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WHEAT GERMINATION AND SEEDLINGS UNDER PEG-INDUCED CONDITONS

KLIJANJE I KLIJANCI PŠENICE U UVJETIMA SUŠE INDUCIRANE PEG-OM

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ABSTRACT

Climate changes are causing frequent drought which leads to shortage of water, reducing wheat production worldwide. The three winter wheat genotypes were examined for their response to drought at the germination and seedling stage. Research was carried out under controlled conditions where polyethylene glycol (PEG 6000) was used in 20% concentration to create artificial drought stress. The results revealed that genotype 'Vulkan' showed maximum germination percentage (85%) in control while the minimum germination percentage was observed in 'Žitarka' (43%) under PEG treatment. At control level seed germination percentage was the higher in all three genotypes compared to drought treatment. 'Vulkan' had the highest shoot length in control plants (14.18 cm) which was significantly reduced in PEG treatment (6.93 cm). Maximum fresh weight of shoots was recorded in 'Vulkan' (1.53 g) followed by 'Antonija' (1.23 g) in control treatment. Under control conditions, genotype 'Antonija' showed maximum root length in control and PEG treatment (14.52 and 12.46 cm, respectively) among other genotypes, followed by 'Vulkan' in control treatment (9.88 cm), while minimum root length was observed in 'Zitarka' in PEG treatment (6.52 cm). The root fresh weight values were decreased with increasing water stress in two wheat genotypes ('Zitarka' and 'Antonija'), while in 'Vulkan' it was increased. Root dry weight decrease was only observed in 'Zitarka'.

Keywords*:* wheat, germination, seedlings, PEG

SAŽETAK

Klimatske promjene uzrokuju čestu sušu koja dovodi do manjka vode, te smanjenja proizvodnje pšenice u svijetu. Tri genotipa ozime pšenice ispitivana su na sušu u stadiju klijanja i klijanaca. Istraživanje je provedeno u

laboratorijskim uvjetima u kojima je polietilen glikol (PEG 6000) korišten u koncentraciji od 20% kako bi se stvorio umjetni stres na sušu. Rezultati su pokazali da je genotip 'Vulkan' pokazao najveći postotak klijavosti (85%) u kontrolnoj skupini, dok je najmanji postotak klijavosti zabilježen u 'Žitarki' (43%) u PEG tretmanu. U kontroli postotak klijavosti sjemena bio je značajno veći u sva tri genotipa, u odnosu na 20% PEG 6000 tretman. 'Vulkan' je imao najveću duljinu izdanaka u kontrolnim biljkama (14,18 cm) što je značajno smanjeno u PEG tretmanu (6,93 cm). Najveća svježa masa izdanaka zabilježena je kod 'Vulkana' (1,5 g), te 'Antonije' (1,23 g) u kontrolnom tretmanu. U kontrolnim uvjetima, genotip 'Antonija' pokazao je najveću duljinu korijena u kontrolnom i PEG tretmanu (14,52 i 12,46 cm) između ostalih genotipova, te 'Vulkan' u kontrolnom tretmanu (9,88 cm), dok je minimalna duljina korijena zabilježena kod 'Zitarke' u PEG tretmanu (6,52 cm). Vrijednosti svježih masa korijena smanjene su u uvjetima suše u dva genotipa pšenice ('Zitarka' i 'Antonija'), dok je u 'Vulkanu' ta masa povećana. U sušnim uvjetima smanjenje suhe mase korijena zabilježeno je samo kod 'Zitarke'.

Ključne riječi: pšenica, klijanje, klijanci, PEG

INTRODUCTION

Due to the climate changes manifested as global warming, drought has become a worldwide problem limiting global crop production (Curtis, 2002) by decreasing average yields of most major crops more than 50% (Boyer, 1982). Reduced water potential is a common consequence of both salinity and drought (Ajirloo et al., 2013). Water deficit affects the seed germination and the growth of seedlings (Van den Berg and Zeng, 2006). Plant growth and development can be negatively impacted together with physiological and biochemical processes in plant as a consequence of water deficit (Osborne et al., 2002). Seed germination is the first stage of growth that is sensitive to water deficit. Drought tolerance at the germination stage is very important because soil moisture at sowing time can result in decrease of seedling emergence consequently affecting yield (Mwale et al., 2003). It was demonstrated that those wheat genotypes which showed drought tolerance at the germination stage exhibited the same tolerance to water deficit under field conditions (Khakwani et al., 2011).

In the seedling stage selection for drought tolerance genotypes can be made by using polyethylene glycol (PEG 6000) for inducing water deficiency stress (Rauf et al., 2006). Due to its high molecular weight this osmotic substance has been used in many studies on drought stress (Jatoi et al., 2014).

The objective of this study was to evaluate several morphological drought resistance traits under water deficiency at seedling stage in three winter wheat genotypes in order to verify whether there were differences in their response to water stress.

MATERIAL AND METHODS

In this study three winter wheat genotypes (‘Zitarka’, ‘Antonija’ and ‘Vulkan’) (Table 1) created at Agricultural Institute Osijek (Croatia) were investigated for their response at seedling stage to drought stress treatment (20% polyethylene glycol, PEG 6000) in order to create artificial stress in comparison to control treatment where only distilled water was applied while drought-exposed plants were watered daily with 20% PEG solution which was applied for 15 days. Thirty seeds in six replications of each genotype were sterilized in 70% ethanol followed by washing with distilled water. Sterilized seeds were grown in petri-dishes (diameter 90 cm) containing layers of filter paper under a 14 h photoperiod, irradiance of 250 $\mu mol^{-2}s^{-1}$ day/night temperature 25/20 ˚C and relative humidity of 60/75%.

Table 1 Origin and year of release of three investigated winter wheat genotypes

Tablica 1. Porijeklo i godina priznanja tri istraživana genotipa pšenice

Genotypes	Origin[a]	Year of release
‘Zitarka’	HR, AIO	1985
'Antonija'	HR, AIO	2011
'Vulkan'	HR, AIO	2009

[a]AIO-Agricultural Institute Osijek

Root and shoot length were measured on 7th and 14th day of experiment.

After 7 days seed germination ratio was calculated:

Seed germination = seeds germinated/total seeds x 100

After 2 weeks seedlings were collected for assessment of morphological parameters. Growth parameters (root and shoot length) were recorded in cm with a ruler after two weeks from sowing. Shoot/roots were separated, dried by tissue paper and weighed to obtained fresh weight. Shoots and roots were dried at 105 °C for 24 hours and weighed again to obtained dry weight.

Statistical analysis

Data were analyzed by using one–way ANOVA analysis and Fisher's LSD test to determine significant differences among genotypes means at significance level of 0.5. All recorded values represent the means of the results of six replications.

RESULTS AND DISCUSSION

Drought is one of the most serious abiotic stress that could influence growth and development of plant. In this research some morphological parameters at seedling stage were investigated by using polyethylene glycol 6000 (PEG 6000) as osmotic agent in three winter wheat genotypes. This experiment with PEG solution showed a significant variation among wheat varieties in recorded morphological parameters. Analysis of variance revealed significant effect of genotypes and water stress on germination percentage, shoot length, shoot FW (fresh weight) and DW (dry weight), root FW and DW, except that water stress did not affect root length between genotypes. Interaction genotype by treatment was statistically significant for all traits, except for germination percentage, shoot FW and root length.

Under the control level, maximum seed germination was recorded in 'Vulkan' (85%) while the minimum seed germination was recorded in 'Zitarka' (67%). Under 20% PEG maximum seed germination was recorded in genotypes 'Antonija' (63%) (Table 2). Germination percentage declined progressively under stressed condition in all three genotypes. Similarly, in the research of Jatoi et al. (2011) 19% PEG caused reduction in germination in comparison to 25% PEG which caused a drastic reduction. Delayed and reduced germination is the result of the water deficiency stress at germination stage. This is in accordance with research of Ashraf and Abu-Shakra (1978) who reported that germination percentage and seedling growth were reported to decrease at low moisture levels.

Table 2 Analysis of variance for germination percentage, shoot and root length, fresh and dry weight of shoots and roots

Tablica 2. Analiza varijance za postotak klijavosti, duljinu izdanka i korijena, svježe i suhe mase izdanka i korijena

Source of variation	Df	MS						
		Germination percentage	Shoot length	Shoot FW	Shoot DW	Root length	Root FW	Root DW
Genotype (G)	2	1013.3 **	10487.4 ***	1.917 ***	0.406 ***	3210.4 ns	3.5807 ***	0.672 ***
Treatment (T)	1	5877.8 ***	10069.0 ***	4.38204 ***	0.053669 ***	15353.9 ***	0.54859 ***	0,649636 ***
G*T	2	136.1 ns	3387.6 **	0.1025 ns	0.2053 ***	693.7 ns	3.6158 ***	0.68735 ***
Error	30	150.6	570.9	0.07	0.002	795.5	0.04	0.002

***,**,* = significant at P < 0.001, 0.01 and 0.05, respectively

Table 3 Reponse of three wheat genotypes to control and 20% PEG treatment on germination percentage, shoot and root length, fresh and dry weight of shoots and roots

Tablica 3. Odgovor tri genotipa pšenice na kontroli i 20% otopini PEG na postotak klijavosti, duljinu izdanka i korijena, suhu i svježu masu izdanka i korijena

Genotype	Treatment	Germination percentage	Shoot length (cm)	Shoot FW (g)	Shoot DW (g)	Root length (cm)	Root FW (g)	Root DW (g)
Zitarka	Control	67.22 ± 3.06 aB	6.30 ± 2.71 aA	0.88± 0.36 aB	0.17± 0.06 aB	6.81± 2.46 aA	0.65± 0.34 aB	0.16± 0.05 aA
	20% PEG solution	42.78 ± 4.12 aA	4.31± 2.37 aA	0.12± 0.08 Aa	0.07± 0.03 aA	6.52± 3.39 aA	0.24± 0.05 aA	0.14± 0.02 aA
Antonija	Control	82.22 ± 1.97 bB	10.55± 0.96 bA	1.23± 0.31 abB	0.19± 0.03 aA	14.52± 3.54 bA	0.81± 0.21 aB	0.19± 0.02 aA
	20% PEG solution	62.78 ± 2.79 bA	9.54± 2.12 bA	0.40± 0.20 bA	0.14± 0.06 bA	12.46± 2.71 bA	0.45± 0.23 Ab	0.23± 0.09 bA
Vulkan	Control	85.00± 2.26 bB	14.18± 1.31 cB	1.53± 0.40 bB	0.27± 0.03 bA	9.88± 2.14 aB	0.71± 0.25 aA	0.17± 0.03 bA
	20% PEG solution	52.22 ± 6.19 abA	6.93 ± 3.77 abA	1.04* acA	0.65* cB	6.57± 2.39 aA	2.226* cB	0.98* cB

Small letters- differences in the same treatment between different wheat genotypes
Capital letters- differences between treatments of same genotype
Values are means of six replicates±standard deviation; *due to lack of tissue there was only one replica

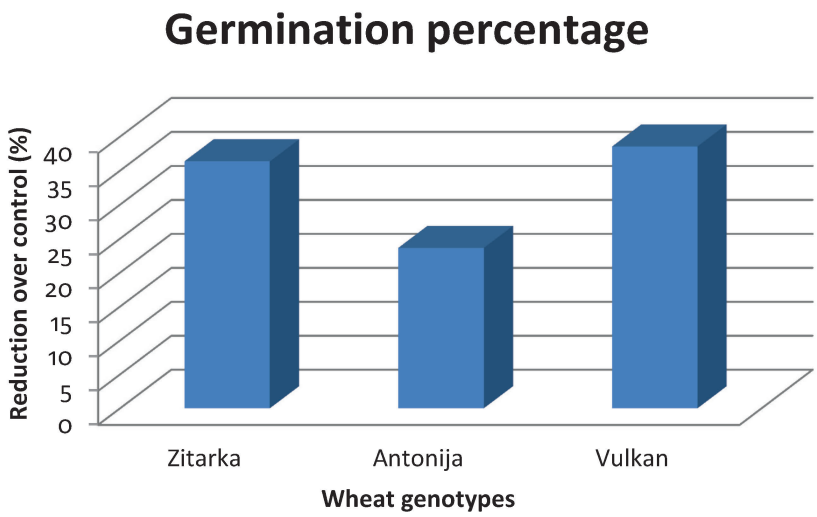

Figure 1 Reduction of germination percentage at PEG treatment over control in three wheat genotypes
Slika 1. Smanjenje postotka klijanja u tretmanu PEG-om u odnosu na kontrolu u tri genotipa pšenice

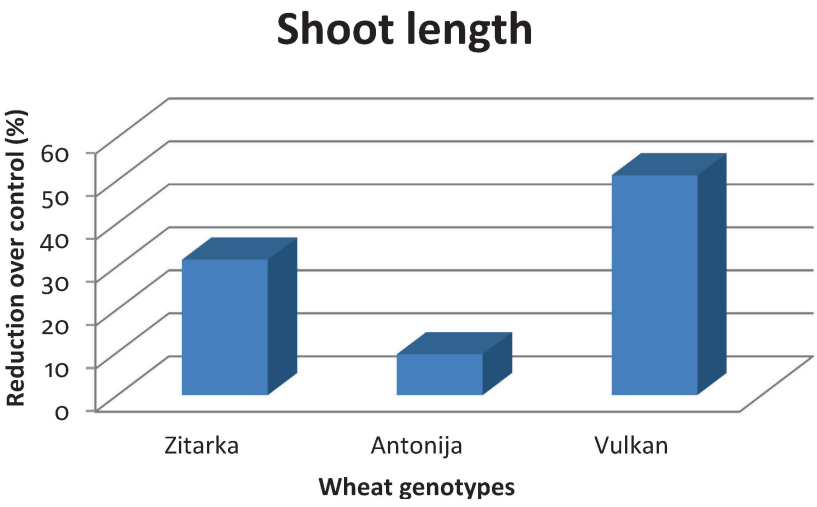

Figure 2 Reduction of shoot length at PEG treatment over control in three wheat genotypes
Slika 2. Smanjenje dužine izdanka u tretmanu PEG-om u odnosu na kontrolu u tri genotipa pšenice

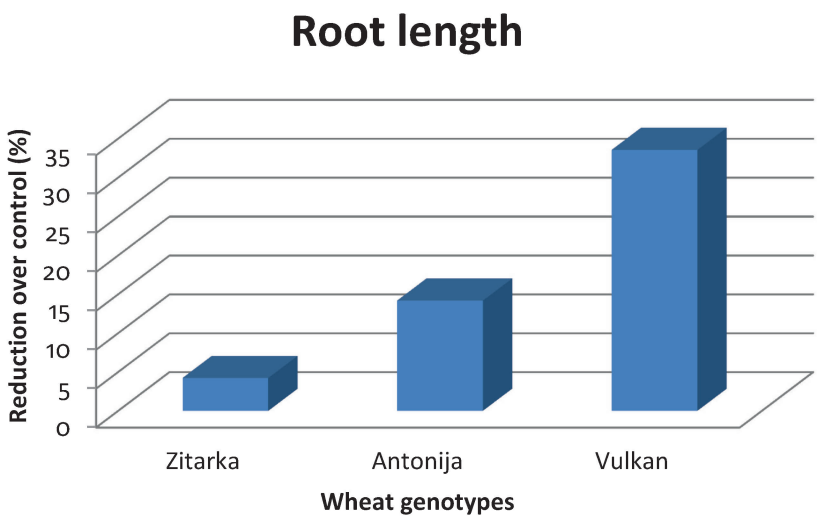

Figure 3. Reduction of root length at PEG treatment over control in three wheat genotypes
Slika 3. Smanjenje dužine korijena u tretmanu PEG-om u odnosu na kontrolu u tri genotipa pšenice

Mean values regarding shoot and root length, as well as values of dry and fresh weight of shoots and roots are presented in Table 3. The highest shoot length (14.18 cm) was recorded in control treatment in 'Vulkan'. There was not any statistical difference between 'Zitarka' and 'Antonija' in control and PEG treatment for shoot length. The least shoot length was recorded in 20% PEG treatment in 'Zitarka' (4.31 cm), followed by control treatment (6.30 cm) of the same genotype. From the results it was observed that 'Antonija' and 'Zitarka' were more drought tolerant. 'Antonija' and 'Zitarka' did not have long shoot and root length but in PEG treatment they did not statistically reduce it.

The shoot fresh weight was highest (1.53 g) in 'Vulkan' in control followed by 'Antonija' (Table 3). Fresh shoot weight was significantly decreased under control when treated with 20% PEG solution in 'Zitarka', 'Antonija' and 'Vulkan'. Sayar et al. (2010) reported that there was a decrease in the seedling fresh weight of both drought tolerant and sensitive durum wheat genotypes as osmotic potential increased.

Root/shoot weight was decreased significantly in 'Vulkan' under drought stress, in comparison to 'Antonija' and 'Zitarka' which retained it at the same significant level. This indicated that 'Antonija' and 'Zitarka' tolerated drought

more successfully. In research of Khakwani et al. (2012) water stress significantly reduced the yield related parameters, such as leaf fresh and dry weight. The decreasing trend in shoot and root dry weight under water deficit was reported previously (Chachar et al., 2014).

The maximum root length (14.52 cm) was recorded in 'Antonija' under control followed by PEG treatment (12.46 cm) (Table 3). The least root length (6.52 cm) was observed in 20% PEG treatment in 'Zitarka' (Table 3). Roots are important in uptaking water and nutrients, perceiving and transducing water deficit to shoots which will trigger different morphological and physiological responses. Root length is considered an important trait in selection of drought resistant genotypes (Turner, 1997) considering morphology and growth rate (Malik et al., 2002). The root length in 'Antonija' in PEG treatment may be associated with elongation during germination. Also, the changes in increment of shoots in stress can result in successful adaptation, although germination was significantly decreased in 20% PEG solution. In the paper of Badiane et al. (2004) it was concluded that dominant drought tolerance genes in root of *Vigna unguiculata* could be activated under stress in the way of reduction of their length. In the study of Jatoi et al (2011) the root length of all genotypes reduced gradually with the PEG concentration. The response of roots to drought stress can be an important trigger to other mechanisms, but also deeper roots will reach available water in the soil which will enable better drought resistance.

Results in Fig 1 indicated that water deficit had a high effect on all three genotypes in comparison with control. Application of water deficit caused 51.14% reduction in 'Vulkan' shoot length in comparison with control as compared to 9.52% in 'Antonija' (Fig 2). However, genotype 'Vulkan' had increment in root FW and shoot and root DW in treated seedlings in comparison with control. Similar trend of root length reduction was observed in the research of Rana et al. (2017).

More or less similar trend was observed in root length parameter. Genotypes 'Zitarka' and 'Antonija' reduced root length (4.24 and 14.20%, respectively) under water deficit in comparison to control (Fig 3). 'Vulkan' decreased it by 33.56% in PEG treatment in comparison with control.

Drought resulted in significant decreases in shoot fresh weight of three genotypes in PEG treatment in comparison with control (Table 3). Root dry weight was at the same level in two treatments in genotypes 'Zitarka' and 'Antonija', except for 'Vulkan' where root DW was significantly increased in PEG treatment in comparison with control (Table 3).

Table 4 Correlation analysis between root and shoot length, and germination percentage

Tablica 4. Usporedna analiza između duljina izdanka i korijena i postotka klijavosti

	Shoot length	Root length
Root length	0.66	
Germination percentage	0.77	0.43

Correlation studies among different traits (Table 4) showed that shoot length exhibited positive but non-significant correlation with root length and germination percentage. Positive correlation (0.43) between germination percentage and root length was also observed. A positive association among mentioned parameters in our research indicated that increase in germination percentage would also increase shoot and root length. Similar was found in the investigation of Ahmad et al. (2013) where root length showed a strong correlation with shoot length and germination percentage as well as shoot length with germination percentage. In the research of Homayoun et al. (2011) it was concluded that drought tolerant genotypes would have better yield potential.

CONCLUSION

In conclusion, water stress significantly reduced seed germination in all three genotypes while shoot and root length was at the same significant level in 'Zitarka' and 'Antonija' in comparison to 'Vulkan' which statistically decreased it in drought stress.

In the first phase of development 'Vulkan' was among the genotypes tested a more tolerant genotype which had the potential to perform better growth under drought conditions.

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