW/SDW, the mean values of the parents and the F1 offspring did not differ significantly either under control conditions or under PEG treatment (Figure 1). Mean values for RDW were significantly different ($p < 0.05$) between parental generation between different treatments (Figure 1). Mean values for RDW/SDW were significantly different ($p < 0.05$) between F1 offspring between different treatments (Figure 1). Of all three traits, the biggest difference between mean values under control conditions and under induced drought stress between parents and F1 offspring was in SDW. Vigorous wheat genotypes have been shown to have higher root biomass than non-vigorous wheat genotypes (Blažić et al., 2024).

Treatment with PEG caused a significant decrease in SDW in the parental and F1 generation, because induced drought stress caused a significant decrease in the SL, which was then reflected in its weight. It has been shown that in dry environmental conditions the aboveground mass of the plant decreases, while the underground mass increases (Nezhadahmadi et al., 2013). The mean values of RDW/SDW were higher under induced drought stress in both the parental generation and F1 generation.

Conclusion

The parents and F1 offspring from all crosses differed the most in DFBR and ASR mean values under control conditions, and in PRL, NSR, SL mean values under induced drought stress. The F1 progeny was heterotic for PRL, NSR, SL, and these traits could have contributed to its greater tolerance to drought stress.

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Author contributions

Conceptualization, D.D.; Methodology, M.B.; Investigation, M.B.; V.K.; Resources, M.B.; D.D.; Writing-original draft, G.B.; Supervision, D.D.; T.Ž.

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PROCENA HETEROTIČNIH OSOBINA KORENA I IZDANKA KLIJANACA HLEBNE PŠENICE U PEG-INDUKOVANOJ SUŠI

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Sažetak

Fenotipizacija poželjnih osobina korena i izdanka je lakša u ranim stadijumima razvoja i može otkriti potencijal za tolerantnost na sušu. Ukupno 11 genotipova hlebne pšenice iz kolekcije od 101 genotipa, sa poželjnim osobinama u pogledu povećane tolerantnosti na sušu, su izabrani za roditelje u 8 ukrštanja. Cilj istraživanja je bio da se utvrde heterotične osobine korena i izdanka 19 genotipova hlebne pšenice iz P i F1 generacija u stadijumu klijanaca, gajenih u hidroponskom eksperimentu u PEG-indukovanoj suši i u kontrolnim uslovima, a mereno je 9 osobina. Ove dve generacije su se najviše razlikovale u prosečnim vrednostima rastojanja do prve grane na primarnom korenu i u uglu seminalnih korenova, u kontrolnim uslovima, kao i u prosečnim vrednostima dužine primarnog korena (PRL), broja seminalnih korenova (NSR), dužine izdanka (SL), u uslovima indukovane suše. F1 potomstvo je ispoljilo heterozis za osobine PRL, NSR, SL. Navedene osobine mogu poslužiti kao pouzdani indikatori povećane tolerantnosti datog genotipa na sušu.

***Ključne reči*:** *Triticum aestivum* L., arhitektura korenovog sistema, nadzemne osobine biljke, ukrštanja, tolerantnost na sušu

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