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THE ROLE OF PHOTOPERIODIC REACTION IN THE FORMATION OF DROUGHT RESISTANCE IN ISOGENIC SOYBEAN LINES

Aim. Analysis of the role of photoperiodic response in the formation of drought tolerance in soybean by determining morphological, physiological and adaptive traits in cultivars and near-isogenic lines (NILs) differing in photoperiod sensitivity. ***Methods.*** Drought tolerance was assessed using a rapid screening approach and vegetation experiments in sand culture. Artificial drought was simulated by germinating seeds in a 10% mannitol solution, while in pot trials plants were grown under different levels of field water capacity (FWC). Seed germination, biomass accumulation, plant growth response at the V3–V4 stage, and free proline content in leaves and roots were measured. ***Results.*** Under both osmotic and soil drought, stress-tolerant soybean genotypes exhibited higher drought resistance, reflected in better seed germination, greater biomass production, enhanced root development, and elevated proline accumulation in treated plants. Photoperiod-neutral genotypes showed the highest drought tolerance, whereas short-day sensitive forms displayed the lowest values. ***Conclusions.*** Drought tolerance level is likely associated with photoperiod sensitivity and the length of the growing season, traits genetically controlled in soybean by the *E*-gene system.

Keywords: *Glycine max* (L.) Merr., NILs, photoperiodic response, *E* genes, express analysis of drought resistance, free proline.

Soybean (*Glycine max* (L.) Merr.) is one of the world's leading leguminous crops, which is important as a source of vegetable protein and oil. In the context of global climate change, particularly the increasing frequency and intensity of droughts, improving the drought resistance of soybean varieties is one of the key tasks of modern plant breeding and physiology (Rasheed et al., 2022, Yerzhebayeva et al., 2024, Staniak et al., 2023). It is known that water deficiency at critical stages of ontogenesis negatively affects the growth, photosynthetic activity, formation of generative organs, and yield of soybeans (Abdullah et al., 2025, Avksentiieva and Taran, 2016, Moloi et al., 2021).

The photoperiodic response is one of the fundamental adaptive traits of soybeans, determining the rate of development, the duration of the vegetative phase, and the time of transition to flowering (Zhai et al., 2022, Zhang et al., 2022). As a typical short-day crop, soybeans are highly sensitive to the ratio of light and dark periods of the day, which is realized through a complex system of phytochromes, cryptochromes, and regulatory genes of photoperiodism (Luo et al., 2022). Changes in photoperiodic conditions can significantly affect plant architecture, root system development (Mwenye et al., 2016), and the balance of assimilates, which indirectly determines the response of plants to abiotic stresses, in particular drought (Sun et al., 2024).

There is growing evidence of a close relationship between photoperiod sensitivity and the adaptive potential of soybeans to water deficiency (González et al., 2023). Regulation of flowering time through photoperiodic response genes can change the “drought avoidance strategy” by shortening or lengthening the growing season, as well as influencing the formation of a deeper and more branched root system. In addition, photoperiod indirectly regulates hormonal balance (abscisic acid, auxins, cytokinins), which plays a key role in controlling transpiration, stomatal conductance, and osmotic adaptation of plants under water stress conditions (Roeber et al., 2022).

Despite a significant number of studies devoted separately to the photoperiodic response and drought tolerance of soybeans, the mechanisms of their integrated action remain insufficiently understood. Data on the role of different types of photoperiodic response of soybean varieties and isogenic lines in the formation of morphophysiological and biochemical traits and markers associated with drought tolerance are particularly limited. In this regard, a comprehensive study of the influence of photoperiodic sensitivity on the realization of the drought-resistant phenotype of soybeans at different stages of ontogenesis is relevant.

The aim of this work is to clarify the role of photoperiodic response in the formation of drought resistance in soybean by analyzing morphological, physiological, and adaptive indicators in varieties and isolines with different types of photoperiodic sensitivity.

Materials and methods

Plant material. The study used varieties and isolines of soybean (*G. max*) from the plant collection of the Department of Plant Physiology and Biochemistry and Microorganisms of V. N. Karazin Kharkiv National University with a specific photoperiodic reaction (Table). The short-day variety Smolyanka, characterized by black seeds, and the neutral-day variety Ambella, included in the State Register of Varieties of Ukraine, isogenic lines NILs of soybeans of the Yuriev plant production institute of the National Academy of Agrarian Sciences of Ukraine (IR): short-day IR-903 and neutral-day IR-907, created in the gene pool of the Harosoy variety, and isogenic lines created in the gene pool of the Clark variety, which differ in the state of individual loci of the E-series genes and photoperiodic response (Agricultural Research Service www.ars.usda.gov).

Research design. Experiments to study the degree of drought resistance were conducted using the express method and under *in vivo* conditions (vegetation experiments).

Screening of the studied varieties and isolines of cultivated soybeans for drought resistance was carried out using the express method based on seed similarity and analysis of seedling biomass. To create conditions of “artificial drought”, seeds were germinated in Petri dishes with the addition of a 10% mannitol solution, while control seeds were germinated in water. Germination was determined according to DSTU 4138:2002.

Vegetation experiments *in vivo* were carried out by cultivating plants in sand culture in 2-liter vegetation vessels (15 plants per vessel) in environmental chamber of the Department of Physiology and Biochemistry of Plants and Microorganisms of V. N. Karazin Kharkiv National University under standard conditions t = 22/20 ± 1 °C (day/night), illumination 15,000 lx, photoperiod 16 / 8 hours (day/night). Karazin under standard conditions t = 22 / 20 ± 1 °C (day/night), illumination 15 thousand lx, photoperiod 16 / 8 hours (day/night). Control plants were grown at 75% of full moisture capacity (FMC), experimental plants – at 35% FMC. The soil was watered to the required moisture capacity values based on the weight of the container. At 6 weeks of age (stage V3–4), morphometric parameters (linear growth and biomass of the above-ground and root systems) were analyzed, and the content of free proline in the leaves and roots of the experimental plants was determined. The proline content was determined by a colorimetric method based on the reaction with ninhydrin reagent in an acidic medium with the addition of glacial acetic acid (Molodchenkova et al., 2025). The optical density of the yellow-brown colored product was measured at λ = 520 nm on a spectrophotometer Shimadzu UV-2600 (Japan). The free proline content was calculated using a calibration curve constructed using L-proline (Khimlaborreaktiv, Ukraine). The degree of drought resistance was calculated as the ratio of the values obtained for the

Table. Soybean varieties and isolines used in the studies

Varieties and NILs	Genotype	Photoperiodic reaction
Smolyanka	–	SDP
Ambella	–	NDP
L-65-3336	*E1 E2 E3 E4 e5 E7*	SDP
L-719-20	*e1 e2 e3 E4 e5 E7*	NDP
IR-903	*E1E2E3*	SDP
IR-907	*e1e2e3*	NDP

Notes: – genotype unknown, SDP – short-day plant, NDP – neutral-day plant.

experimental plants to those of the control plants, expressed as a percentage. The experiments were conducted in three biological series, the results were statistically processed using the pairwise comparison method, and the significance of differences was assessed at $p \leq 0.05$. The tables and graphs show the average values of drought resistance indicators, their standard deviations, and the number of samples ($M \pm SD$, $n = 20$). Excel-Microsoft 365 and Statistica 5.0 software were used for statistical analysis and data visualization.

Results and discussion

Analysis of drought resistance results based on seed germination showed that the studied soybean varieties and isolines differ greatly in terms of drought resistance (Fig. 1). The lowest drought resistance indicators were demonstrated by the Smolyanka soybean variety and the L-65-3336 isoline – 46.13% and 48.58% respectively, while the Ambella variety showed a drought resistance level of 60%. while other isolines are characterized by significantly higher drought resistance indices: IR-903 – 82.66%, IR-907 – 89.79%, L-719-20 – 92.84%. It should be noted that the photoperiodic response of varieties and NILs clearly determines the difference between the degree of drought resistance in each comparison pair, and regardless of the absolute indicators, the results demonstrate significant differences between the studied varieties and isolines. According to the results of the express analysis, photoperiodically neutral plants always show a higher degree of drought resistance compared to short-day experimental plants.

The results of the analysis of drought resistance based on biomass accumulation as an integral marker of growth response demonstrate greater resistance of this indicator to the effects of “artificial drought” compared to germination – the degree of drought resistance in all varieties and NILs was within the range of 81.91–92.23%. At the same time, there is also a clear trend in the difference in drought resistance of plants with different photoperiodic responses. Again, photoperiodically neutral varieties and isolines are more resistant compared to short-day photoperiodically sensitive cultivated soybean plants.

An integral indicator of plant life processes in various environmental conditions, including drought, is the course of biosynthetic processes, which is characterized by biomass accumulation and growth response. The results of determining this indicator in a model vegetation experiment showed that under the influence of drought (35% of the FMC), all varieties and isogenic lines of soybeans experience a significant decrease in linear parameters and biomass accumulation in the aboveground and underground parts of the experimental plants (Fig. 2). The degree of drought resistance in terms of length is higher than in terms of biomass accumulation. The lowest drought resistance indicators are shown for the same samples as in the express method analysis – the Smolyanka, Ambella, and short-day isoline L-65-3336 varieties. Comparing the degree of drought resistance of the aboveground and underground parts of the experimental plants, it can be noted that the root system in all studied varieties and NILs for both growth response indicators (linear parameters and biomass accumulation) is more resistant compared to the aboveground part of cultivated soybean plants. Analyzing

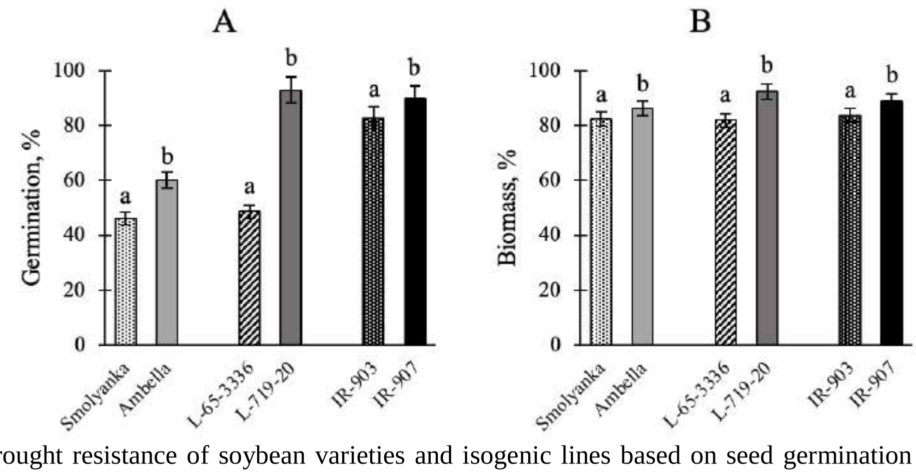

Fig. 1. Drought resistance of soybean varieties and isogenic lines based on seed germination (A) and biomass accumulation (B), %. Different letters indicate significant differences between SDP and NDP.

the relationship between the photoperiodic response of varieties and NILs of cultivated soybeans and the degree of drought resistance in vegetation experiments with different levels of substrate moisture supply, we also observe a clear pattern: neutral-day experimental plants are characterized by a higher level of drought resistance compared to photoperiodically sensitive short-day soybean samples.

However, isolines IR-903 and IR-907 do not show significant differences in drought resistance in terms of biomass and shoot length, but in the root system, the differences between the IR-903 KDR and IR-907 NDR isolines are significant, which once again emphasizes the greater sensitivity and importance of the root system in the formation of the complex drought resistance of soybean crops.

Among the mechanisms that ensure plant resistance to drought, the process of biochemical adaptation through the synthesis of “compatible osmolytes”, including the amino acid L-proline, is very important. Free proline under stress is multifunctional; it is a marker and protector in conditions of water deficiency, performing the functions of an osmolite, membrane protector, antioxidant, energy source, transporter, and nitrogen reserve, etc. (Molodchenkova et al., 2025, Nguyen et al., 2020).

According to the results of our experiments, the synthesis and accumulation of proline is not organospecific in the experimental samples of cultivated soybeans – the degree of drought resistance based on proline accumulation is practically the same in the leaves and roots of soybean varieties and NILs, which once again confirms the non-specificity and marker status of this trait in a stress state. Drought resistance according to this indicator is high and ranges from 82.70% to 93.56% (Fig. 3). Analysis of the results confirms the same pattern regarding photoperiodic sensitivity and drought resistance of cultivated soybean plants. The photoperiodically neutral variety Ambella and isoline L-719-20 are more resistant to soil drought compared to the short-day variety Smolyanka and isoline L-65-3336. No significant differences in leaves and roots were found between the IR-903 and IR-907 isolines, which were created in the Harosoy variety gene pool.

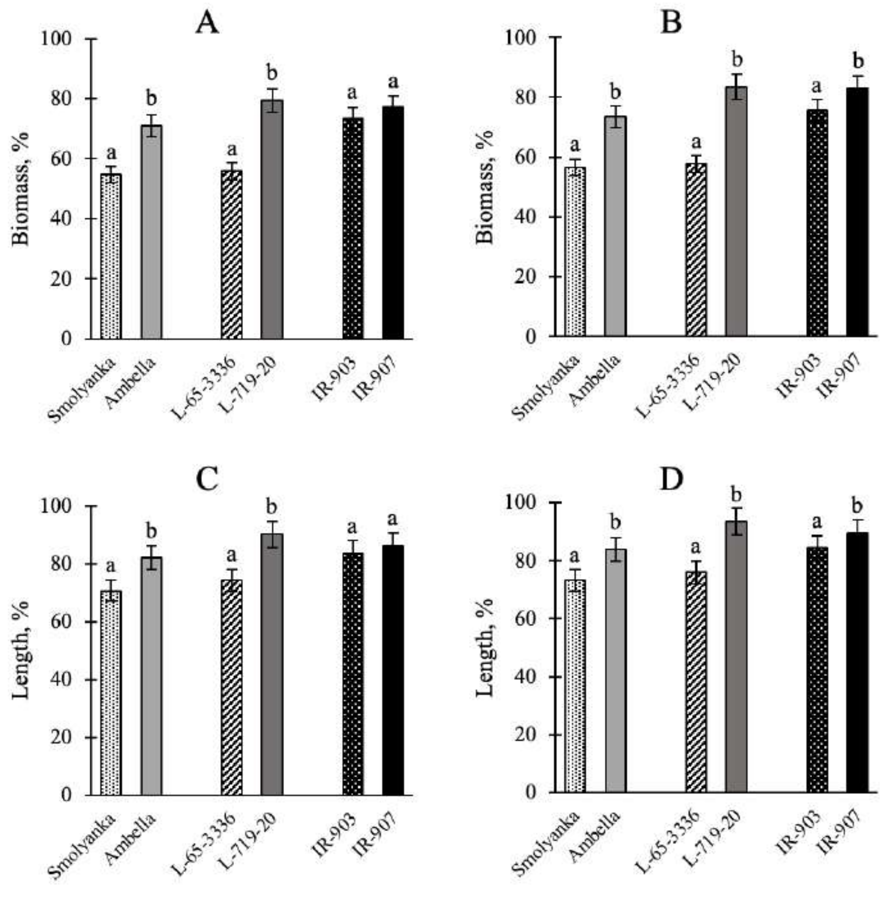

Fig. 2. Drought resistance of soybean varieties and isogenic lines based on plant growth response indicators in a vegetation experiment: shoot biomass (A) and root biomass (B), shoot length (C) and root length (D), %. Different letters indicate significant differences between SDP and NDP.

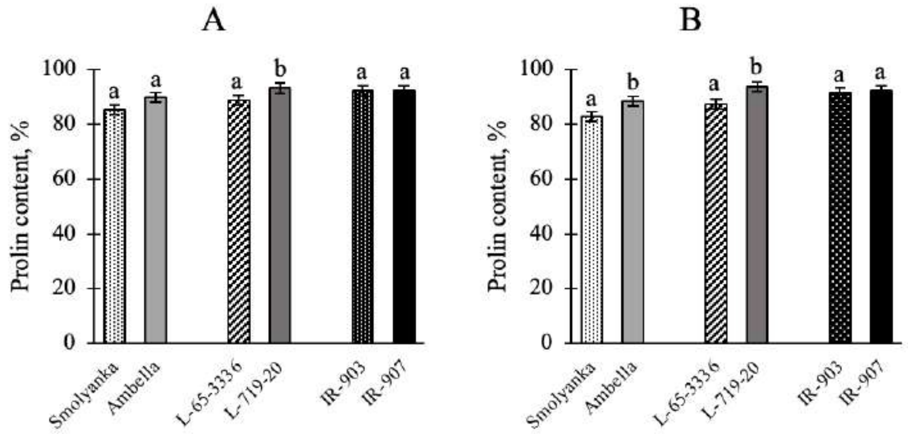

Fig. 3. Drought resistance of soybean varieties and isogenic lines based on the content of free proline in leaves (A) and roots (B), %. Different letters indicate significant differences between SDP and NDP.

Therefore, based on the results of analyses of drought resistance using the rapid method and *in vivo* in vegetation experiments for all pairs of comparisons of varieties and NILs of cultivated soybeans, a general trend was identified: photoperiodically neutral varieties and isolines exhibit higher drought resistance compared to short-day samples. Our previous studies (Avksentiieva and Taran, 2016) also showed the same pattern for soft wheat isolines, which is a long-day crop in terms of photoperiodic response. Thus, photoperiodically neutral (insensitive) plants show a higher degree of adaptability and drought resistance compared to photoperiodically sensitive plants, regardless of their photoperiodic response – short-day or long-day plants. Photoperiodically neutral varieties and NILs of cultivated soybeans have a shorter growing season and greater ecological plasticity, which allows plants to adjust their ontogenetic development depending on water supply conditions, unlike photoperiodically sensitive ecotypes, whose development is determined by the length of the photoperiod.

Conclusions

1. During the rapid analysis, differences in drought resistance between varieties and NILs were identified using the E-gene system isolines. It is possible that genes controlling photoperiod sensitivity indirectly influence the expression of drought resistance genes in cultivated soybeans.

2. Under conditions of artificial and soil drought, it was found that stress-resistant soybean samples are characterized by higher drought resistance indicators in terms of seed germination, plant biomass accumulation, enhanced root system growth, and increased proline content in the aboveground and below-ground parts of the test plants.

3. Analysis of the degree of drought resistance of varieties and isolines using various methods showed that the influence of the genotype on the manifestation of stress resistance does not depend on the phase of plant ontogenesis.

4. The maximum drought resistance indicators were characteristic of photoperiodically neutral varieties and isolines, while the minimum indicators were characteristic of varieties and lines with a short-day photoperiodic response.

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РОЛЬ ФОТОПЕРІОДИЧНОЇ РЕАКЦІЇ У ФОРМУВАННІ ПОСУХОСТІЙКОСТІ ІЗОГЕННИХ ЛІНІЙ СОЇ

Мета. Аналіз ролі фотоперіодичної реакції у формуванні посухостійкості сої шляхом визначення морфологічних, фізіологічних і адаптивних показників у сортів та ізогенних ліній (NILs) з різним типом фотоперіодичної чутливості. ***Методи.*** Аналіз посухостійкості проводили експрес-методом і за вегетаційних експериментів у піщаній культурі. Для створення умов «штучної посухи», насіння пророщували з додаванням 10% розчину манітолу, в умовах вегетаційних дослідів рослини культивували за різних умов повної вологоємності (ПВ). Аналізували схожість насіння, накопичення біомаси, ростову реакцію рослин (стадія V3–V4), вміст вільного проліну у листках і коренях рослин. ***Результати.*** В умовах штучної і ґрунтової посухи встановлено, що стрес-стійкі зразки сої культурної характеризуються вищими показниками посухостійкості за параметрами схожості насіння, накопичення біомаси, зростанням кореневої системи і підвищеним вмістом проліну у дослідних рослинах. Максимальними показниками посухостійкості характеризувалися фотоперіодично нейтральні рослини, мінімальними – з короткоденною фотоперіодичною реакцією. ***Висновки.*** Припускається, що рівень посухостійкості може бути пов’язаний з фотоперіодичною чутливістю й ознакою тривалості періоду вегетації, які детерміновані у сої системою *E*-генів.

Ключові слова: *Glycine max* (L.) Merr., NILs, фотоперіодична реакція, гени *E*-серії, експрес-аналіз посухостійкості, вільний пролін.

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