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Evaluation of pea germplasm for drought resistance

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Abstract

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Drought is a serious threat to grain pulse production worldwide. One of the most widely grown grain legumes in the world is pea (*Pisum sativum* L.). Twenty-four pea varieties from diverse ecological and geographical origins, types, and breeding years were evaluated under laboratory conditions to define further the drought resistance level of the pea source material. PEG-6000 concentrations of 8.6% and 10% were used for the evaluation of drought resistance of pea accessions. Four cultivars (Kamelot, Baryton, Mascara, and Kharkivskyy yantarnyy) were selected. They outperformed the control and will be used as sources of drought resistance in further breeding work. It is also important to note the accessions Tsarevich and Malakhit, which showed a stable root length at the level of the control at both concentrations of PEG-6000.

Keywords: Pisum sativum L.; polyethylene glycol; PEG-6000; concentration; differentiation; accessions

Introduction

Today's world is facing climate change, food shortages, water scarcity, and population growth. Grain legumes are important sources of protein, essential micronutrients, and vitamins for human nutrition. Climate change, including drought, is a serious threat to grain legume production worldwide. Drought stress is a persistent challenge for crops and has been identified as a significant constraint on global agricultural productivity. Its intensity and severity are predicted to increase shortly. Drought tolerance in legumes is one of the most important ways to provide healthy food for people under modern conditions (Nadeem et al., 2019).

Leguminous crops are susceptible to drought conditions. Even crops grown in arid regions, which are relatively drought-tolerant (such as chickpeas and lentils), show a significant reduction in productivity under the influence of drought. For example, cowpea is drought-tolerant, but under African conditions, a water deficit reduced the average grain yield per plant by 67.28% (Fatokun et al., 2012). The most prominent effects of drought stress include reduced germination, stunted growth, severe damage to the photosynthetic apparatus, reduced net photosynthesis, and reduced nutrient uptake. Understanding the effects, mechanisms, and agronomic and genetic basis of drought is essential for sustainable management to mitigate the devastating effects of drought in legumes (Nadeem et al., 2019).

Yield loss in grain legumes varies from species to species and even from variety to variety within a species, depending on the severity of drought stress and several other factors, such as phenology, soil texture, and agro-climatic conditions. Legumes exhibit various tolerance mechanisms, including morphological, physio-biochemical, and molecular ones. Legumes can adapt to drought stress by changing their morphology, physiology, and molecular mechanisms. Improved root system architecture, reduced number and size of leaves, stress-induced phytohormones, stomatal closure, antioxidant defense system, solute accumulation (e.g., proline), and altered gene expression play a crucial role in drought tolerance. Further research should be conducted to enhance drought-tolerant traits in grain legumes, aiming to prevent losses during drought (Khatun et al., 2021). Increasing the root-shoot ratio and proline content were universal strategies of sugar beet germplasm to overcome drought stress (Tan et al., 2023).

Peas (*Pisum sativum*) are one of the most widespread legumes in the world. It occupies an area of 7.04 mln ha of sowing data from FAOSTAT in 2021, with a total production of 12.4 mln t (FAOSTAT, 2023). It is a grain legume native to the temperate zone that is rich in protein, fiber, micronutrients, and bioactive compounds, which can be beneficial to human health. Pea is one of the most sensitive legume crops to moisture deficiency. It is less drought-resistant than other legumes, such as chickpeas or lentils. Drought, corresponding to a soil water potential of -0.64 MPa, reduced the leaf water potential of pea to -1.83 MPa and resulted in a 23.9% reduction in dry matter yield. This compares with chickpea, which has a reduction of only 14.5% under the same conditions (Amede and Schubert, 2003). In the reduction of pea yield due to drought, the intensity and duration of the stress are crucial factors. Strategies such as screening, breeding, and marker-assisted selection can be used to manage drought tolerance in pea. As a result, various biotechnological approaches have led to the development of drought-tolerant pea varieties (Bagheri et al., 2023).

Pea production is characterized by significant yield variability between years, between fields, and even between fields in the same small area in a given year. Spring pea crops are most affected by water stress and high temperatures in dry years. Winter peas can partially escape the effects of drought, high temperatures, and root diseases during the reproductive phase of the crop cycle. However, suppose winters are mild, without progressive freezing temperatures that allow frost acclimation. In that case, the available varieties are not sufficiently frost-resistant and are susceptible to aerial diseases, such as Ascochyta blight and bacterial blight. Breeding of new varieties better adapted to different stresses is underway in national and European projects (Bénézit et al., 2017).

Many scientific projects aim to screen the local pea genofond and research new sources for breeding drought-resistant pea varieties, which is a priority in various countries, as noted by Iglesias-García et al. (2017) in the Mediterranean.

The development of drought-resistant pea varieties is one of the trends in modern breeding, objectively justified by climate change and significant crop losses due to drought.

Semi-leafless cultivars (*afila*) are primarily used in major pea production areas, including Western Canada, the European Union, Australia, and Ukraine. This type of leaf has much less lodging and is easier to harvest. However, the development of new cultivars using only semi-leafless peas could lead to a reduction in genetic diversity (Tran et al., 2023). Varieties of peas with standard leaf types exhibit higher levels of productivity and drought resistance; therefore, we need to identify sources to create new varieties with standard leaf types and enhanced drought resistance. Water use by semi-leafless peas (*Pisum sativum* L.) is usually lower than that of conventional peas because of their reduced leaf surface area. This suggests that semi-leafless peas would be less sensitive to drought because drought would occur later (Baigorri et al., 1999).

Pea genotypes with regular and reduced leaf (stipule morphology) were grown under drought stress conditions. Significant differences in dry matter accumulation were found between the two irrigation regimes. The physiological response varied accordingly. Under stress conditions, genotype *af*, in which the leaflets are transformed into tendrils, showed a faster CO_2 exchange rate, a lower stomatal resistance, and a lower canopy temperature than normal-leaf genotypes. This resulted in lower soil water use but higher pod dry matter accumulation, which doubled in the last 2 weeks. This behaviour was also favoured by a leaf dry matter accumulation, in terms of quantity and duration, that was significantly higher than that shown by normal-leaf genotypes. In addition, *af* plants showed a higher leaf-to-stem ratio and negligible senescence (Alvino and Leone, 1993).

Drought is a major constraint on crop yields and is becoming more frequent in the context of climate change. Drought can affect growth, plant water relations, and water use efficiency. There are several methods for assessing drought resistance. These are both direct field assessments conducted under natural drought conditions and laboratory assessments conducted under controlled conditions. These methods are often used in combination to check and clarify the results. A collection of cowpeas from the International Institute of Tropical Agriculture was evaluated for drought tolerance in Africa under field conditions by stopping irrigation. As a result, six lines were identified that could serve as sources for breeding drought-tolerant genotypes (Fatokun et al., 2012). To understand the mechanisms of drought tolerance in cassava, field trials were conducted under drought conditions in Kenya. Drought stress reduced yield by 59%, the number of edible storage roots by 43% and leaf retention by 50% on average. Phenotypic and molecular patterns associated with drought tolerance were identified at early stages of water deficit (Orek et al., 2020).

Various mathematical evaluation indices are also used to determine the level of influence of the stress factor. All these methods provide important information, allowing for the study of resistance mechanisms and the identification of sources for breeding, as well as the evaluation and selection of drought-tolerant and high-yielding lablab accessions in Tanzania's dry farming systems, based on drought tolerance indices and field performance. Thus, new effective methods for breeding drought stress-tolerant accessions with high seed yield were determined (Missanga et al., 2023).

A combination of field and laboratory experiments was conducted to evaluate the drought tolerance of soybean at different growth stages. A relative drought index and regression model were used to identify drought-tolerant cultivars during the vegetative and reproductive growth stages. Variation in the drought tolerance coefficient could explain 73.70% of the total variation at the vegetative growth stage. This suggests that these traits are closely related to plant drought tolerance (Yan et al., 2020).

Germination in an osmotic solution is one of the most convenient and standard methods for plant growth. Most researchers applied germination to PEG-6000. Tamindžić et al.'s (2021) study aimed to estimate genetic variability among four pea cultivars, determine seed physiological quality, and initial growth of garden pea (*Pisum sativum* L.) under drought stress. Drought stress significantly affected germination and the other traits, with the stress effects being proportional to the level of stress applied (Tamindžić et al., 2021).

Chaghakaboodi and Dogan (2021) used five drought tolerance indices to identify drought-tolerant genotypes in oilseed rape. Measurement of cell membrane stability using polyethylene glycol (PEG) was used as a drought tolerance test. The results of the analysis of variance showed a significant difference for all the indices. Based on these indices, soybean genotypes were divided into four groups according to drought resistance. Polyethylene glycol and cell membrane stability assays were used to evaluate drought and heat tolerance, respectively, in 14 wheat lines based on seedling traits and molecular analysis (El-Rawy and Youssef, 2014).

Different concentrations of PEG-6000 were used for different crops in different countries. Drought stress resistance of rice in the Republic of Iraq by treatment with 10% PEG (Al Azzawi et al., 2020). The identification of drought tolerance in sugar beet was tested under simulated conditions. Seven days and 9% PEG treatment were the optimal conditions for evaluation, under which more phenotypic indicators showed significant differences in the drought tolerance coefficient (Tan et al., 2023). PEG-6000 solution concentrations of 3%, 5%, and 7% were used for the evaluation of chickpea drought resistance by Asati et al. (2023). A working solution of PEG-6000 with concentrations of 15.0% and 19.5% was used for lentils (Vus et al., 2021).

The seeds of the pea cultivars were subjected to water stress induced by the polyethylene glycol PEG 6000 at three levels of stress (0, -0.15, -0.49, and -1.03 MPa). The results suggest that the osmotic potential of -0.49 MPa may be the threshold of sensitivity for germination in pea varieties (Tamindžić et al., 2021). A concentration of 8.6% PEG-6000 was found to be the highest possibility of differentiation for pea accessions in Ukraine (Vus et al., 2020).

This study aimed to determine the optimum concentration of PEG-6000 to assess drought resistance in peas under laboratory conditions, with the primary objective of identifying sources of drought resistance in the working collection and evaluating new breeding lines.

Materials and Methods

This research was performed at the research area of Plant Production Institute (PPI) in V. Ya. Yuriev, Kharkiv, Ukraine (PPI NAAS) – Elitne village, Kharkivskyi district, Kharkivska region (49°59′31″N, 36°26′55″E; 95 m above sea level), in 2019. Accessions from the working collection of the Legume Crops Laboratory of the Plant Production Institute "V. Ya. Yuriev" (Kharkiv, Ukraine) (PPI) from the 2019 harvest were used in the experiment. A total of 24 pea varieties from different ecological and geographical origins, types, and breeding years were studied. All Ukrainian accessions (18 genotypes) were the result of PPI's breeding, while the others were commercial varieties from the European Union (Table 1).

To verify the previously described method, the resistance of 24 pea genotypes to drought was assessed using a PEG-6000 solution with a concentration of 8.6%, as determined in previous research. The control was germinated in distilled water. Germination was carried out in a thermostat at a temperature of t = 21°C. On the seventh day, the shoot length (in mm) and root length (in mm) were measured for both the control and experimental plants. Data are presented as a percentage relative to the control plants.

$$D = \frac{y}{x} \times 100\%,$$

x – the median of the seedling length on the control,

y – the median of the seedling length on the PEG-6000 solution.

The number of seeds in each variant of the study was 25 seeds, with two repetitions.

Statistical analysis and the figures were created using R software version 4.2.2 (R Core Team, 2023) carried out using the with the help of the following packages: «tidyverse» and «rlang» (Henry and Wickham, 2023), «openxlsx» (Schauberger and Walker, 2022), «ggplot2» (Wickham, 2016).

Table 1. List of accessions studied

Genotype	Leaf-type	Country of origin
Baryton	afila	Germany
Kamelot	afila	Czech Republic
Chekryhinskyy	afila	Ukraine
Damir 2	afila	Germany
Deviz	afila	Ukraine
Efektnyy	afila	Ukraine
Hayduck	afila	Ukraine
Hlyans	afila	Ukraine
Gotik	afila	Czech Republic
Zekon	afila	Czech Republic
Tsarevich	afila	Ukraine
SL_12_20	afila	Ukraine
SL_11_25	afila	Ukraine
Otaman	afila	Ukraine
Oplot	afila	Ukraine
Metzenat	afila	Ukraine
Mascara	afila	Germany
Intensyvnyy 92	wild type	Ukraine
Kharkivskyy etal-onnyy	afila	Ukraine
Kharkivskyy yan-tarnyy	wild type	Ukraine
Korvet	afila	Ukraine
Magelan	afila	Ukraine
Magnat	afila	Ukraine
Malakhit	afila	Ukraine

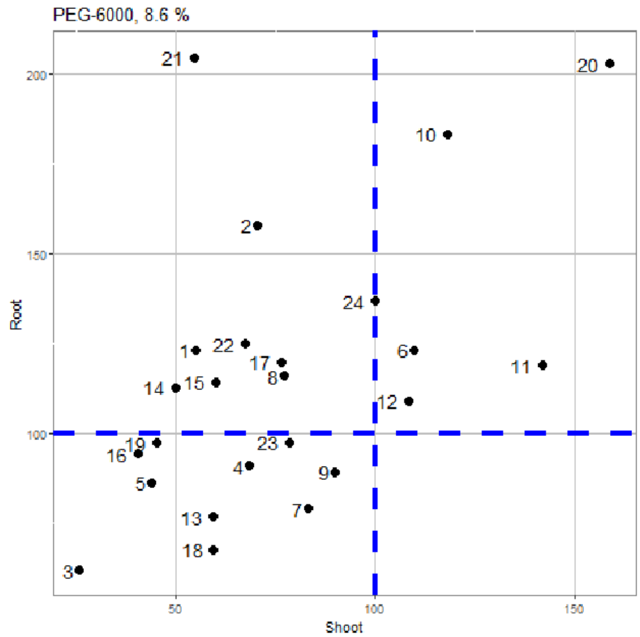

Fig. 1. Seedling length relative to the control under 8.6 % concentration of PEG-6000, % to control: (Genotypes:1 – Baryton, 2 – Chekryhinskyy, 3 – Damir 2, 4 – Deviz, 5 – Efektnyy, 6 – Gotik, 7 – Hayduck, 8 – Hlyans, 9 – Intensyvnyy, 10 – Kamelot, 11 – Kharkivskyy etalonnyy, 12 – Kharkivskyy yantarnyy, 13 – Korvet,14 – Magelan, 15 – Magnat, 16 – Malakhit, 17 – Mascara, 18 – Metzenat, 19 – Oplot, 20 – Otaman, 21 – SL_11_25, 22 – SL_12_20, 23 – Tsarevich, 24 – Zekon; dashed lines – 100 % relative to control)

Results

The germination of 24 accessions of the working pea collection of the Legume Breeding Laboratory (PPI) on a solution of PEG-6000 8.6% to assess drought resistance showed that the ratio of the length of roots and shoots as a percentage of the control varied significantly (Figure 1). The range of root length variation was from 62.00% (Damir-2) to 204.55% (SL 11-25), with an average of 116%. In 14 genotypes, an increase in root length was observed under PEG-6000 conditions compared to the control.

According to the median length of the seedlings, inhibition of growth processes under the influence of the osmotic solution was noted, with an average decrease of 77% compared to the control. The diapason of variation for this feature ranged from 25.93% (Damir 2) to 158.82% (Otaman). Five genotypes exceeded the control for both indicators (root and shoot length).

Although exposure to PEG-6000 solution (8.6%) caused a depression of shoot growth in most accessions, some genotypes showed an increase in this indicator. Therefore, we decided to conduct an additional experiment with a more rigorous background to compare the depression indicators in PEG-6000 solutions with concentrations of 8.6% and 10.0%. Fourteen accessions, including two from the group with increasing growth and five each from the other two groups, were selected for further study. The experiment was conducted using the same method with seeds from the same 2019 crop, employing a PEG-6000 concentration of 10%. This concentration was found to be the most differentiating for drought-resistant chickpea (Vus et al., 2020).

When pea seeds were germinated in a PEG-6000 solution with a concentration of 10.0% (which was calculated for chickpea culture), we observed a significant depression of the «stem length» indicator. The increased level of osmotic stress allowed for more precise differentiation of the accessions (Figure 2).

Shoot length ranged from 19.05 % (Magelan) to 51.61 % (Malakhit) of the control. Six genotypes also reduced root

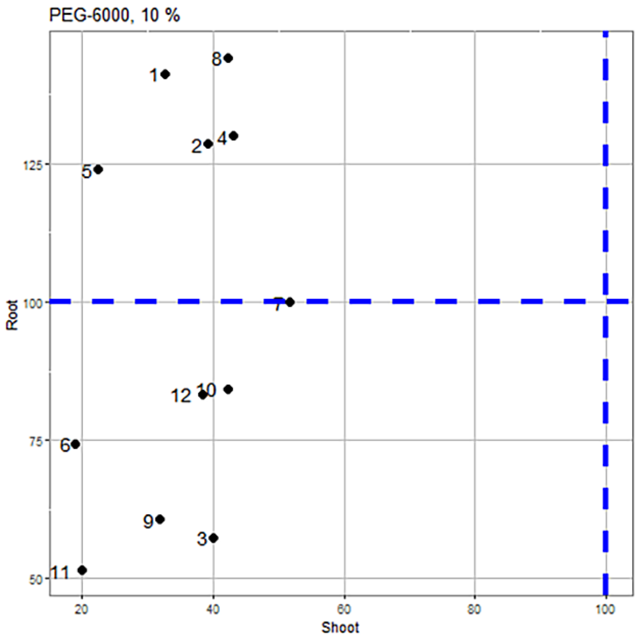

Fig. 2. Seedling length relative to the control under 10 % concentration of PEG-6000, % to control: (Genotypes:1 – Baryton, 2 – Damir 2, 3 – Efektnyy, 4 – Kamelot, 5 – Kharkivskyy yantarnyy, 6 – Magelan, 7 – Malakhit, 8 – Mascara, 9 – Oplot, 10 – SL_11_25, 11 – SL_12_20, 12 – Tsarevich; dashed lines – 100 % relative to control)

length from 51.46 % (SL 12-20) to 84.29 % (SL 11-25) of the control.

A comparison of the mean length of roots and shoots relative to the control of pea accessions at two concentrations of PEG-6000 is shown in Figure 3. A significant suppression of shoot growth was observed under the influence of PEG-6000. Out of 14 genotypes, only two (Kharkivskyy yantarnyy and Kamelot) showed a slight excess over the control at the concentration of 8.6%. All other varieties reduced shoot length. For root length, the pea accessions had a significantly higher variability. After comparing them with the control, we selected four varieties (Kamelot, Baryton, Mascara, and Kharkivskyy yantarnyy) that outperformed the control and will be used as sources of drought resistance in further breeding work. It is also important to note the accessions Tsarevich and Malakhit, which had a stable root length at the control level at both concentrations of PEG-6000. In particular, the variety Kharkivskyy yantarnyy should be determined. This variety, with a standard leaf type, exhibited stimulation of root growth in response to the stress factor at both concentrations. At a concentration of 8.6%, it even showed enhanced stimulation of root growth.

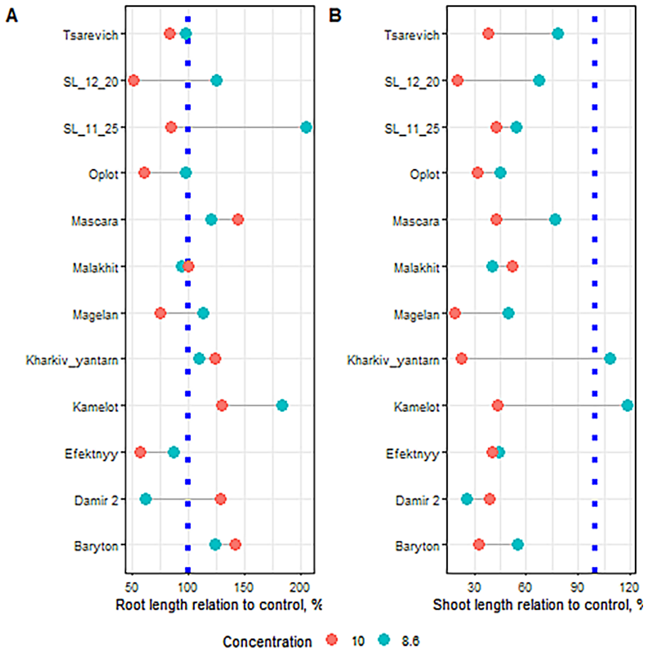

Fig. 3. Comparison of relative length of roots (A) and shoots (B) of pea accessions influenced by different concentrations of PEG-6000, % of control

Discussion

Our studies on pea germination in a PEG-6000 solution demonstrated a higher level of sensitivity of pea sprouts to water stress compared to their roots. This finding aligns with the data presented by Kausar et al. (2023). Drought stress is often exacerbated by climate change and is a significant constraint on agricultural production. The ability of plants to maintain higher productivity under drought stress depends on their ability to withstand drought stress and subsequently recover. Drought stress leads to a significant reduction in shoot and root length at the beginning of the vegetation period, resulting in a reduced number of leaves, plant height, and a decrease in the number of pods and seeds, ultimately leading to a loss of yield (Kausar et al., 2023).

We noted genotypes that exhibited a significant stimulation of root growth under the influence of a stress factor, which is due to the physiological response of peas to a moisture deficit during germination and their rapid search for a source of moisture, thereby ensuring further development. A robust root system is one of the mechanisms of legumes' drought resistance (Amede and Schubert, 2003). Drought resulted in a significant reduction in the root and shoot length of wheat genotypes. However, the root/shoot ratio increased under drought stress.

Root length showed a highly significant negative correlation with the Drought Susceptibility Index (DSI) under drought conditions (El-Rawy and Youssef, 2014).

Positive root hydrotropism of *Pisum sativum* L. (Tsuda et al., 2003; Miyazawa and Takahashi, 2020). When exposed to water stress induced by PEG 6000, the growth of pea epicotyls was dramatically reduced. Although variability was observed between cultivars, the degree of inhibition was proportional to the concentration of PEG. Differences in the osmotic adjustment or turgor maintenance capacity of each cultivar could account for the intraspecific variability in growth under water stress. The results obtained suggest that measurements at early stages of development can be used to identify drought-tolerant genotypes, and that the use of PEG as a marker of drought tolerance may aid in identifying such genotypes (Sánchez et al., 2004).

As a result of the experiment conducted and the analysis of the data obtained, we concluded that the PEG-6000 solution with a concentration of 8.6% is the best for evaluating pea accessions for drought resistance. This finding aligns with the data obtained by Sai Kachout and co-authors, who determined a PEG-6000 concentration of 8.0% (Sai Kachout et al., 2021). The use of a 10.0% PEG-6000 solution is more effective for evaluating the critical level of drought resistance in peas.

One of the most effective methods for evaluating drought resistance in peas is to combine laboratory and field research with the use of mathematical indices. Thus, the variety Magnat was determined in our research as relatively drought-resistant (root length 114.29% to the control by concentration 8.6%), was selected as a source of drought resistance in NCPGRU with a drought tolerance index of 1.2 by long-term (2006-2015) field research (Kobyzeva et al., 2016).

Two of the accessions studied were new breeding lines to evaluate their level of drought resistance. It was found that line SL11-25 is more drought-resistant, with the highest relative root length (204.55% on 8.6% PEG-6000) and 84.29% on 10% PEG-6000, and a stable level of shoot length at both concentrations of PEG-6000 (54.55% and 42.31%, respectively).

Conclusions

Therefore, we can conclude that the concentration of 8.6% is more discriminating, allowing us to select drought-resistant samples as a source for selection and to evaluate the drought resistance of the newly created selected material. The 10% concentration of PEG-6000 has a significant inhibitory effect on development and can only be used for extreme evaluation purposes.

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