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Effects of osmotic stress on root development in winter wheat (*Triticum aestivum* L.) genotypes with diverse drought tolerance

Az ozmotikus stressz hatásainak vizsgálata eltérő szárazságtűrő-képességű őszi búza genotípusok gyökérfejlődésére

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ABSTRACT

Climate change results in more frequent and severe drought periods that highlight the importance of breeding aiming to identify drought-tolerant wheat lines, thus increasing food security. One of the most important components of surviving the drought period could be the dynamics of root development, the shape of the root structure, which helps the plant through more efficient water absorption. This experiment investigated the root development of 18 winter wheat genotypes at the seedling stage in a hydroponic system. The seedlings were grown in Hoagland's solution, to which, in the case of the treated group, polyethylene-glycol (PEG-6000) was added at a concentration of 18%, osmotically inhibiting their water absorption. After four days of treatment, the root parameters were measured using the WinRHIZO Pro system. As a result of PEG treatment, the total root length and surface area of all varieties significantly decreased. The greatest reduction was measured in the Babuna and Bayraktar varieties, where the reduction in length exceeded 90% (91.8% and 93.5%, respectively), while in the case of four varieties (Disponent, Aura, Salamouni and Scirocco) the reduction didn't even reach 50%. All four varieties can be classified into middle and late-ripening groups.

Keywords: osmotic stress, drought tolerance, polyethylene glycol, cereal, wheat seedling, root development, climate change

KIVONAT

Az egyre gyakoribb és súlyosabb aszályos periódusokat eredményező éghajlatváltozás felértékeli azt a kutatói-növénynemesítői törekvést, mely jobb szárazságstressz-tűrő búza vonalak azonosítását és előállítását célozza, növelve ezzel a termésbiztonságot. Az aszályos időszak átvészelésének egyik legfontosabb összetevője lehet a gyökérfejlődés dinamikája, a gyökérstruktúra kialakulása. Kísérletünkben 18 őszibúza fajta csíranövénykori gyökérfejlődését vizsgáltuk hidropóniás rendszerben. A csíranövényeket Hoagland oldatban neveltük, melyhez a kezelt csoport esetében 18% töménységben polietilén glikolt (PEG-6000) adtunk, ozmotikusan gátolva a vízfelvételüket. Négy napos kezelést követően a gyökérparamétereket WinRHIZO Pro gyökérszkenner és szoftver segítségével mértük. A PEG kezelés hatására valamennyi fajta összesített gyökérhossza és gyökérfelülete csökkent. Legnagyobb mértékű csökkenést a Babuna és Bayraktar fajtáknál mértük, ahol a csökkenés mértéke a 90%-ot is meghaladta (91,8%, 93,5%), míg négy fajta esetében (Disponent, Aura, Salamouni és Scirocco) a csökkenés mértéke az 50%-ot sem érte el. Mind a négy fajta közép és késő érésű csoportba sorolható.

Címszavak: ozmotikus stressz, szárazságtűrés, polietilén glikol, gabona, búzacsíranövény, gyökérnövekedés, klímaváltozás

INTRODUCTION

The increasing frequency and intensity of drought events, exacerbated by climate change, present a significant threat to global agriculture. Drought is one of the major stress factors that limit cereal production worldwide and may affect about 40–60% of the world's agricultural lands (Farkas et al., 2021). Among the primary cereal crops, winter wheat (*Triticum aestivum* L.) has a critical role in human and animal nutrition, contributing approximately 20% of global caloric intake (D'Odorico et al., 2014). However, wheat as well as other small grain cereal species like barley and oats are sensitive to water deficits, particularly during key developmental stages such as germination and grain filling. The negative consequences of drought stress have made it a primary target for research into stress tolerance mechanisms (Peršić et al., 2022).

Drought stress significantly impairs wheat productivity, primarily by limiting water availability for physiological processes (Abido and Zsombik, 2020). The ability of a wheat genotype to survive and thrive under such conditions is intricately tied to its root system architecture (RSA)(Hoad et al., 2001).

Root system performance under drought varied significantly by genotype (Varga et al., 2015). Key RSA traits such as root depth, root length density, surface area, and xylem diameter are instrumental in enhancing drought tolerance by improving water uptake from deeper soil layers (Djanaguiraman et al., 2019). Varieties with longer and deeper root systems performed better under water-limited conditions (Varga et al., 2022) and had been shown to exhibit yield advantages under water-limited conditions, with increases in thousand-kernel weight and grain yield of up to 35% and 38%, respectively, compared to shallow-rooted varieties (Li et al., 2021; Li et al., 2024).

The genetic basis for RSA traits has been explored extensively, leading to the identification of several quantitative trait loci (QTLs) associated with drought tolerance (Ober et al., 2021; Tibor Kiss, 2016). These studies have uncovered genes influencing root morphology and other traits critical for drought adaptation, including root length, dry weight, and surface area (Peršić et al., 2022). For instance, genome-wide association studies (GWAS) have enabled the mapping of genetic factors controlling seedling-stage drought tolerance, providing valuable insights for breeding programs aimed at enhancing wheat's resilience to drought stress (Zhang et al., 2015).

Controlled environment studies, such as those utilizing hydroponic systems and polyethene glycol (PEG)-induced osmotic stress, can be essential for early phenotyping of RSA traits. PEG reduces the osmotic potential of the growth medium, simulating drought conditions and enabling the study of genotypic responses under water-limited scenarios (Peršić et al., 2022; Tabatabai et al., 2022). Such methods could be able to allow researchers to identify genotypes with favourable RSA traits, which can be further exploited in breeding programs (Robin et al., 2021; Schierenbeck et al., 2023).

In addition to morphological traits, physiological and biochemical mechanisms play a pivotal role in drought tolerance. PEG-induced stress has been shown to trigger root system adaptations such as increased lateral root outgrowth and premature differentiation of root apical meristems, enhancing water absorption under drought-like conditions (Djanaguiraman et al., 2019; Robin et al., 2021). Concurrently, osmolyte accumulation and the activation of antioxidant defences have been observed as key adaptive responses (Peršić et al., 2022). However, in some crops like barley, PEG-based osmotic stress treatments may result in root anatomical changes that are not representative of field conditions, suggesting limitations in PEG-based screening in certain species (Blum, 2011; Töpfer et al., 2024)

This growing collection of studies highlights the critical importance of understanding the role of root system architecture in the field of drought stress tolerance and its genetic foundations to develop wheat varieties capable of maintaining productivity under dry periods. By combining advanced phenotyping methods with molecular breeding techniques, researchers aim to create high-performing cultivars that are well-suited to the challenges of a changing climate.

The current study aimed to screen the early rooting habits and osmotic stress reactions of a genotypes assessment showing a wide genetic variability and characterized by the Molecular Breeding Department, HUN-REN Centre for Agricultural Research for various drought tolerance-related phenotypic parameters, and mapped those genetic backgrounds based on a 25K single-nucleotide polymorphism (SNP) panel.

MATERIALS AND METHODS

To achieve the defined goal, 18 genotypes were selected for this study. To get the widest picture out of the possibilities, the genetic origins were previously studied, along with the thermal time (earliness), country of origin, and, of course, the drought stress tolerance they showed in field trials (under rainout shelter) and the final list was decided accordingly(Kiss, 2016.).

Based on drought stress tolerance, the genotypes were ranked in three categories. The first table displays the varieties, arranged by the countries of origin and marked with category-specific colors (Table 1).

The drought stress tolerance was determined by the rate of biomass and yield reduction under the rainout shelter.

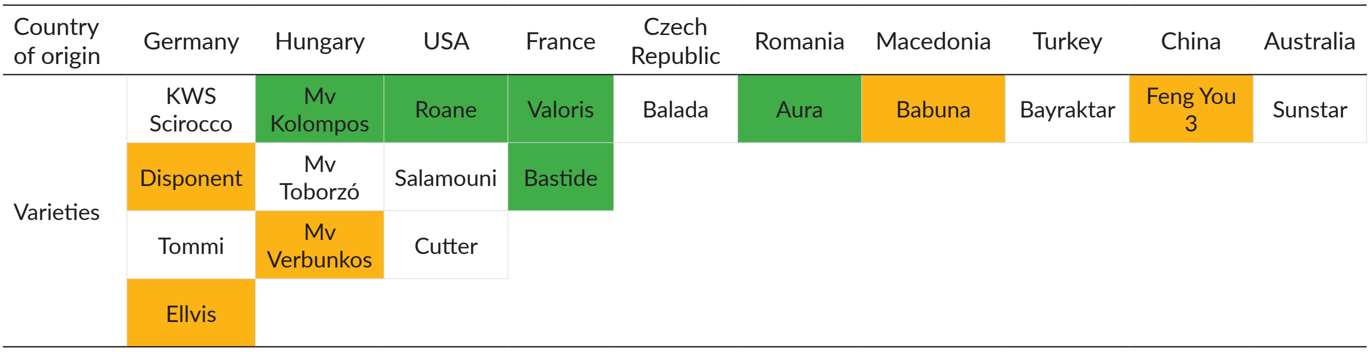
The study was carried out in the Fitotron of the Agricultural Institute in Martonvásár. Seeds were surface-sterilized in a 0.5% Neomagnol solution for 3 minutes, followed by triple rinsing with distilled water. Seeds were germinated on moistened filter paper in Petri dishes at 25 °C.

Uniform seedlings in eight replications per genotype were transferred to modified 8×12 Polymerase Chain Reaction (PCR) plates. The bottoms of the plates were removed in a manner that allowed unrestricted root growth while preventing seed displacement. The PCR plates were placed over glass tanks with a depth of 14 cm, filled with full-strength Hoagland solution up to the level of the plates. Osmotic stress was simulated by adding 18% polyethene glycol (PEG-6000) to the solution of the stress treatment group. The tanks were placed in a growth chamber (POL-EKO ST 500 Cooled Incubator) and illuminated using four LED lights (Dee-50a Blue White, 14W) with a photon flux density of 165 µmol/m² per second. The photoperiod was set to 16 hours per day, with the temperature maintained at 25 °C, 70% relative humidity, and continuous circulation of the nutrient solutions.

After 96 hours (Figure 1), the seedlings were removed from the PCR plates, and their root systems were separated.

Root parameters were digitized and measured using a WinRHIZO Pro root scanning system and software (Regent Instruments Ltd, Canada) (Figure 2). Measurements included total root length, average root diameter, and root surface area for each seedling. Statistical analyses were performed using the 'agricolae' (de Mendiburu, 2021)

Table 1. Countries of origin and drought stress tolerance of the varieties used in this study



Country of origin	Germany	Hungary	USA	France	Czech Republic	Romania	Macedonia	Turkey	China	Australia
Varieties	KWS Scirocco	Mv Kolompos	Roane	Valoris	Balada	Aura	Babuna	Bayraktar	Feng You 3	Sunstar
	Disponent	Mv Toborzó	Salamouni	Bastide						
	Tommi	Mv Verbunkos	Cutter							
	Ellvis									

The colours indicate the group of drought stress tolerance, determined based on the variety and field experiments: green - tolerant, white - intermediate, and gold – sensitive.

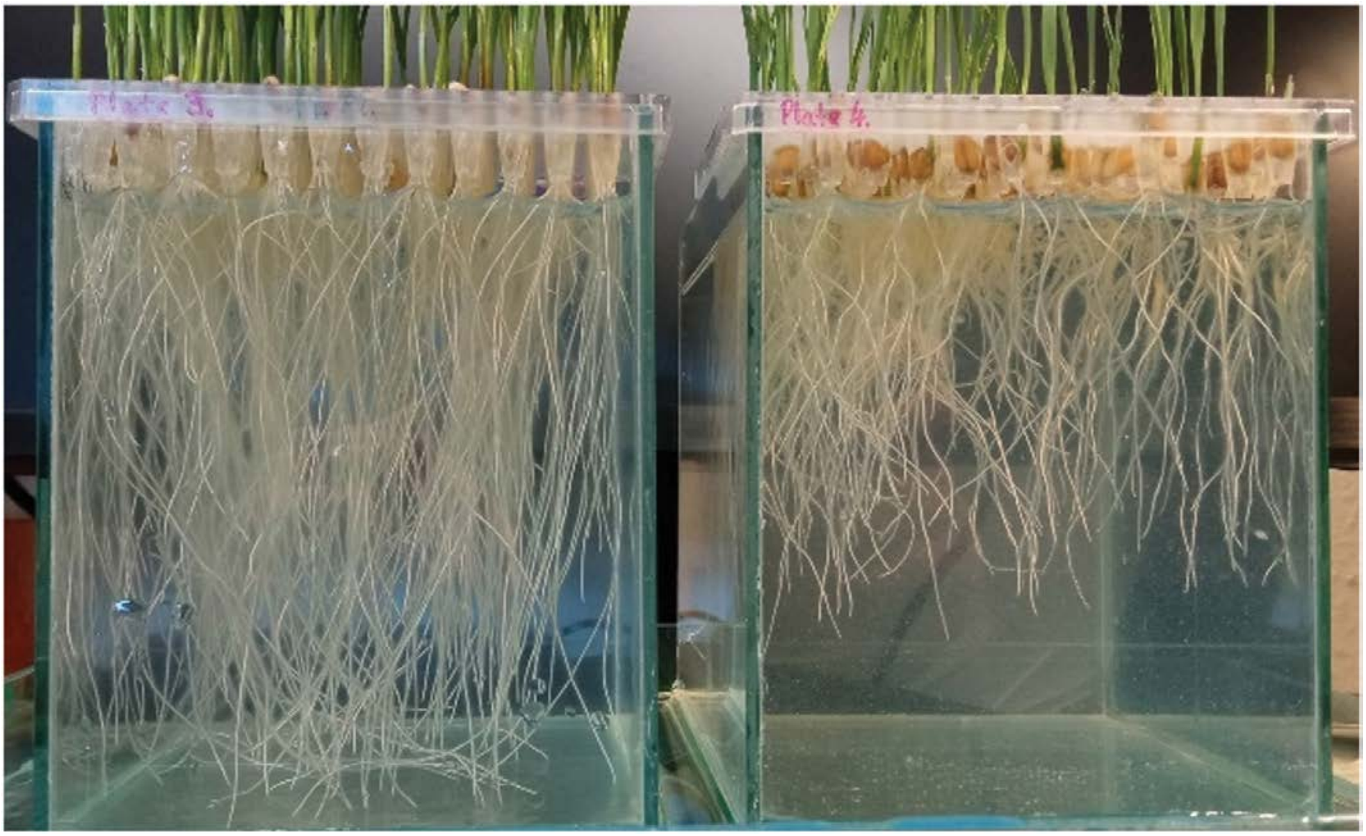


Figure 1. Plants at the end of the trial Control (left) and PEG treated (right)

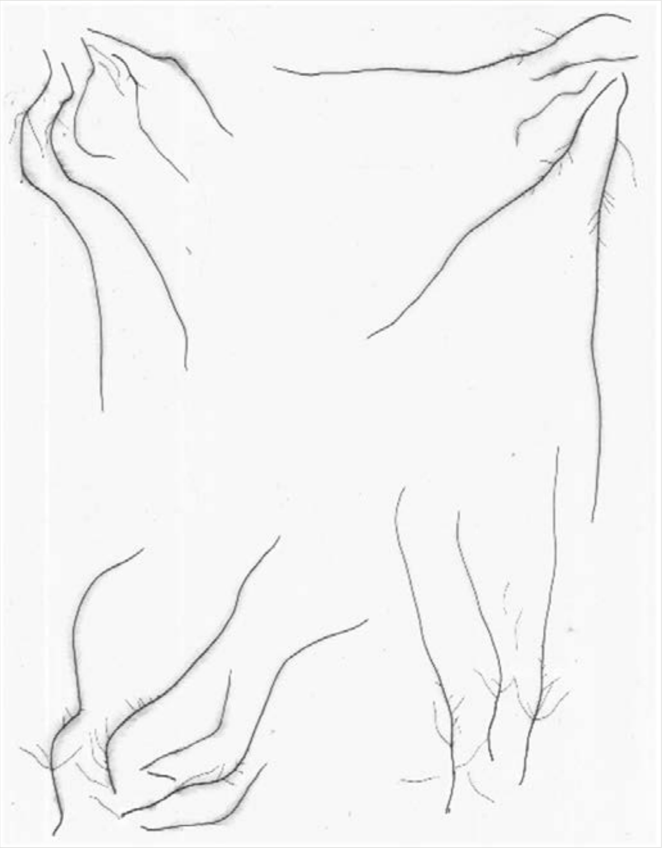


Figure 2. Digitalized image of four seedling root

package of the R statistical software (R Core Team, 2021). Within treatments, genotype means were compared using the LSD test at a significance level of ($P<0.05$). Differences in root diameter between treatments within the same genotype were evaluated using T-tests at a significance level of ($P<0.05$).

RESULTS

PEG-induced osmotic stress treatment significantly reduced root length and root surface area across all genotypes (Table 2). Consequently, significant differences among genotypes were only examined within the same treatment, because the impacts of the stress treatment resulted in a more intensive response of the varieties than the variability in genotypic differences. The osmotic stress treatment decreased root length by 70.2% on average of the 18 varieties; however, the range of the root length reduction was between 45.54% and 93.5%.

Under control conditions, the genotypes Mv Toborzó, Mv Kolompos, and Cutter exhibited the longest root lengths, whereas the shortest total root length was recorded for the Balada genotype. There were no significant differences recorded between Disponent, Babuna, Aura, Bastide, Salamouni and Valoris regarding those root lengths. The varieties with the longest root system belonged to the intermediate and drought-stress tolerant groups; however, in the simulation experiment, genotypes could have been identified as belonging to each stress-tolerant group developing a short root system (Table 2).

Under PEG-6000 treatment, root length generally decreased for each variety. The longest roots were observed for Disponent, whose genotype was ranked among the varieties having the shortest root length under control conditions. There were some genotypes whose root length did not differ significantly from Disponent; however, under control conditions, those had moderate or short root length such as Scirocco, Sunstar, Aura, Bastide and Salamouni. The shortest root length under osmotic stress conditions was observed for Bayraktar whose genotype did not significantly differ from Babuna, Balada, Tommi, Verbunkos, Valoris, Feng-you-3 and Roane. Only two genotypes, Toborzó and Cutter, were ranked among the varieties with the highest root length both under control and PEG-induced stress conditions and three varieties (Balada, Babuna and Valoris) were consequently grouped into the classes having a short root structure.

As a result of the osmotic stress, the most substantial reductions in root length were observed in Bayraktar, Babuna, Mv Verbunkos, Tommi, and Mv Kolompos, by 93.5%, 91.2%, 87.6%, 86.7% and 84.4%, respectively. The decrease in root length induced by PEG-6000 application was less intensive (between 45.5% and 50.2%) for varieties Disponent, Aura, Salamouni, Scirocco and Bastide, which varieties generally performed with short root lengths under control conditions (Table 2).

The average root diameter exhibited limited variability among genotypes under control conditions, with an average root diameter was 0,635 mm and 0.655 mm under control and drought-stressed conditions, respectively (Table 2). However, significant differences were detected between the varieties. Scirocco (0.733 mm), Salamouni (0.714 mm), and Aura (0.694 mm) had significantly larger root diameters than the other genotypes. Valoris and Cutter have thinner roots (0.571 and 0.572 mm, respectively); however, there are many other varieties whose root diameter did not differ significantly from these varieties (Mv Toborzó, Mv Kolompos, Elvis, Bayraktar, Mv Verbunkos, Babuna and Balada).

Overall, the osmotic stress induced a 3.2% increase in root diameter on average for each genotype, but the more evident impact of the stress conditions was that it increased the variability in root diameter due to differential responses among genotypes. For instance, genotype Mv Kolompos, Valoris, Elvis, Fengyou, and Valoris exhibited a significant increase in root diameter (36.1%, 22.3%, 16.7% and 16.2%, respectively 16.2%), whereas a significant reduction was observed in Bayraktar (8.4%) and Scirocco (14.2%). Under stress conditions, the root diameter of Mv Kolompos was significantly higher than that of all other genotypes, resulting in a smaller reduction in root surface area for this genotype compared to the reduction in root length.

In terms of root surface area, the osmotic stress induced a significant reduction in the average of each genotype (69.8%). When plants were grown under control conditions, the highest root surface area values were calculated in genotypes Mv Toborzó (26.74 cm^2), Mv Kolompos (25.98 cm^2), and Cutter (22.19 cm^2); however, the root surface area of Scirocco did not differ significantly from these genotypes. Scirocco belongs to the varieties with intermediate root length under control conditions; however, the largest root diameters observed in this variety resulted in the total root surface being as high as that of the varieties having the longest root system. The lowest surface area was recorded in the Balada genotype; its root surface area was less than 7 cm^2, but the root surface areas of Valoris, Salamouni, Bastide, Babuna and Disponent did not significantly differ from Balada (Table 2). The reactions of the genotypes under PEG-induced osmotic stress showed a large variability, and the plant's responses to unfavourable conditions were independent of the rooting habits observed under optimum conditions.

Table 2. Changes in root length, root surface area, and root diameter of wheat seedlings grown under control conditions and polyethene glycol (PEG-6000)-induced osmotic stress

	Root length (cm)		Diameter (mm)		Root surface (cm^{2})	
Variety	Control	Peg	Control	Peg	Control	Peg
MV Toborzó	142,954^{a}	43,372$^{a\text{-}d}$	0,594^{de}	0,595^{e}	26,74^{a}	8,02$^{a\text{-}d}$
MV Kolompos	134,800^{a}	21,032^{ef}	0,614^{cdeB}	0,836^{aA}	25,98^{ab}	4,8^{de}
Cutter	123,318^{ab}	47,164^{ab}	0,572^{e}	0,606^{de}	22,19^{abc}	8,94^{abc}
Roane	111,108^{bc}	25,533$^{c\text{-}f}$	0,629^{cd}	0,677$^{b\text{-}e}$	21,88^{bc}	4,89^{cde}
Elvis	107,162^{bc}	27,508$^{b\text{-}f}$	0,613^{cdeB}	0,715^{bcA}	20,58^{cd}	6,22$^{a\text{-}e}$
Bayraktar	106,941^{bcd}	6,997^{f}	0,625^{cdeA}	0,572^{eB}	20,94^{bcd}	1,26^{e}
MV Verbunkos	106,208^{bcd}	13,213^{f}	0,606^{cde}	0,603^{de}	20,12^{cd}	2,54^{e}
Feng you 3	101,313^{bcd}	23,970^{def}	0,642^{cdB}	0,745^{bA}	20,45^{cd}	5,06$^{b\text{-}e}$
Tommi	98,237^{cd}	13,054^{f}	0,652^{bc}	0,636^{cde}	20,13^{cd}	2,56^{e}
KWS Scirocco	96,051^{cde}	48,357^{ab}	0,733^{aA}	0,628^{cdeB}	22,18^{abc}	9,36^{ab}
Sunstar	94,465^{cde}	38,215$^{a\text{-}e}$	0,646^{bc}	0,602^{de}	18,96^{cd}	7,63$^{a\text{-}d}$
Disponent	92,949$^{c\text{-}f}$	50,616^{a}	0,636^{cd}	0,599^{de}	18,56^{cde}	9,51^{a}
Babuna	88,151$^{c\text{-}f}$	7,760^{f}	0,594^{de}	0,645$^{b\text{-}e}$	16,49^{de}	1,52^{e}
Aura	84,753^{def}	44,829^{abc}	0,694^{ab}	0,631^{cde}	18,8^{cd}	8,88^{abc}
Bastide	81,532^{def}	40,538$^{a\text{-}e}$	0,658^{bc}	0,702^{bc}	16,86^{cde}	9,02^{abc}
Salamouni	74,296^{ef}	38,31$^{a\text{-}e}$	0,714^{a}	0,695^{bcd}	16,64^{de}	8,23$^{a\text{-}d}$
Valoris	71,158^{ef}	20,756^{ef}	0,571^{eB}	0,698^{bcdA}	12,78^{e}	4,56^{de}
Balada	35,523^{f}	9,917^{f}	0,633^{cde}	0,612^{cde}	6,97^{e}	1,91^{e}
Average	97,273	28,952	0,635	0,655	19,292	5,828

In the indices, different lowercase letters indicate significant differences among genotypes within the same treatment based on the LSD test ($P<0.05$), while uppercase letters denote significant differences in root diameter between the two treatments within the same genotype, as determined by t-tests ($P<0.05$). The colors indicate the group of drought stress tolerance determined based on the variety and field experiments: green - tolerant, orange - transitional, and gold – sensitive.

The smallest root surface area was observed in Bayraktar, Babuna, Balada, Mv Verbunkos and Tommi. All these genotypes belonged to the drought-sensitive or intermediate drought-stress tolerance group, but the root surface of Mv Kolompos and Valoris ranked as drought-stress tolerant varieties, which were statistically in pair with the abovementioned genotypes. The largest reductions in root surface area under osmotic stress were recorded for Bayraktar (94%), Babuna (90.8%), Mv Verbunkos (87.4%), Tommi (87.3%), and Mv Kolompos (81.5%). Only two genotypes, Bastide and Disponent, showed less than a 50% reduction in root surface area under osmotic stress compared to the control. Under stress conditions, the root surface areas of these genotypes did not differ significantly from those of the high-performing genotypes under control conditions (Mv Toborzó and Cutter).

DISCUSSION

The results of this study provide critical insights into the response of winter wheat genotypes to osmotic stress, particularly concerning root system architecture. The findings align with and expand upon existing research on the role of RSA traits in drought tolerance. While PEG-induced osmotic stress significantly reduced root length and surface area across all genotypes, the degree of reduction varied considerably, highlighting the genetic diversity in drought response. This variability is in accordance with the findings of previous studies, underscoring the complexity of drought tolerance mechanisms and their genetic basis (Peršić et al., 2022; Tabatabai et al., 2022).

Impact of osmotic stress on root traits

The most significant reductions in root length and surface area were observed in genotypes such as Babuna and Bayraktar, which exhibited declines exceeding 90%. These findings align with prior research demonstrating the sensitivity of certain wheat genotypes to osmotic stress (Zia et al., 2021). Conversely, genotypes such as Disponent, Aura, and Salamouni showed more moderate reductions, suggesting inherent resilience. Suggestion in this case cannot be compared to field experiences: While Disponent appeared to be sensitive to drought stress, Salamouni was semi-sensitive (transitional) and Aura had shown good resistance during field trials.

Similar patterns have been documented in studies emphasizing the role of RSA traits, including deeper roots and higher root length densities, in mitigating the effects of water deficits (Nehe et al., 2021; Paeßens et al., 2019).

These differential responses reflect the critical role of genetic and phenotypic diversity in RSA traits under drought conditions. For instance, deeper-rooted genotypes are better equipped to access water from lower soil layers, a trait previously associated with improved performance under drought stress (Hoad et al., 2001; Paeßens et al., 2019).The stability of root surface area in genotypes like Disponent and Scirocco under PEG treatment further supports their potential crossing partners to improve the drought resilience of progenies.

Physiological adaptations and biochemical responses

The observed reductions in root parameters, particularly in susceptible genotypes, may be attributed to the physiological stress imposed by PEG, which simulates water deficits by reducing osmotic potential. Such stress conditions can lead to premature differentiation of root apical meristems and reduced root elongation, as reported in earlier studies (Peršić et al., 2022). However, the relative stability of root traits in resilient genotypes may result from adaptive mechanisms such as enhanced lateral root growth and osmolyte accumulation, which have been shown to improve water uptake and drought tolerance (Djanaguiraman et al., 2019).

Comparison with field performance

Interestingly, some genotypes that performed poorly under PEG-induced osmotic stress have been noted for their field-level drought tolerance. This apparent discrepancy highlights the importance of environmental interactions in shaping drought responses. While PEG effectively simulates drought stress, it does not account for soil-based factors such as microbial interactions and nutrient dynamics, which may contribute to the resilience observed in field conditions (Blum, 2011). Thus, the genotypes showing strong performance under PEG treatment, such as Disponent, Aura or Bastide or did poorly like Bayraktar, Babuna and Verbunkos represent promising candidates for further testing under field conditions.

Implications for breeding programs

The variability in RSA traits observed here is consistent with the findings of QTL mapping and GWAS studies that identify genetic loci associated with root traits under drought conditions (Nouraei et al., 2024; Wang et al., 2024). Incorporating these genotypes into breeding programs offers an opportunity to leverage molecular markers for RSA traits, enabling the development of cultivars tailored to specific drought-prone environments.

The significant reduction in root length and surface area across most genotypes underscores the need for targeted breeding strategies focused on enhancing RSA traits. According to these genotypes - like Disponent and Scirocco -, which maintained higher root length and surface area under stress, and also the ones with the lowest decrease – like Disponent, Salamouni or Bastide - can be the ideal candidates for breeding programs aimed at improving drought resilience. These findings are in line with previous research suggesting that deeper and more extensive root systems are critical for sustaining yield under water-limited conditions (Djanaguiraman et al., 2019). However, previous drought simulation experiments under real field conditions showed that the most promising variety, according to the results of this recent study– Disponent – had shown high sensitivity to drought. This suggests that this method alone cannot be applied with high certainty for pre-selection based on drought tolerance. However, in conjunction with further comparisons and studies, it can serve as an important and rapid guiding tool.

Limitations and future directions

While PEG-based systems provide a controlled environment for assessing drought responses, they may not fully replicate the complexity of field conditions. Future studies should combine controlled and field-based evaluations to validate these findings and identify genotypes that perform consistently across environments. Additionally, further exploration of the molecular pathways underlying RSA traits could enhance the efficiency of breeding programs (Peršić et al., 2022).

CONCLUSION

PEG-induced osmotic stress can be an effective method for assessing drought tolerance among winter wheat genotypes at the seedling stage, revealing significant variability in root system architecture (RSA) traits. Root length and surface area were the most affected parameters, with reductions ranging from 45.5% to over 90% depending on genotype. Disponent, Aura, and Salamouni exhibited greater resilience under stress, maintaining relatively stable root parameters, whereas Babuna and Bayraktar experienced the most substantial declines, suggesting their early-stage sensitivity. These findings highlight the potential of using RSA traits, particularly root length and surface area, as early indicators for drought tolerance in breeding programs. However, discrepancies between PEG-induced stress responses and field performance underscore the need for integrative evaluations that combine controlled and field-based testing. Further comparisons are needed to determine how applicable the effects of PEG-induced osmotic stress on root growth during the seedling stage are for pre-selection purposes. The study supports the role of RSA as a key factor in drought resilience and offers valuable insights into the development of wheat varieties adapted to water-limited environments.

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