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Oryginalny artykuł naukowy
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Ocena obiektów cebuli (*Allium cepa* L.) pod względem tolerancji na suszę w fazie kiełkowania i wzrostu siewek

Evaluation of onion (*Allium cepa* L.) accessions for drought tolerance at germination and seedling stages

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Celem badań była ocena wpływu niedoboru wody na zdolność kiełkowania nasion i wzrost siewek cebuli oraz identyfikacja obiecujących obiektów do wykorzystania w przyszłych programach hodowli nowych odmian cebuli tolerancyjnych na stres suszy. Oceniono 150 linii/odmian cebuli pochodzących z polskich i zagranicznych firm hodowlano-nasiennych oraz z Banku Genów Instytutu Ogrodnictwa w Skierniewicach. Doświadczenia prowadzono w warunkach laboratoryjnych indukując stres suszy przez zastosowanie 18% poliglikolu etylenowego (PEG_{8000}) w fazie kiełkowania nasion i 10% PEG w fazie siewek. Badane obiekty cebuli wykazały istotne zróżnicowanie pod względem tolerancji na stres suszy, potwierdzone wysokimi wartościami współczynnika zmienności (CV) dla ocenianych parametrów: maksymalny procent skiełkowanych nasion (35,3%), świeża masa siewek (38,5%), długość liścieni siewek (42,9%) i długość korzeni siewek (56,3%). Zanotowano, że niedobór wody najmniej ograniczył kiełkowanie nasion o 23%, następnie długość korzeni o 36%, a najbardziej świeżą masę siewek i długość liścieni, odpowiednio o 52% i 53,5%. Stwierdzono silną korelację (r = 0,81-0,86) między cechami opisującymi wzrost siewek w warunkach suszy, wskazując, że deficyt wody redukuje wzrost całych siewek. Natomiast zależności pomiędzy nimi a kiełkowaniem w stresie suszy okazały się nieistotne (r = 0,19-0,24). Spośród 150 obiektów cebuli, genotyp 171026 wykazał wysoki poziom tolerancji na suszę zarówno w fazie kiełkowania, jak i w fazie siewek.

Słowa kluczowe: *Allium cepa*, glikol polietylenowy (PEG), niedobór wody, tolerancja

The aim of this study was to assess the effect of water deficiency on germination ability and growth of onion seedlings, and identify promising accessions for use in future breeding programs of new onion varieties with drought tolerance. We evaluated 150 onion lines/cultivars derived from Polish and foreign breeding and seed companies and from the Gene Bank of the Institute of Horticulture in Skierniewice. Experiments were performed under laboratory conditions inducing drought stress by using 18% polyethylene glycol (PEG_{8000}) in the seed germination phase and 10% PEG in the seedling phase. Significant differences in reaction to drought stress were observed among onion accessions, confirmed by the high values of the coefficient of variation (CV) for the assessed parameters: maximum percentage of seedling (56.3%). The received results showed, that water deficiency reduced seed germination the least by 23%, then the root length by 36%, and the fresh weight of seedling and cotyledon length by 52% and 53.5%, respectively. A high correlation (r = 0.81–0.86) between the features describing the growth of seedlings under drought conditions was found, indicating that water deficit reduced growth of whole seedlings. However, no correlation (r = 0.19–0.24) between them and germination under drought conditions was observed. Among 150 onion accessions, genotype 171026 showed a high level of drought tolerance, both in seed germination stage and in seedling growth stage.

Keywords: *Allium cepa*, polyethylene glycol (PEG), tolerance, water deficit

Introduction

Onion is one of the most important vegetable species in terms of yield volume, both in Poland and in the world. Poland, with an onion yield of over 650,000 tonnes/year, is the third largest onion producer in the EU, following the Netherlands and Spain (EUROSTAT 2017). Onion ranks first in terms of the acreage of vegetables in Poland (GUS 2018). This species is particularly sensitive to drought stress at the stage of seed germination and seedling emergence, but also at the stage of bulb formation (Addai et al., 2014; Hanci and Cebeci, 2015). Water deficiency at the seed germination stage delays emergence, reduces the number of plants per unit of area and, consequently, reduces the yield. Periods of drought before and during the formation and further development of bulbs inhibit their growth, resulting not only in reduced yield, but also lower storage quality. The best way to protect onion crops

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against the negative effects of drought is breeding varieties with increased tolerance to drought stress. This is particularly important considering the forecasted greater water shortage caused by progressive climate change (Ziernicka-Wojtaszek, 2015).

In recent years, the Institute of Horticulture (IO) in Skierniewice started research, where the first step was to develop a method of testing onion at the stage of seed germination and seedling growth using polyethylene glycol (PEG), an osmotically active substance lowering water potential (Michel, 1983). PEG is a polymer inducing osmotic stress and has been used for *in vitro* testing of drought tolerance in different species of plants: maize (Hohl and Schopfer, 1991), pine (Zhu et al., 2006), wheat (Kolasińska, 2009), sorghum (Bibi et al., 2012), rice (Wang et al., 2013), lentil (Muscolo et al., 2014), tomato (Brdar-Jokanović and Zdravković, 2015), and cucumber (Kłosińska et al., 2016).

Considering the wide range of registered onion varieties (57 varieties in the COBORU register, about 1200 varieties in the EU register) and the collection of onion accessions in the Gene Bank of the Institute of Horticulture, there is a high probability of identifying genotypes with good tolerance to water deficit. Therefore, a study was conducted to evaluate the effect of PEG-induced drought stress on germination capacity and seedling growth in 150 onion lines/varieties of different origins.

Material and Methods

The research material consisted of 150 onion accessions sourced from Polish and foreign breeding and seed companies, and from the Gene Bank of the Institute of Horticulture in Skierniewice (tab. 1). Laboratory experiments were conducted in 2019 at the seed germination stage and seedling growth stage. Drought stress was induced with polyethylene glycol (PEG_{8000}) reducing water potential, according to an optimised method developed at the Institute of Horticulture in Skierniewice (Kłosińska et al., 2019).

Seed germination stage

Seeds of 150 onion accessions were placed on Petri dishes lined either with filter paper soaked in 4 ml of 18% PEG or 4 ml dH_2O (control). The test was performed in triplicates, each with 50 seeds in two combinations (18% PEG and dH_2O). Petri dishes were kept in a dark growth chamber at a temperature of 18°C. The number of germinated seeds was counted daily from sawing for 14 days, and the following parameters were calculated: gMAX (maximum % of seeds germinated), and % of seeds germinated in 18% PEG compared to control.

Seedling stage

Germinated seeds (length 10-15 mm) representing 150 onion accessions were placed on Petri dishes lined either with filter paper soaked in 4 ml 10% PEG or 4 ml dH_2O (control). The test was performed in triplicates, each with 10 seeds in two combinations (10% PEG and dH_2O). Petri dishes were placed in a walk-in growth chamber at a temperature of 24°C/day and 20°C/night, and a 12h L:12h D photoperiod. After 12 days seedlings were assessed for the length of the roots and cotyledons, as well as fresh weight.

Data were processed to calculate mean values for the traits of the investigated onion accessions exposed to drought stress and their controls. The effect of water deficit on the value of parameters expressed in % relative to control was analysed

Tabela 1
Table 1

Pochodzenie i liczebność badanych obiektów cebuli

Origin and number of onion accessions used in study

Pochodzenie Origin	Liczba obiektów Number of accessions
Gene Bank, Institute of Horticulture, Skierniewice	34
Polan, Kraków	22
Plantico, Zielonki	59
Spójnia, Nochowo	20
PNOS, Ożarów Mazowiecki	2
Bejo Zaden	8
Enza Zaden	2
Nunhems Bayer	2
Syngenta	1

for each onion line/variety. Coefficients of variation (CV) and correlation coefficients (r) were calculated.

Results and Discussion

Germination and seedling growth are the most critical stages in the life cycle of plants (Ahmed et al., 2009). The exposure of seeds to stress, including drought, has a destructive effect on the formation of seedlings and often further plant development (Albuquerque and Carvalho, 2003). In our study drought was induced with 18% PEG at the germination stage and 10% PEG at the seedling stage. PEG in both concentrations significantly reduced the germination capacity (tab. 2), but also inhibited the growth of seedlings (tab. 3). Significant differences in response to drought stress were observed between onion accessions, as confirmed by the high values of the coefficient of variation (CV) for the assessed parameters: maximum % of germinated seeds (35.3%), fresh weight of seedlings (38.5%), cotyledons length of seedlings (42.9%), and root length of seedlings (56.3%) (Tabs. 2, 3).

The seeds of 150 tested onion varieties/lines showed good germination capacity under the control conditions (0% PEG), and the mean gMAX (maximum % of seeds germinated) was 76.1% (tab. 2). The effect of 18% PEG used in our study was previously tested on cucumber by Zhang et al. (2013) and Kłosińska et al. (2016). However, our experiments revealed that onion at the germination stage

Tabela 2
Table 2

Maksymalny % kiełkowania nasion (gMAX) 150 obiektów cebuli w warunkach kontrolnych (0% PEG) i stresu suszy (18% PEG) oraz liczba skiełkowanych nasion w 18% PEG względem kontroli w %

Maximum % of seeds germinated (gMAX) for 150 onion accessions under control treatment (0% PEG) and drought stress (18% PEG); and number of seeds germinated under 18% PEG treatment relative to control in %

Parametr Parameter	gMAX (%)		% kontroli % control
	kontrola control	stres stress	
Średnia Mean	76.1	58.6	77.0
Zakres Range	11.3-100	4.7-96.7	7.5-139.3
CV* (%)	23.0	35.3	30.0

**współczynnik zmienności; the coefficient of variation*

Tabela 3
Table 3

Świeża masa, długość liścieni i długość korzeni siewek 150 obiektów cebuli w warunkach kontrolnych (0% PEG) i stresu suszy (10% PEG) oraz wartości tych parametrów w 18% PEG względem kontroli w %

Fresh weight, cotyledon length and root length of seedlings of 150 onion accessions under the control treatment (0% PEG) and drought stress (10% PEG); and values of these parameters under the 10% PEG treatment relative to control in %

Parametr Parameter	Świeża masa siewek Fresh weight of seedlings			Długość liścieni siewek Cotyledon length of seedlings			Długość korzeni siewek Root length of seedlings		
	kontrola control (mg)	stres stress (mg)	% kontroli % control	kontrola control (cm)	stres stress (cm)	% kontroli % control	kontrola control (cm)	stres stress (cm)	% kontroli % control
Średnia Mean	37.0	17.5	48.1	4.8	2.2	46.5	4.3	2.7	64.0
Zakres Range	24.0-55.5	7.4-35.9	19.3-89.6	2.6-7.6	0.7-5.1	15.2-112.1	1.4-7.4	0.5-7.7	16.7-149.5
CV* (%)	18.8	38.5	40.6	20.3	42.9	44.8	29.6	56.3	48.1

**współczynnik zmienności; the coefficient of variation*

is more sensitive to drought stress than cucumber. For six onion accessions, the mean rate of germination under stress conditions was 10 to 36.3% higher compared to controls (tab. 4), which might indicate their high tolerance to drought. There were three lines most sensitive to water deficit at the germination stage, and the % of seeds germinated 14 days after treatment with 18% PEG ranged from 7.5 to 12.7% compared to controls (tab. 4).

Due to the different vigour of control seedlings the effect of 10% PEG on the value of investigated parameters was analysed separately for each line/variety. In this process we selected onion lines characterized by extreme responses to drought stress. In 33 onion genotypes the root length was 90-149.5% of that measured for controls, including 14 accessions for which the root length in seedlings exposed to 10% PEG was 110% of the control (tab. 4). Interestingly, there were three genotypes (ZS 113, ZS 109 and 171026) for which the root length measured in seedlings exposed to drought stress was 49.5, 42.4 and 33.6% greater compared to controls, which might indicate high tolerance to drought, as suggested by only a slight decrease in the fresh weight noted in these genotypes. On the other hand, the greatest reduction in root length, by over 80% compared to the control, was found for six accessions (tab. 4). We also investigated the effect of 10% PEG on cotyledon length in onion seedlings. In two lines, ZS/105 and ZS/11, cotyledons were 12 and 11% longer compared to controls, while in another 148 lines drought stress inhibited the growth of cotyledons. The strongest inhibition (more than 80% of controls) was observed for six lines (tab. 4). In all onion accessions treatment with 10% PEG also reduced the fresh weight of seedlings. Among 150 evaluated accessions, reduction in fresh weight compared to the control (10-24%) was lowest for 12 accessions, and highest (80%) for two genotypes, NOE/61 and 171009.

The mean values for all lines indicated that drought stress was associated with a 23% lower seed germination capacity (tab. 2), 36% lower root length, 52% lower fresh weight of seedling, and 53.5% lower cotyledon length (48 and 46.5% of control, respectively) (tab. 3). Many reports have emphasized that drought reduces the growth of the aboveground parts more than the growth of roots in various species: beans (Turkan et al., 2004), melon (Tuna et al., 2010), tomato (Prokic and Stikic, 2012), and sorghum (Bibi et al. 2012), which was also confirmed in our study on onion. Therefore, inhibited growth of the aboveground parts and increased growth of roots is one of the most important indicators of tolerance to drought stress.

Our findings suggest that parameters such as seed germination, fresh weight of seedlings, and length of root and cotyledons can be used for the fast selection of genotypes tolerant to drought in the early stage of growth. A tested line was classified as either tolerant or sensitive to drought stress if the analysis showed at least one clearly positive or negative parameter for this line (tab. 4). Among 150 onion accessions we selected 23 tolerant and 13 sensitive lines/varieties, while the remaining 114 accessions were characterized by a moderate tolerance or sensitivity to drought. The highest number of tolerant accessions (14) were sourced from Plantico, and this might result from the large overall number of accessions provided by this company (59; 40% of all tested). The second highest number of tolerant accessions were sourced from the Gene Bank (6), and the lowest number of tolerant accessions were sourced from Spójnia, Polan and PNOS (tab. 4). Among all the 23 tolerant lines, as many as 20 had good or very good tolerance to drought at the germination stage, and the germination capacity ranged from 77.4 to 136.3% of the control (tab. 4). However, two of these genotypes (ZS/131 and 171007) with the highest germination capacity under conditions of water deficit (21.4 and 36.3% higher compared to control) were characterised by a strongly reduced fresh weight of seedlings, and length of cotyledons and roots (63.3 to 73.4%). A similar correlation was found for the majority of sensitive lines, which also had good germination parameters (73.4 to 105.6% compared to controls) and were characterized by strongly inhibited seedling growth. Among sensitive lines, six were sourced from the Gene Bank, three from Plantico, three from Spójnia and one from Polan (tab. 4). The selected lines characterised by an extreme response to drought stress will be used to investigate physiological and biochemical factors determining tolerance to water deficit in onion.

The correlation coefficient (r) was calculated to identify the relationship between the studied features and drought stress (tab. 5). A strong correlation was found between fresh weight of seedlings and the length of cotyledons (r=0.8645) and the length of roots (r=0.8322) in response to drought. There was also a significant correlation between the length of cotyledons and roots (r=0.8116). These data are consistent with findings reported by Kłosińska et al. (2016), who observed the same correlation for cucumber seedlings treated with 18% PEG. Similar findings were also reported for melon (Kavas et al., 2013), and the study revealed

Tabela 4
Table 4

Charakterystyka tolerancyjnych i wrażliwych obiektów cebuli w warunkach stresu suszy (18% PEG - faza kiełkowania; 10% PEG – faza siewek) względem kontroli w %

Characteristics of tolerant and sensitive onion accessions under drought stress conditions (18% PEG – germination stage; 10% PEG – seedling stage) relative to control in %

Obiekt Accession	Pochodzenie Origin	Kiełkowanie % kontroli Germination % control	Świeża masa siewek % kontroli Fresh weight of seedlings % control	Długość liścieni siewek % kontroli Cotyledon length of seedlings % control	Długość korzeni siewek % kontroli Root length of seedlings % control
			Tolerancyjne Tolerant		
ZS/105	Plantico, Zielonki	80.6	80.3	112.1	124.2
ZS/109	Plantico, Zielonki	89.1	77.5	64.4	142.4
ZS/110	Plantico, Zielonki	100.8	75.6	64.4	121.4
ZS/111	Plantico, Zielonki	81.6	88.2	68.5	120.0
ZS/113	Plantico, Zielonki	65.3	83.0	72.8	149.5
ZS/115	Plantico, Zielonki	90.8	37.6	81.0	112.5
ZS/117	Plantico, Zielonki	91.0	67.0	111.0	104.7
ZS/118	Plantico, Zielonki	64.1	57.4	70.7	113.2
ZS/119	Plantico, Zielonki	81.6	69.2	90.9	120.5
ZS/131	Plantico, Zielonki	121.4	26.6	29.7	36.7
ZS/134	Plantico, Zielonki	116.7	61.8	61.5	94.7
ZS/135	Plantico, Zielonki	112.8	57.8	69.6	84.4
ZS/138	Plantico, Zielonki	85.9	83.4	80.2	116.1
ZS/158	Plantico, Zielonki	86.2	58.6	65.2	113.9
171007	Bank Genów, IO	136.3	29.6	31.0	33.6
171011	Bank Genów, IO	91.7	85.4	66.2	91.0
171017	Bank Genów, IO	102.4	88.7	79.0	86.1
171018	Bank Genów, IO	103.1	89.6	79.9	116.9
171026	Bank Genów, IO	112.2	82.0	75.8	133.6
171099	Bank Genów, IO	63.5	86.8	71.9	116.9
NOE/64	Spójnia, Nochowo	92.6	82.2	71.9	87.9
P/37	Polan, Kraków	77.4	52.8	54.6	115.5
Bolero F_1	PNOS, Ożarów Maz.	115.3	39.2	35.3	40.1
			Wrażliwe Sensitive		
ZS/46	Plantico, Zielonki	8.1	53.8	49.3	36.9
ZS/159	Plantico, Zielonki	12.7	38.3	39.2	47.0
ZS/181	Plantico, Zielonki	7.5	41.4	33.8	35.3
171009	Bank Genów, IO	73.4	19.7	15.2	18.0
171035	Bank Genów, IO	74.4	25.6	21.3	16.7
171036	Bank Genów, IO	82.6	28.3	16.4	22.1
171040	Bank Genów, IO	105.6	25.2	18.7	24.1
171042	Bank Genów, IO	83.4	25.7	25.8	18.6
171051	Bank Genów, IO	95.7	23.5	19.0	21.5
NOE/59	Spójnia, Nochowo	67.5	27.9	18.7	19.9
NOE/60	Spójnia, Nochowo	83.4	23.4	16.9	21.3
NOE/61	Spójnia, Nochowo	84.9	19.3	23.0	18.2
P/32	Polan, Kraków	77.6	35.0	22.7	18.2

Tabela 5
Table 5

Współczynnik korelacji (r) pomiędzy badanymi cechami 150 obiektów cebuli w warunkach stresu suszy (18 % PEG)

Correlation coefficient (r) between the studied features of 150 onion accessions under drought stress conditions (18% PEG)

Parametr Parameter	Kiełkowanie Germination (%)	Świeża masa siewek Fresh weight of seedlings (mg)	Długość liścieni siewek Cotyledon length of seedlings (cm)	Długość korzeni siewek Root length of seedlings (cm)
Kiełkowanie Germination (%)	1	0.2363^{ns}	0.1919^{ns}	0.2116^{ns}
Świeża masa siewek Fresh weight of seedlings (mg)	0.2363^{ns}	1	0.8645*	0.8322*
Długość liścieni siewek Cotyledon length of seedlings (cm)	0.1919^{ns}	0.8645*	1	0.8116*
Długość korzeni siewek Root length of seedlings (cm)	0.2116^{ns}	0.8322*	0.8116*	1

**istotne na poziomie α = 0,01; significant at α = 0.01*

a strong correlation between the length of roots and the length of aboveground parts in response to drought stress. On the other hand, the correlation between germination capacity and the parameters describing the growth of seedlings (fresh weight, length of roots and cotyledons) in response to drought stress was insignificant. This is confirmed by the low values of the correlation coefficient, i.e. r=0.2363, r=0.2116, and r=0.1919 respectively (tab. 5). Our findings suggest that the mechanisms determining tolerance to drought stress at the germination stage might be different from those that determine this trait in seedlings. A similar hypothesis was proposed by Kłosińska et al. (2016) in their studies on cucumber.

Due to the high variability in tolerance to water deficit, the evaluated onion accessions will be further investigated for genomic polymorphism using the DArTseq platform. Our research also allowed for the selection of accessions that can be used as a valuable starting material in breeding programmes to create new onion varieties tolerant to drought. We identified six lines/varieties of onion tolerant to drought at the germination stage: ZS/131, ZS/134, ZS/135, 171007, 171026 and Bolero F_1, as well as nine lines/varieties tolerant to drought at the seedling stage: ZS/105, ZS/109, ZS/110, ZS/111, ZS/113, ZS/138, 171018, 171026 and 171099. These accessions were sourced from Plantico, the Gene Bank, and PNOS (Bolero F_1). One genotype, 171026, deserves special attention as it was distinguished by a high tolerance to drought both at the germination and seedling stages.

Conclusions

1. The assessed onion accessions differed significantly in terms of tolerance to drought stress at the germination and seedling stages. For this reason they are a valuable material for genotyping to identify regions of the genome responsible for tolerance to water deficit in onion.
2. The increase in root length stimulated by drought stress might indicate tolerance to water deficit in onion.
3. There was a strong correlation between the fresh weight of seedlings and the length of their cotyledons and roots in response to drought stress. On the other hand, the correlation between germination capacity and the parameters describing the growth of seedlings (fresh weight, length of cotyledons and roots) in response to drought stress was insignificant. This might indicate that the tolerance of onion at different stages of growth is determined by entirely different mechanisms.
4. We selected onion accessions that can be a valuable material for use in programmes aimed at breeding onion varieties tolerant to drought stress.

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