

GENETIC ABILITY AND SEEDLING DROUGHT CHARACTERIZATION OF WINTER WHEAT LINES IN CROATIA

**GENETSKA SPOSOBNOST I KARAKTERIZACIJA OTPORNOSTI
NA SUŠU KLIJANACA LINIJA OZIME PŠENICE U HRVATSKOJ**

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ABSTRACT

Wheat is one of the most common and important cereals in Croatia. Therefore, high selection pressure at wheat breeding program is continually placed on disease, drought and lodging resistance, heading date and end-use quality. The aim of the current study was to evaluate the wheat grain productivity and quality, as well as response of wheat seedlings to drought. Five lines of winter wheat with reference variety Kraljica were used for evaluation of desired traits. In general, investigated wheat lines had such good characters as early maturity, high yield, they were smaller in test weight and protein content, but with higher sedimentation value, dough energy and extensibility, compared to reference variety. Overall, lines Osk.4.330/6-18, Osk.3.530/59-18, Osk.4.354/12-18 out yielded the reference variety with regard to the grain yield. According to the results collected in this research, wheat lines differences in germination energy and seedling growth affected by drought were obtained. All lines could offer farmers tolerance to mild drought during sowing and will achieve high yields. Nevertheless, stability and drought tolerance of investigated winter wheat lines in different environments have to be checked in multi-location trials.

Keywords: drought, yield performance, quality traits, wheat

SAŽETAK

Pšenica je jedna od najčešće zasijanih i najvažnijih žitarica u Hrvatskoj. Stoga se u programu oplemenjivanja pšenice kontinuirano stavlja veliki selekcijski pritisak na otpornost na bolesti, sušu, polijeganje, datum klasanja i krajnju kvalitetu zrna. Cilj ovog istraživanja bio je ocijeniti produktivnost i kvalitetu zrna pšenice, kao i odgovor klijanaca pšenice na sušu. Za procjenu željenih svojstava korišteno je pet linija ozime pšenice i kontrolna sorta

Kraljica. Uzimajući u obzir ispitivane parametre, linije pšenice u ovom istraživanju imale su određene dobre karakteristike kao što su rano sazrijevanje i visok prinos zrna, bile su manje hektolitarske mase i sadržaja proteina, ali s većom vrijednošću sedimentacijske vrijednosti, energijom tijesta i rastezljivosti u odnosu na kontrolnu sortu. Općenito gledano, linije Osk.4.330/6-18, Osk.3.530/59-18, Osk.4.354/12-18 imale su veće vrijednosti prinosa zrna u odnosu na kontrolnu sortu. Prema rezultatima prikupljenim u ovom istraživanju dobivene su razlike između linija pšenice u energiji klijanja i rastu klijanaca u uvjetima suše. Navedene linije mogle bi poljoprivrednicima ponuditi tolerantnost na blagu sušu tijekom sjetve i postići visoke prinose. Ipak, stabilnost i otpornost na sušu istraživanih linija ozime pšenice u različitim okolinama potrebno je istražiti u pokusima koji uključuju više lokacija.

Ključne riječi: suša, uspješnost prinosa, svojstva kvalitete, pšenica

INTRODUCTION

Increased biotic and abiotic stresses occurring as a consequence of climate change are a threat to the already decreasing wheat productivity (Suzuki et al., 2014). According to Liu et al. (2016) for every 1 °C increase in temperature, global wheat yields are predicted to decline by 4.1 – 6.4%. By 2050 increase of more than 60% in global grain production will be needed, as a consequence of food demand by population growth (Godfray et al., 2010). Therefore, the main targets of wheat breeding programs should be focused on increasing grain production limits. Also, food security would be enhanced by expanding the numbers of cereal species (Henry, 2021). The main objectives of the winter wheat breeding program at Agricultural Institute Osijek are good quality and high yields, early maturity including some other characteristics such as disease and drought resistance. The major threat in yield production are various pests and pathogens which cause considerable losses every year where fungal diseases cause the highest damage. Despite the fact that the most important is the resistance of the genotype, most of wheat varieties were produced with high inputs of fungicides for plant defense against biotic stress.

Besides biotic stress, the concurrent occurrence of abiotic stresses such as drought and heat is more and more common due to global warming (Mittler, 2006). Hence, these climatic changes will have a dramatic effect on agriculture especially in the regions with water shortage and high temperatures (Rinaldi, 2009). Therefore, the development of new wheat varieties with high water use efficiency is crucial for producing optimum yield in marginal rainfall conditions. According to Micky and Aldesuquy (2017) in certain

developmental stages wheat appears to be more sensitive to osmotic damage. However, it was demonstrated that wheat genotypes that showed drought tolerance at the germination stage exhibited the same tolerance to water deficit under field conditions (Khakwani et al., 2011).

In order to obtain appropriate levels of yield and quality, investigated winter wheat lines and reference variety were screened without usage of fungicide to find the best suited varieties for disease stressed conditions. In addition, a seedling test for drought resistance was performed in controlled conditions.

MATERIALS AND METHODS

Plant material and field experiment

Five winter wheat lines with reference variety Kraljica were studied in the field trial in vegetative season 2019/2020 at Osijek (45°27' N, 18°48' E) in Croatia, where soil type is eutric cambisol. The plants were sown in October in the experimental plot area of 7.56 m² in four replicates. The average annual precipitation in vegetative period in 2019/2020 was 408.6 mm and the average annual temperature was 11.1 °C. Insecticides and herbicides were applied as needed, without usage of fungicides. In the field trial, traits as heading date, resistance to lodging and plant height were evaluated. After harvest at the beginning of July, grain yield (t ha⁻¹), test weight (kg hl⁻¹) and 1000 kernel weight (g) were obtained.

Technological and rheological quality

The quality tests described in this section are standardized testing procedures commonly used for quality control purposes. After milling the grain samples protein and wet gluten content, gluten index, Zeleny sedimentation volume and falling number were obtained by ICC method No. 155, 116/1 and 107/1, respectively. Farinograms were obtained using method for the Brabender Farinograph (HRN ISO 5530-1:1999). Extensogram test was conducted using method for the Brabender Extensograph (HRN ISO 5530-2:1999).

Experiment with drought on seedlings

The growth chamber experiments were conducted to evaluate drought resistance in seedlings stage by usage of polyethylene glycol-6000 (PEG6000) solutions as the moisture stress inducing media. Seedlings were grown in trays and water stressed up to seven days with 16 h light period at 25 °C and 8 h dark

period at 20 °C, with a constant relative humidity of 60%. Drought-exposed plants were watered daily with 10 ml of 10% and 20% PEG solution, while in the control treatment only distilled water was applied. After seven days germination energy, growth parameters, as well as relative water content (RWC) were calculated. Germination energy was defined as the percentage of germinated seeds in a given sample within the seven days. Growth parameters (root and shoot length) were recorded in mm with a ruler after seven days of different treatments. RWC was calculated by the formula: $RWC (\%) = (FW - DW) / (TW - DW) * 100$ where FW was a fresh weight of leaf tissue which upon weighting was submerged in distilled water for 24 h to reach turgid weight (TW). Dry weight (DW) of tissue was obtained by drying leaf discs at 105 °C for 24 h.

Statistical analysis

All recorded values for the grain yield represent the means of the results of four replicates whereas standard error was calculated, and for the other traits field sample from four replicates was mixed as one and trait was measured in one replicate. For drought experiment, 15 seedlings were taken for the calculation of mean value of investigated traits.

Statistica 12 Software was used to perform Fisher's LSD test to detect significant differences between treatments at a significance level $p < 0.05$ for each line/variety separately.

RESULTS AND DISCUSSION

The investigated winter wheat lines were selected by the pedigree method over few years of selection and tested in varietal experiments in the field trial in vegetative season 2019/2020 at one location. Climate changes enhance the importance of drought stress in wheat growing regions of the world where drought stress caused a high yield decline in recent years. We want to create drought-tolerant varieties and therefore, it is essential to primarily understand response of wheat plants in water-deficient conditions at different stages of growth.

Agronomical and morphological traits

Osk.3.530/59-18 (12.75 t ha⁻¹) had the highest grain yield line. The lowest grain yield was recorded for line Osk.4.312/10-18 (11.55 t ha⁻¹) with the lowest test weight. Test weights ranged between 78.5 (Osk.4.312/10-18) to 82.2 kg hl⁻¹ (Kraljica). The tallest line Osk.4.354/12-18 had the highest 1000 kernel weight (43 g). Although reference variety Kraljica (a compromise between yield and

proteins, with good baking quality) had the highest test weight, it did not give the highest yields, where the lowest resistance to lodging could be one of the reasons for not performing the best yields. Feng et al. (2019) reported that changing weather patterns such as rain, wind, and hail storms have made the current varieties more susceptible to lodging, leading up to 80% yield losses. Test weight as the specific volume was in good range in all wheat lines. According to Mecha et al. (2017) by selection on this trait together with grain filling period, number of productive tillers per plant, spike length, number of spikelets per spike, number of kernels per spike, 1000 kernel weight, biomass yield per plot and harvest index, there is also a possibility to increase grain yield of bread wheat.

Five wheat lines had the similar heading date as reference variety Kraljica, except line Osk.4.330/6-18 with three days later heading date. Investigated five lines were early maturing, similar to reference variety Kraljica, and thus well suited to short seasons. It is more and more important to grow winter wheat varieties that are not late-maturing as harvest delays or terminal heat stresses can often occur (Mondal et al., 2016). Among wheat lines, the maximum plant height (96 cm) was recorded in line Osk.4.354/12-18, and minimum plant height (85 cm) was recorded in line Osk.4.312/10-18, the same as for reference variety Kraljica. All lines together with Kraljica had red colored seed (Table 1). Plant height was measured from soil surface to the base of the ear head of main shoot at maturity stage. It should be in relation to the plant architecture, lodging resistance and yield performance (Wang et al., 2017).

Table 1 Agronomical and morphological traits of five winter wheat lines and reference variety Kraljica

Tablica 1. Agronomska i morfološka svojstva pet linija ozime pšenice i kontrolne sorte Kraljica

Variety/Line	GY*	SE	TW	1000KW	HD	LOD	PH	SC
Osk.3.530/59-18	12.75	2.27	79.5	40	5.05.2020.	1	86	Red
Osk.4.330/6-18	12.62	2.37	81.6	39	8.05.2020.	0	89	Red
Osk.4.354/12-18	12.62	4.59	81.9	43	5.05.2020.	0	96	Red
Kraljica	12.54	5.42	82.2	40	5.05.2020.	1,5	85	Red
Osk.4.324/5-18	12.52	5.71	80.1	37	5.05.2020.	0	89	Red
Osk.4.312/10-18	11.55	5.11	78.5	37	6.05.2020.	0	85	Red

*GY-grain yield in t ha⁻¹ ±SE-standard error for the gran yield, TW-test weight in kg hl⁻¹, 1000KW-1000 kernel weight in g, HD-heading date, LOD-lodging evaluated as 1-9 (0-no lodging, 9-fully lodged plants at the plot), PH-plant height in cm, SC-seed color (soaked in 1 M NaOH)

Technological and rheological quality

To meet specifications of mill and bakery industry in grain and flour, quality testing is necessary to evaluate dough and gluten strength properties. Results from these tests have a direct relationship to finished product quality. In the current research, the lowest protein content and wet gluten content were recorded for line Osk.4.324/5-18 (12.8 and 22.9%), while reference variety Kraljica had the highest values of those two parameters (14.5 and 28.7%). The same line Osk.4.324/5-18, together with line Osk.3.530/59-18 had the highest sedimentation value (49 ml). Gluten index ranged between 93 (Osk.4.330/6-18) to 99 (Kraljica, Osk.4.324/5-18 and Osk.4.312/10-18). Line Osk.3.530/59-18 (463 s) had the highest falling number, followed by reference variety Kraljica (450 s).

Line Osk.4.354/12-18 had a high yield potential and also maintained a higher grain protein content than other lines with similar yield potential. Four wheat lines had very good sedimentation values, compared to reference variety, thus showing good gas retention in dough stability and baking volume. Usually, sedimentation value of flour depends on the wheat protein composition and is mostly correlated to the protein content (Hrušková and Faměra, 2003), which was not the case in the current study. It was observed that reference variety Kraljica had the highest wet gluten content. It was previously concluded that the higher a flour protein content, the higher the gluten formation (Baslar and Ertugay, 2011). Further, all tested wheat lines were selected for low pre-harvest sprouting, where falling number as the level of alpha amylase activity, was within acceptable limits.

Knowing the technological properties of the flour is also not enough to fully characterize end-use quality of wheat. Therefore, it is useful to obtain rheological properties of the dough. Reference variety Kraljica (58.3%) and line Osk.4.354/12-18 (58.2%) had the best water absorption, belonging to quality groups A2 and A1 thus showing good end-use quality properties. Line Osk.4.330/6-18 exhibited the lowest dough energy and extensibility. Line Osk.4.354/12-18 had the highest dough energy, and the best extensibility was obtained in line Osk.4.312/10-18 (Table 2). The flour with good bread-making properties has higher water absorption, takes longer to mix and is more tolerant to over-mixing than poor-quality bread flour (William, 2001). All lines, except Osk.4.330/6-18, had higher energy values showing the greater gas holding capacity and fermentation tolerance of the dough where no difficulties in bread making should be obtained. Similar behavior of those lines was obtained for dough extensibility. According to Anderssen et al. (2004) rheological parameters can be used to determine the processability of wheat to different products.

Table 2 Technological and rheological parameters of five winter wheat lines and reference variety Kraljica

Tablica 2. Tehnološki i reološki parametri pet linija ozime pšenice i kontrolne sorte Kraljica

Variety/Line	P	SED	VG	GI	FN	WA	QG	E	Ext
Osk.3.530/59-18	13.2	49	26.6	97	463	55.5	B2	90	155
Osk.4.330/6-18	13.1	42	26.9	93	369	56.1	B2	-	-
Osk.4.354/12-18	14.3	46	24.6	97	376	58.2	A1	98	159
Kraljica	14.5	44	28.7	99	450	58.3	A2	86	155
Osk.4.324/5-18	12.8	49	22.9	99	354	51.2	B1	96	160
Osk.4.312/10-18	13.2	48	24.4	99	374	53.1	B2	91	175

*P-protein content in %, SED-sedimentation value in ml, VG-wet gluten content in %, GI-gluten index, FN-falling number in s, WA-water absorption in %, QG-quality group (ranking A1-C1), E-energy in cm², Ext-extensibility in mm

Drought-related traits in seedling

During germination wheat plants are very sensitive and drought stress can delay or inhibit germination processes, leading to potential yield loss due to reduced cropping density (Almansouri et al., 2001). In general, both solutions (PEG 10 and 20%) affected seedling growth parameters (shoot and root lengths), compared to controlled treatment. Relative water content (RWC) and germination energy were influenced to a lesser extent.

The highest germination energy had reference variety Kraljica and Osk.4.330/6-18 (91.25%) in controlled treatment, Kraljica in PEG 10% treatment (92.5%) and line Osk.4.330/6-18 in PEG 20% treatment (81.25%) (Figure 1). Germination energy differed among wheat lines, where Osk.4.530/6-18 showed the lowest value in PEG 10% treatment. The line Osk.4.330/6-18 had the highest germination energy in PEG 20% treatment, compared to other lines. According to the results of Duan et al. (2017) germination of wheat seeds could be inhibited by drought stress, where germination energy significantly decreased with the increase of drought degree in most wheat lines. In the current research the germination energy of Osk.4.330/6-18 and reference variety Kraljica was stronger, compared to other lines, but only Osk.4.330/6-18 and Osk.4.312/10-18 significantly reduced germination energy in PEG 20%, compared to controlled treatment.

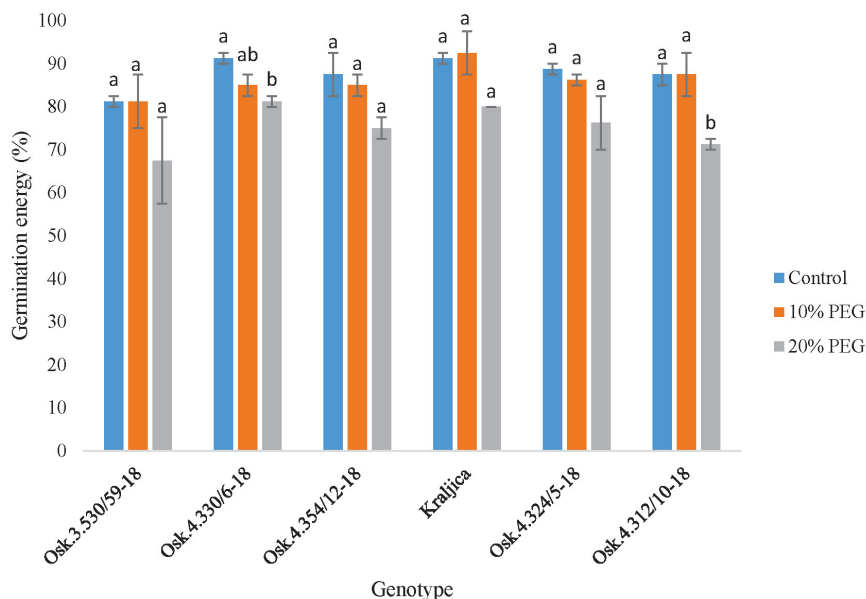


Figure 1 Germination energy of five winter wheat lines and reference variety Kraljica
Slika 1. Energija klijanja pet linija ozime pšenice i kontrolne sorte Kraljica

All lines and reference variety Kraljica significantly reduced shoot length in PEG 20% treatment, compared to PEG 10% and controls (Figure 2). The highest reduction of shoots in 10% PEG solution, compared to controlled treatment, was recorded in line Osk.4.354/12-18 (13.04%), while the length of the roots was increased in all wheat varieties, where line Osk.3.530/59-18 was showing the highest increase among other varieties (67.33%), followed by line Osk.4.324/5-18 (58.36%) (Figure 3). The same significant level of root length was kept in lines Osk.4.354/12-18 and Osk.4.312/10-18 between control and PEG 10% treatment. The highest reduction of shoots was recorded in line Osk.3.530/59-18 (37.54%) in 20% PEG treatment, while the highest reduction of the roots had line Osk.4.354/12-18 in 20% PEG treatment (27.5%). Lines Osk.4.330/6-18 and Osk.3.530/59-18, as well as reference variety Kraljica increased length of the roots in 20% PEG treatment (17.13, 14.41 and 1.39%, respectively), compared to seedling in controlled conditions. Liu et al. (2013) reported that wheat seedling growth indices decreased under drought stress. In the current research it was different where lines Osk.4.330/6-18,

Osk.3.530/59-18 and Osk.4.324/5-18 showed the increase of shoots length in PEG 10% treatment, with the highest reduction of shoots in PEG 20% treatment, compared to controlled treatment. The same lines had the highest increase of root length in PEG 10% treatment with lower increase in PEG 20% treatment, except Osk.4.324/5-18 line which showed root reduction in PEG 20% treatment. According to Xu et al. (2015) alterations in root system architecture aid in short-term adaptation to water deficit. Root length at the seedling stages of the plant is a key genetic trait for increasing yield under drought conditions (Shahbazi et al., 2012). Also, this trait was the most variable trait, as according to previous study the length of wheat seminal roots was mainly affected by the presence of another wheat plants (Finch et al., 2017) and that could be the case in the current research for the roots in petri dishes.

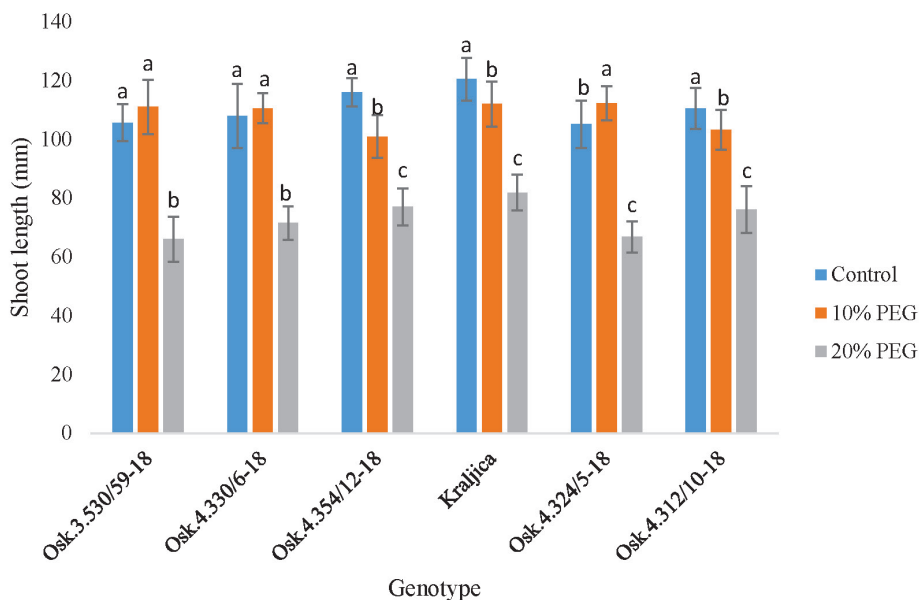


Figure 2 Shoot length of five winter wheat lines and reference variety Kraljica at 7th day of the experiment

Slika 2. Duljina izdanka pet linija ozime pšenice i kontrolne sorte Kraljica na 7. dan eksperimenta

Three lines (Osk.3.530/59-18, Osk.4.330/6-18 and Osk.4.324/5-18) significantly reduced relative water content (RWC) in PEG 20% treatment, compared to control. RWC ranged between 88.0% (Osk.4.324/5-18) to 104.7% (Osk.4.312/10-18) in controlled treatment, while in 10 % PEG treatment that range was from 82.4 (Osk.4.354/12-18) to 89.6% (Osk.4.312/10-18), and in 20% PEG solution it ranged from 70.02 (Osk.4.324/5-18) to 95.5% (Kraljica) (Figure 4). The line Osk.4.312/10-18 (with the lowest yield) had the highest RWC in controlled and PEG 10% treatment, but not in PEG 20% treatment thus showing that increased drought stress will influence RWC. Datta et al. (2011) applied both normal and water-deficient conditions to wheat genotypes and observed that genotypes performed better under environments which had optimum RWC and root and shoot length, which were considered drought-tolerant genotypes. In the current research all varieties retained good RWC when grown under mild drought conditions (PEG 10%). Similar results were obtained by Tahara et al. (1990) in winter wheat varieties as the high-yield selections maintained a significantly higher RWC than the low-yield selections.

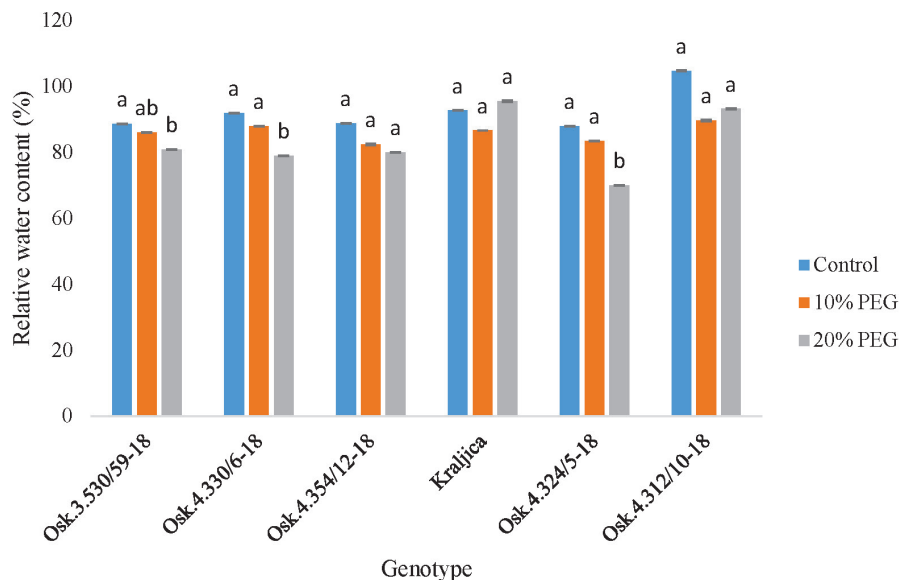


Figure 3 Root length of five winter wheat lines and reference variety Kraljica at 7th day of the experiment

Slika 3. Duljina korijena pet linija ozime pšenice i kontrolne sorte Kraljica na 7. dan eksperimenta

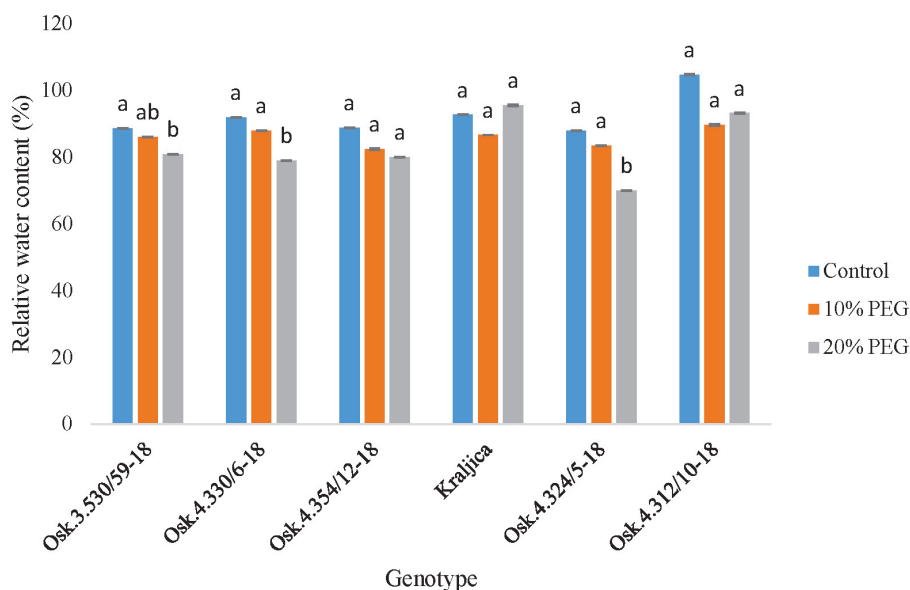


Figure 4 Relative water content of five winter wheat lines and referent variety Kraljica
Slika 4. Relativna vlaga pet linija ozime pšenice i kontrolne sorte Kraljica

CONCLUSIONS

Although created winter wheat lines have been tested for production in Croatia, multi-location trials need to be set up in next seasons. Multi-location testing of those lines will allow a better understanding of the genotype×environment (G×E) interactions related to grain yield. The current results demonstrate that advanced winter wheat lines perform well compared to reference variety but have yet to be completed this year. Continued emphasis has been placed on selecting breeding lines with superior quality and disease resistance where line Osk.4.354/12-18 showed good performance.

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