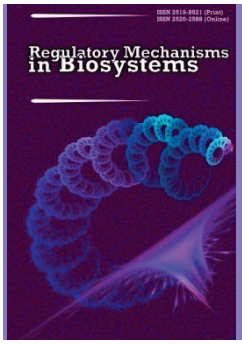

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Influence of *Bradyrhizobium japonicum* on the growth parameters and formation of the assimilation apparatus in *E*-gene isogenic lines of soybean

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The study investigated the impact of the interaction between soybean and rhizobia on the assimilation apparatus functioning and biomass accumulation in different soybean lines with varying photoperiod sensitivity. Nearly isogenic lines (NILs) of soybean were used, with genes *E1*, *E2*, and *E3* in different allelic states: Clark (*e1E2E3*), L80-5879 (*E1e2e3*), L63-3117 (*e1e2E3*), and L71-920 (*e1e2e3*). The experimental group for each line was treated with *Bradyrhizobium japonicum* 634b. Plants were grown under natural long-day conditions (16 hours). Growth indicators of the studied lines, such as relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), and specific leaf area (SLA), were analyzed, as well as the content of chlorophylls A and B in the V3 and V5 developmental stages. The results demonstrate that the influence of rhizobia on the functioning of the assimilation apparatus and biomass accumulation depends on the soybean line genotype. In the study, RGR, which characterizes the biomass accumulation rate, has similar trends to those observed with NAR, characterizing the assimilation apparatus's functioning. However, each line showed its own tendencies. For instance, in the short-day variety Clark, under bacterial influence, the value of RGR and NAR decreased. Additionally, LAR and SLA values indicated a reduction in the total photosynthetic surface area and leaf dry matter. Bacterial inoculation did not significantly affect the content of photosynthetic pigments in Clark leaves. Another short-day line, L80-5879, showed no significant impact of bacterial inoculation on biomass accumulation. However, soybean interaction with *Bradyrhizobium japonicum* 634b led to a decrease in leaf surface area and dry matter content. Probably, bacterial inoculation supported assimilation processes by increasing auxiliary chlorophyll *b* in photosystem I. A general trend of significant RGR reduction in neutral-day soybean lines, L63-3117 and L71-920, was identified under bacterial influence. The interaction with rhizobia differently affected LAR and SLA values, indicating distinct adaptive mechanisms to the interactions. In conditions of almost zero plant biomass accumulation, *Bradyrhizobium japonicum* 634b caused a decrease in the total photosynthetic surface area and chlorophyll *a* and *b* content in the L63-3117 line. In L71-920, bacterial inoculation had no effect on the total photosynthetic surface area, while leaf dry matter and photosynthetic pigment content decreased. The obtained results demonstrate that interaction with rhizobia can influence the functioning of the assimilation apparatus in soybeans with varying photoperiod sensitivity that is determined by genotype. It is important in improving soybean productivity and its application in agricultural practices.

Keywords: Glycine max; rhizobia; *E*-loci; photoperiod sensitivity; growth analysis; chlorophyll *a* and *b*; inoculation; leaf area.

Introduction

Soybean (*Glycine max* (L.) Merr) is an important agricultural crop, the production of which contributes significantly to satisfying the demand for protein and oil. The nutritional qualities of soybean make it an essential component of consumption not only for humans but also for animals. Therefore, research aimed at increasing the yield, studying the course of developmental phases, and the adaptation of soybean in variable environmental conditions is fundamentally and economically justified (Pagano et al., 2016). The growth and development of soybean are influenced by a wide range of factors, the sensitivity and response to which are genetically determined. In other words, the ontogenetic development of the plant and its productivity are determined by the interaction of genotype and the environment. In general, genotype, the environment, and their interaction determine the manifestation of all physiological and morphological characteristics that influence plant productivity (Rani et al., 2023). The plant's productivity is ensured by a certain level of assimilation processes that influence its physiological state at each stage of ontogenetic development. Simultaneously, the level of assimilation processes depends on the functioning of the assimilation apparatus, which is understood as the organs that ensure the processes of photosynthesis. The primary organ responsible for the plant's assimilation processes is the leaves. Therefore, their morphometric parameters, structure, and content of photosynthetic pigments – all of these factors influence biomass accumulation, energy expenditures, and, ultimately, the development and productivity of the plant (Weraduwage et al., 2015; Liu et al., 2020; Wang et al., 2023). Thus, the parameters of the assimilation apparatus can be influenced by a wide range of factors and their interaction with the plant's genotype. A significant factor with the potential to exert such an influence is the photoperiod (Roeber et al., 2022; Osnato et al., 2022), capable of regulating the timing of flowering and, consequently, influencing the growth, development, and productivity of the plant. This influence is logical, as varying day lengths impact light availability, and consequently, energy provision. The sensitivity of soybean to the photoperiod is genetically determined (Zheng et al., 2021). Genes that determine sensitivity to the photoperiod include early flowering genes (*E*-genes), among which genes from *E1* to *E9*, as well as *J*, *Dt1*, and *Dt2*, are identified (Zhang et al., 2021; Zimmer et al., 2021).

It is noteworthy that, as of today, the precise structure, regulatory system, and function are not known for every gene. However, it has been established that flowering is facilitated by the *GmFT2a* gene and its homologs (Sun et al., 2011). Currently, Kong et al. (2014) and Zhao et al. (2016) have determined that the *E9* gene corresponds to the *GmFT2a* gene, the dominant state of which induces flowering. Simultaneously, the function of other *E*-series genes is directed towards modulating the expression of *GmFT2a*. It has been determined that the *E1* gene encodes a functional protein containing a *B3* domain – a conservative region exclu-

sively found in transcription factors. Numerous studies have revealed that under long-day conditions, the dominant state of the *E1* gene represses the expression of the *GmFT2a* (*E9*) gene (Xia et al., 2012). Simultaneously, strong expression of the *E1* gene leads to a reduction in leaves and their curling at the unrolled unifoliolate leaves stage (VC), through negative regulation of genes associated with leaf development (Li et al., 2021). Other genes – *E3* and *E4*, encoding homologous forms of the phytochrome A photoreceptor, are capable, under long-day conditions, of repressing the induction of the flowering gene *GmFT2a*, through positive regulation of the *GmTOE4a* gene, which acts as a repressor of *GmFT2a* (Zhao et al., 2015). Additionally, phytochromes play a role in perceiving signals of red and far-red light, crucial in early plant development and manifested in photomorphogenic responses (Tripathi et al., 2019). The *E2* gene is an ortholog of the *GIGANTEA* gene in *Arabidopsis thaliana*, which also inhibits the expression of the *GmFT2a* gene under long-day conditions (Tsubokura et al., 2013). Furthermore, this gene may participate in regulating the plant's circadian rhythm, determining resistance to cold and drought (Mishra & Panigrahi, 2015). Thus, the response to the photoperiod determines not only the ontogenetic development of the plant but also physiological changes in developmental phases. In terms of photoperiodic response, soybean is facultative short-day, meaning it can flower under both short and long days (with a duration increased vegetative development period) (Taniguchi et al., 2020).

Another environmental factor influencing plant growth and development is interaction with microorganisms. Symbiotic relationships with representatives of the order Rhizobiales, capable of atmospheric nitrogen fixation and supplying it to plants, are highly specific for soybean (Wani & Gopalakrishnan, 2019; Hu et al., 2023). In this context, interactions with rhizobia extend beyond simple mineral nutrient intensification. The development of such interactions involves genes in both plants and bacteria (Fagorzi et al., 2021). It is logical to assume that a broad spectrum of other factors, including photoperiod, can influence the development of such interactions. Indeed, Wang et al. (2021) demonstrated that the products of *GmFT2a* (*E9*) and *GmSTF3* gene expression may participate in initiating nodule formation during interactions with rhizobia and proposed a hypothesis of light-induced nodule formation. Other authors suggested a symbiotic pathway regulating the expression of the *GmFT2a* gene under low nitrogen conditions during interactions with representatives of the order Rhizobiales. Yun et al. (2023) identified that combinations of symbiotic miR172c and local miR172c, induced by fixed nitrogen, induce *GmFT2a* expression by repressing *GmTOE4a*. In essence, they proposed a new symbiotic pathway for flowering induction.

Therefore, research on the role of soybean genotype in the interaction with rhizobia and the impact of these interactions on the formation and functioning of the assimilation apparatus is relevant. Thus, the aim of our study was to determine the influence of the interaction between cultivated soybean and rhizobia on changes in the assimilation apparatus and biomass accumulation, serving as a marker for the intensity of assimilation processes, in soybean lines with varying sensitivity to photoperiod.

Material and methods

Plant and bacterial materials. In the study, nearly isogenic lines (NILs) for photoperiod sensitivity genes (*E*-series) of soybean (*Glycine max* (L.) Merr) were used. These lines were developed based on the Clark variety and were provided by the National Center for Plant Genetic Resources of Ukraine (Kharkiv). Isogenic lines have the same genotype but differ in the state of one or more loci, making them a convenient model for studying the control of *E*-series genes in physio-biochemical processes in response to photoperiod. For the experiment, lines with known allele states of genes *E1-E5*, *E7* (Tasma & Shoemaker, 2003) were selected, where genes *E1*, *E2*, and *E3* were in different allelic states (Table 1). The photoperiodic response of the lines was determined based on their genotype. The seed germination energy in all lines was the same – 99%.

Inoculation was performed with the strain *Bradyrhizobium japonicum* (Kirchner) 634b, provided by the Institute of Agricultural Microbiology and Agro-Industrial Production of the National Academy of Agrarian Sciences of Ukraine in Chernihiv. This strain is active, virulent, and has been the subject of numerous studies (Melnykova & Kots, 2019). To obtain a bacterial culture for inoculation, the bacteria were cultivated on a medium for slow-growing nodulating bacteria on pea broth with the following composition (g/L): sucrose – 5.0 (Hlr LLC, China); glucose – 10.0 (Hlr LLC, Ukraine); $(NH_4)_2SO_4$ – 1.0 (Merk, Germany); KH_2PO_4 – 0.5 (Hlr LLC, China); K_2HPO_4 – 0.5 (Hlr LLC, China); $MgSO_4 \cdot 7H_2O$ – 0.2 (Merk, Germany); $CaCO_3$ – 0.2 (Hlr LLC, Ukraine); NH_4MoO_4 – 0.05 (Merk, Germany); pea broth (100 g of peas boiled in 1 liter of tap water for 30–40 minutes), agar – 12.0, pH = 6.8, sterilization regime – 1 atm, 30 minutes (Kozar et al., 2012). For bacterial inoculation, a cell suspension was prepared by washing the bacterial culture with a NaCl solution (0.8%, Hlr LLC, Ukraine) on solid medium. The number of cells used for seed inoculation was determined photometrically on a KFK-2M using the optical density of the solution at 600 nm. A calibration graph was constructed by determining the cell count using the Vinogradsky-Shulgin-Brid method.

Table 1
Isogenic lines (NILs) for genes controlling photoperiod sensitivity in soybeans, created in the genotype of the Clark variety

Isolines	Allelic state of genes in genotype	Photoperiodic reaction
Clark	*e1E2E3E4e5E7*	short-day plant
L80-5879	*E1e2e3E4e5E7*	short-day plant
L63-3117	*e1e2E3E4e5E7*	day-neutral plant
L71-920	*e1e2e3E4e5E7*	day-neutral plant

Experiment design. The field experiment was conducted on the experimental plot of the Department of Plant Physiology and Biochemistry at V. N. Karazin Kharkiv National University in 2021. Prior to seed sowing for each line, the seeds were sterilized with a 10% hydrogen peroxide solution (Hlr LLC, Ukraine) for 30 minutes. Subsequently, half of the seeds from each line were inoculated with a bacterial suspension at a concentration of 108 cells/ml, calculated at 300,000 cells per seed (experimental). The other half of the seeds were treated with sterile distilled water (control). Manual sowing was performed at the end of May in five biological replicates for each line. Twenty seeds of plant material were sown for each biological replicate. Plant fertilization and bacterial treatments were not applied. Plants were grown under natural long-day conditions (in the city of Kharkiv, 50° N – 16 hours). Morphometric and gravimetric parameters necessary for growth analysis indices were determined at the third true leaf stage (V3) and the fifth true leaf stage (V5). To determine pigment content, 2–3 fully developed leaves in the V3 and V5 stages were collected at 12:00 pm and fixed at 110 °C in a thermostat for 30 minutes.

The onset of the phenological phase was recorded when more than 50% of plants in each variant entered the V3 or V5 development phase. Depending on the line, the V3 phase corresponded to 35–42 days, and the V5 phase to 47–55 days.

Growth analysis. For the calculation of relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), and specific leaf area (SLA), the necessary parameters were immediately determined after collecting the plant material. This included the number of leaves per plant and leaf area using leaf image analysis (image processing). After drying the plants, the dry mass of leaves and the overall plant was determined. The calculation of indices was performed using the following formulas, according to Hunt (2017).

Determination of chlorophyll a and b content. The pigment content was determined using the Lichtenthaler & Wellburn (1985) method. The average sample of each biological replicate for each line was analyzed twice, resulting in a total dataset for each line containing 10 data points (5 biological replicates × 2 analytical replicates). For this, dry plant material was ground in a porcelain mortar with sand and extracted with the addition of 10 mL of 96% ethyl alcohol. The precipitate was separated by centrifugation for 20 minutes at 3000 rpm. The optical density of the extract was recorded at 649 and 665 nm. The total chlorophyll content was calculated using formulas for ethyl alcohol (96% v/v, Medlev LCC, Ukraine).

Bioinformatic methods. To analyze the obtained data, the localization and expression conditions of genes *E1–E3* were determined for the model soybean variety Williams.82 using the databases SoyBase (http://soybase.org), Phytozome (https://phytozome-next.jgi.doe.gov), and the visualiza-

tion tool ePlant Soybean from the University of Toronto (https://bar.utoronto.ca/eplant_soybean) (Waese et al., 2017).

Statistical data analysis. The obtained data were analyzed using Statistica 10 software (StatSoft Inc., 2011, USA). We calculated standard mean values (x) and standard deviation (SD). Differences between groups were determined using Tukey's test, where the differences were considered reliable at $P < 0.05$ (taking into account Bonferroni correction).

Results

Growth analysis. The calculation of the relative growth rate (RGR) showed a significant decrease in the indicator for bacterial inoculation in almost all lines, except for L80-5879, which has the *E1* gene in a dominant state (Table 2). This means that biomass accumulation occurs with reduced speed in the Clark variety under bacterial inoculation, compared to the control. Lines L63-3117 and L71-920, which are neutral-daylength under bacterial inoculation, almost do not accumulate biomass over the studied period (V3-V5). At the same time, in the control variant, the highest values of the indicator are observed in the Clark variety (with the *E2E3* genes present in the genotype) and the neutral-daylength line L63-3117 (with the *E3* gene present in the genotype). Similar trends are observed for the net assimilation rate (NAR) – a significant reduction in biomass accumulation in all variants, except for L80-5879 (Table 2).

The calculated values of the leaf area ratio (LAR) in the V3 phase show a significant decrease in the indicator under bacterization conditions in all lines, except for L71-920, which has recessive alleles *e1-e3*. In the V5 phase, we observe a slightly different trend – significant decrease in the LAR is present only in the lines L80-5879 and L63-3117 (Table 3). When comparing the changes in the indicator between the V3 and V5 development phases in the control variant, a significant increase in LAR is noted in the short-day line L80-5879 and the neutral-day L71-920.

Under bacterization, an increase in the indicator during the V5 development phase compared to the V3 phase is observed in the Clark variety and the lines L80-5879 and L71-920. The neutral-day line L63-3117 reduces leaf area ratio in the V5 stage (Table 3).

Table 2

Effect of *Bradyrhizobium japonicum* (Kirchner) 634b on the relative growth rate and net assimilation of soybean (*Glycine max* (L.) Merr) nearly isogenic lines (NILs) for *E*-genes) (n = 10, x ± SD)

Isolines	Relation growth rate, mg/(g × day)		Net assimilation rate, mg/(cm^2 × day)	
	without treatment	bacterization	without treatment	bacterization
Clark	75.9 ± 2.1	17.2 ± 0.4*	0.563 ± 0.021	0.131 ± 0.004*
L80-5879	31.8 ± 1.0	28.9 ± 0.9	0.196 ± 0.008	0.205 ± 0.012
L63-3117	54.8 ± 1.7	1.52 ± 0.02*	0.342 ± 0.014	0.0101 ± 0.0003*
L71-920	24.4 ± 0.4	0.39 ± 0.01*	0.152 ± 0.012	0.0025 ± 0.0001*

Note: comparisons were made within each isogenic soybean line between the variant with bacterization and without it (control) for each of the parameters; average value in column "Bacterization" for each of isogenic lines that is marked with symbol * significantly differ from average value in column "Without treatment", based on Tukey's test ($P < 0.05$) with Bonferroni correction.

Table 3

Influence of *Bradyrhizobium japonicum* (Kirchner) 634b on the leaf area ratio and specific leaf area of isogenic soybean lines (NILs) at development stage V3 and V5 (n = 10, x ± SD)

Isolines	Leaf area ratio, cm^2/g of plant			
	development stage – V3		development stage – V5	
	without treatment	bacterization	without treatment	bacterization
Clark	146.8 ± 5.2^a	118.7 ± 3.0^b	137.1 ± 7.3^{ac}	132.5 ± 3.4^c
L80-5879	163.7 ± 5.7^a	140.1 ± 3.8^b	181.2 ± 2.9^c	151.5 ± 3.2^d
L63-3117	159.4 ± 3.6^a	144.4 ± 5.1^b	165.6 ± 5.1^a	119.7 ± 2.7^c
L71-920	145.2 ± 4.5^a	149.2 ± 5.1^a	176.9 ± 4.2^b	171.3 ± 4.5^b
	Specific leaf area, cm^2/g of leaves			
Clark	395.2 ± 6.4^a	270.6 ± 7.1^b	339.9 ± 14.0^c	341.0 ± 7.1^c
L80-5879	396.1 ± 6.6^a	327.4 ± 9.3^b	375.5 ± 4.5^c	362.6 ± 8.8^c
L63-3117	378.9 ± 3.9^a	375.4 ± 8.9^{ba}	366.4 ± 8.1^b	278.3 ± 9.1^c
L71-920	324.5 ± 6.0^a	351.5 ± 4.5^b	391.5 ± 6.5^c	426.7 ± 6.4^d

Note: firstly, comparisons were made within each isogenic soybean line at developmental stages V3 and V5 between the bacterized and untreated (control) variants; secondly, comparisons were made within each isogenic soybean line between developmental stages V3 and V5 in the control and experimental variants; different letters indicate values that differed one from another reliably within one line of the table for each of parameters, according to the results of comparison using the Tukey test ($P < 0.05$) with Bonferroni correction.

A decrease in specific leaf area (SLA) in the V3 phase under bacterization was observed in the Clark variety and the L80-5879 line compared to the control. In the V5 phase, bacterization in the Clark variety does not lead to significant changes compared to the untreated variant. Instead, a significant decrease in specific leaf area is observed in the L80-5879 and L63-3117 lines. The L71-920 line under bacterization demonstrates a significant increase in this indicator both in the V3 and V5 phases (Table 3). Compared with the V3 phase in the control variant, a decrease in specific leaf area is observed in all lines except L71-920, which increases the SLA in V3-stage. Under bacterization, an increase in the indicator is noted in short-day lines (Clark variety and L80-5879) and the neutral-day line L71-920. Meanwhile, the neutral-day line L63-3117 shows a decrease in specific leaf area (Table 3).

The chlorophyll a and b content in the leaves of soybean. In the V3 development phase, bacterization did not lead to a significant change in chlorophyll *a* content in the leaves of the Clark variety (Fig. 1A) and the short-day line L80-5879 (Fig. 1B). However, in the neutral-day lines, bacterization had diverse effects: in the line with the dominant state of the *E3* gene L63-3117, there was a significant increase in chlorophyll a (Fig. 1C), while in the L71-920 line, with the recessive state of genes *e1-e3*, bacterization led to a decrease in chlorophyll *a* (Fig. 1D). In the V5 phase, bacterization did not result in a significant change in the pigment in the Clark variety. Instead, in the short-day line L80-5879, prior inoculation significantly increased the amount of chlorophyll *a*. In other lines with a photoperiodically neutral reaction, a significant reduction in the pigment was noted. Comparing the dynamics of chlorophyll *a* content between the V3 and V5 developmental phases, in the control variant, a decrease in the amount of pigment is observed in the L80-5879 and L71-920 lines, while under bacterization, a decrease in the amount of chlorophyll *a* is noted only in the neutral-day lines L63-3117 and L71-920.

In the V3 phase under bacterization, a decrease in chlorophyll *b* is observed only in the L71-920 line, which has recessive genes *e1-e3*, compared to the control (Fig. 1D). In all other lines, bacterization leads to a significant increase in chlorophyll *b* content (Fig. 1A–C). In the V5 phase, neutral-day lines under bacterization demonstrate a significant reduction in pigment content (Fig. 1C, 1D), while among short-day lines, only in L80-5879, is there a significant increase in chlorophyll *b* content (Fig. 1B).

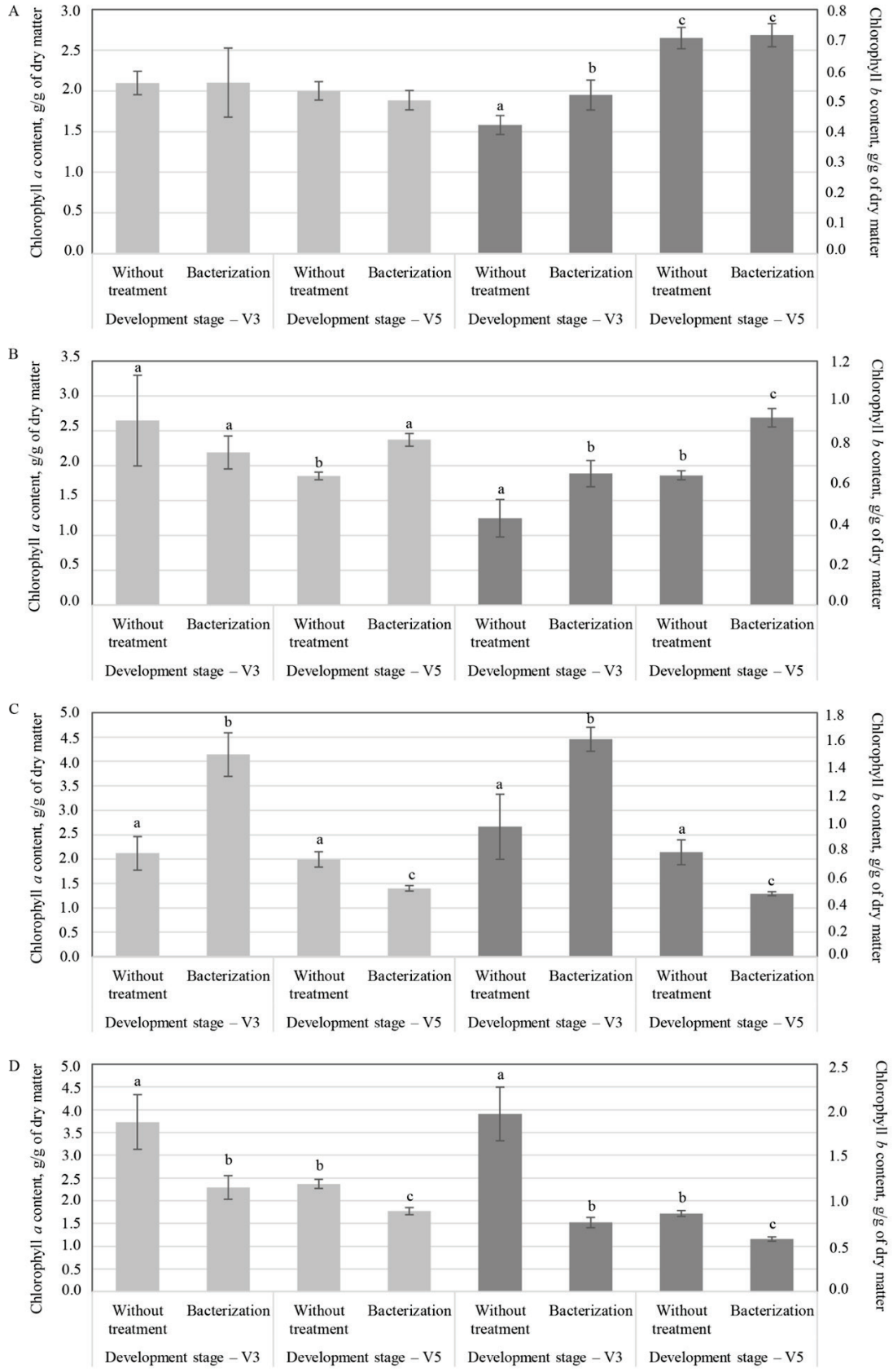

Fig. 1. The impact of bacterization on the content of chlorophyll *a* and *b* in leaves of isogenic soybean lines (NILs) with *E*-genes at different developmental stages (mg/g of dry matter, n = 10, x ± SD): Isolines: *A* – Clark variety; *B* – L80-5879; *C* – L63-3117; *D* – L71-920; light gray bar chart – content of chlorophyll *a*; dark gray bar chart – content of chlorophyll *b*; firstly, comparisons were made within each isogenic soybean line at developmental stages V3 and V5 between the bacterized and untreated (control) variants; secondly, comparisons were made within each isogenic soybean line between developmental stages V3 and V5 in the control and experimental variants; different letters indicate values that differed one from another reliably within one isolines of soybean for each of parameters, according to the results of comparison using the Tukey test ($P < 0.05$) with Bonferroni correction; if the letter indices are absent, then no significant differences were found within one isoline for each of parameters

The general trend in the dynamics of chlorophyll *b* content was observed in experimental variants. Short-day lines increase pigment content in leaves in the V5 phase, while neutral-day lines decrease it, compared to the V3 phase.

Discussion

Changes in biomass accumulation. Relative growth rate (RGR) is an integral growth indicator defining the rate of accumulation of newly formed dry biomass per unit of already developed biomass over a certain period (Hunt, 2017). The value of this parameter significantly depends on the initial plant mass indicators (Lamont et al., 2023). The short-day soybean variety Clark demonstrates the highest relative growth rate in the control variant (Table 2). Conversely, under *Bradyrhizobium japonicum* (Kirchner) 634b interaction, we observe a significantly reduced biomass accumulation rate, but simultaneously higher than in the neutral-day lines. It is worth noting that all plants were cultivated under long-day conditions (16 hours of light), explaining this response due to the plant's sensitivity to the photoperiod. Under the influence of a long photoperiod, short-day plants extend the vegetative phase, resulting in delayed flowering, thus facilitating biomass accumulation and increased morphometric parameters. At the same time, another short-day line, L80-5879, shows no significant changes in biomass accumulation rate compared to the control variant under *B. japonicum* (Kirchner) 634b interaction. According to our results, in the *B. japonicum* (Kirchner) 634b treatment variant, neutral-day lines almost do not accumulate biomass during the V3-V5 development period (Table 2). Thus, in the V3 phase under bacterial treatment, the lines L63-3117 and L71-920 formed sufficient biomass for further vegetation and transition to the generative phase of development.

Another indicator, net assimilation rate (NAR), determines the biomass accumulation per unit leaf area over a certain period. Thus, this parameter characterizes the biomass accumulation by the photosynthetic surface of the plant (Li et al., 2016). The same dependencies and trends were observed for this indicator as for the relative growth rate (Table 2). This can be explained by the fact that NAR is a component of RGR, which can be calculated as the product of net assimilation and leaf area ratio: RGR = LAR × NAR. Since NAR is a component of RGR, and considering similar trends, a correlation analysis between the indicators is not reasonable (Lamont et al., 2023). However, taking into account the similar tendencies, we can conclude that net assimilation plays a significant role in biomass accumulation, as confirmed by Li et al. (2016). The obtained results of net assimilation (Table 2) can be explained by both morpho-anatomical features of the leaves, such as different leaf surface areas or leaf structure, and the amount of chlorophylls *a* and *b*. Indicators characterizing the photosynthetic surface of the plant include the leaf area ratio and specific leaf area. The leaf area ratio shows the total photosynthetic surface per plant, while the specific leaf area is determined by the concentration of dry matter in them (i.e., the number of cells, their structure, and the photosynthetic apparatus).

Changes in accumulation apparatus. The enlargement of leaf area, and consequently, the total photosynthetic surface, enhances light absorption and is a crucial parameter in determining plant productivity. However, such an increase does not have linear correlations with biomass augmentation, as it depends on the carbon (carbohydrate) distribution among different plant organs (Weraduwage et al., 2015). Our results confirm the absence of a linear relationship between the rate of biomass accumulation and leaf area.

In the short-day soybean variety Clark, we observe no significant changes in leaf area ratio between the V3 and V5 developmental stages in the control variant, despite its having the highest relative growth rate. However, under *B. japonicum* (Kirchner) 634b interaction, a significant increase in leaf area is noted in the V5 developmental stage, while the relative growth rate decreases. This discrepancy can be explained by different carbohydrate distributions. Our earlier findings (Hlushach et al., 2023) demonstrated a reduction in the transport form of carbohydrates in leaves under bacterial treatment in both V3 and V5 phases, suggesting a flow of assimilates to the attracting centers – symbiotic nodules and meristems. Furthermore, over the V3-V5 developmental period, the control variant shows a decrease in specific leaf area, while under bacterial treatment, an increase is observed. Specifically, in the V3 phase, a significant reduction in LAR and SLA is noted in bacterial treatment compared to the control. However, in the V5 phase, no significant difference is observed between the experimental and control variants. The formula for calculating specific leaf area (SLA) reveals its dependency on the overall photosynthetic surface and leaf mass (Hunt, 2017). Thus, an increase in total leaf area leading to enhanced SLA may indicate a decrease in dry matter content in the leaves, reflecting structural changes in the organ. The dynamics of the content of photosynthetic pigments (chlorophyll *a* and *b*) in Clark cultivar leaves are also interesting. There is no significant difference in chlorophyll a content between the V3 and V5 phases, in both the control and experimental variants. Simultaneously, bacterial treatment does not result in a significant difference in chlorophyll *a* content in the V3 and V5 phases. Regarding chlorophyll *a* content, an increase is observed between the V3 and V5 phases in both the control and experimental variants. In the bacterial treatment variant, there is an increase in pigment content in the V3 phase and no significant difference in the V5 phase compared to the control. It is worth noting that chlorophyll is a pigment crucial for photosynthesis, determining the assimilatory metabolism. Two main types of chlorophyll are distinguished: *a*, as the primary chlorophyll located in the reaction centers of Photosystems II and I, and *b*, the auxiliary chlorophyll found in light-harvesting complexes (Esteban et al., 2014). Considering the overall trend in chlorophyll *b* dynamics, as presented in the results, it can be inferred that the sensitivity of the line to the photoperiod dictates the trend in changes in this pigment's quantity. The increase in chlorophyll *b* content, without altering the amount of chlorophyll *a*, indicates the plant's need for an additional energy source to support assimilatory processes. Thus, considering the relative growth rate, our results suggest rapid biomass accumulation in the Clark cultivar due to increased photosynthetic surface area and dry matter content in leaves in the control variant. Under bacterial treatment, such a fast increase in leaf mass and total leaf area is not observed. This is evidenced by the lack of dynamics in chlorophyll *a* quantity in the V3 and V5 phases in both the control and experimental variants. Therefore, assimilation processes are facilitated only through morpho-anatomical changes in leaf surface and an increase in the content of auxiliary chlorophyll *b* in light-harvesting complexes, leading to a reduction in biomass accumulation rate under bacterial treatment.

In the short-day soybean line L80-5879, when comparing the leaf area ratio between the V3 and V5 phases, an increase in LAR is observed in both the control variant and treatment with *B. japonicum* (Kirchner) 634b. However, during the V3 and V5 developmental phases under bacterial treatment, a significant reduction in leaf area ratio is noted compared to the control. Therefore, over the V3-V5 development period, there is an increase in both plant biomass and the overall photosynthetic surface. However, under *B. japonicum* (Kirchner) 634b interaction, the increase in total leaf area occurs at a slower rate. Calculated specific leaf area values in the V3 and V5 phases demonstrate a reduction in SLA under bacterial treatment compared to the untreated variant. In the control variant, SLA decreases over the V3-V5 development period, while under bacterial treatment, it increases. This suggests a relatively lower dry matter content in leaves under bacterial treatment compared to the control. Growth rate indicators show an identical rate of biomass accumulation both with and without bacterial treatment. Obviously, the reduced morpho-anatomical indicators (total leaf area, leaf weight) in the experimental variant cannot achieve identical growth rate indicators. This could be attributed to differences in carbohydrate distribution. However, in previous studies on the L80-5879 line, we demonstrated no significant difference in the content of transport forms of carbohydrates under bacterial treatment compared to the control. Therefore, we assume an increased intensity of assimilatory processes, supporting biomass accumulation in the bacterial treatment variant. A marker for enhanced assimilatory processes can be observed in the dynamics of chlorophyll *a* and *b* quantities. Under bacterial treatment, no significant changes occur in the quantity of chlorophyll *a* between the V3 and V5 developmental phases, while in the control variant, there is a substantial decrease in the amount of this pigment in the V5 phase compared to the V3 phase. Comparing the control and experimental variants, no significant difference is observed in the quantity of chlorophyll *a* in the V3 phase, but in the V5 phase, a significant decrease in chlorophyll *a* is noted in the control variant compared to the experimental. The dynamics

of chlorophyll *b* content in L80-5879 leaves differ slightly. In both the control and experimental variants, a significant increase in chlorophyll *b* occurs in the V5 developmental phase compared to the V3 phase. However, in these same phases, a significantly higher quantity of chlorophyll *b* is observed under bacterial treatment compared to the control. Thus, during the V5 developmental phase in the control variant, there is a decrease in the main chlorophyll *a* compared to the V3 phase. However, sufficient assimilatory processes are maintained through a rapid increase in the overall photosynthetic surface and an increase in auxiliary chlorophyll *b*. Meanwhile, under *B. japonicum* (Kirchner) 634b interaction, the increase in total leaf area occurs at a slower rate. The high level of assimilatory processes is supported by a constant level of chlorophyll a and an increase in chlorophyll *b* content.

In the neutral-day soybean line L63-3117, the indicator of LAR in the control variant remains significantly unchanged during the V5 developmental phase compared to the V3 phase. However, under bacterial treatment, a reduction in this indicator occurs during this period. Furthermore, in both the V3 and V5 developmental phases, the leaf area ratio is lower under interaction with *B. japonicum* (Kirchner) 634b compared to the control variant. A significant decrease in specific leaf area is observed in the V5 phase compared to the V3 phase in both the control and experimental variants. Simultaneously, no bacterial action is observed in the V3 phase, but there is a decrease in SLA in the V5 phase compared to the control. The reduction in the leaf area ratio under bacterial treatment can be explained by a decrease in the total photosynthetic surface and the absence of significant changes in the overall plant mass. This may occur due to the slower development of the 4th and 5th leaves in the bacterial treatment variant, resulting in a reduction in assimilation process intensity. Therefore, under bacterial treatment, there is a very slow biomass accumulation during the V3-V5 development period. Earlier studies on the L63-3117 line identified a decrease in the quantity of oligosaccharides under interaction with *B. japonicum* (Kirchner) 634b during the V3 and V5 developmental phases (Hlushach et al., 2023). It is worth noting that in the V3 developmental phase under bacterial treatment, the line showed the highest content of chlorophyll *a* and *b*. However, in the V5 phase, a significant decrease occurs, which could also be a marker of decreased assimilation processes. In contrast, the control variant did not have significant changes in pigment quantity during of the V3-V5 developmental phases.

In both the control and experimental variants of the neutral-day soybean line L71-920, an increase in leaf area ratio is observed during the V5 developmental phase compared to the V3 phase. Simultaneously, no significant impact of bacterial treatment on this indicator is noted in both the V3 and V5 phases compared to the control. There is a substantial increase in specific leaf area during the V3-V5 developmental period, both in the control and bacterial treatment. Additionally, under bacterial treatment, a significant increase in this indicator is observed in both the V3 and V5 phases compared to the control. Considering the almost negligible biomass accumulation, it can be concluded that certain anatomical and morphological changes occur during the V3-V5 development period. This includes an increase in the overall photosynthetic surface, both with and without bacterial treatment. Importantly, the increase in leaf surface area occurs without a corresponding increase in leaf dry mass in both the experimental and control variants (Table 2). The significantly higher SLA value in the V5 developmental phase under bacterial treatment indicates that the interaction with *B. japonicum* (Kirchner) 634b leads to a reduction in the dry matter content in leaves compared to the control variant. Structural changes in leaves impact the content of chlorophylls *a* and *b*. We identified a common trend of a significant decrease in the content of chlorophylls *a* and *b* in the leaves of the L71-920 line during the V3-V5 period, both in the control and experimental variants. Interestingly, under bacterial treatment, there is a substantial reduction in the content of photosynthetic pigments in both the V3 and V5 phases, which may contribute to the decrease in assimilation processes in this variant. This observation aligns with the reduction in the quantity of carbohydrate transport forms under bacterial treatment, as reported in previous studies.

In our study, no general trends were identified for each photoperiod sensitivity group based on the indicators, indicating the genotypic sensitivity of each line to the interaction with *B. japonicum* (Kirchner) 634b under long-day conditions. General trends were only determined for chlorophyll *b* content during the V3-V5 development period; short-day lines increased pigment content in experimental variant, while neutral-day lines decreased.

Genotype of isolines analysis. Currently, there is no direct evidence of the influence of *E1-E3* genes (on which the experimental lines differ) on the establishment of the "plant-microorganism" interaction. However, using the Phytozome database, the possibility of *E2* gene expression (dominant state present in the Clark variety) in roots and symbiotic nodules was identified. Also, the homologous *GIGANTEA* gene in *Arabidopsis thaliana* is known to regulate lateral root development (Singh, 2022), potentially impacting mineral nutrient uptake. This regulation occurs through modulation of the auxin signaling pathway, a crucial hormone in symbiotic nodule formation (Wang, 2019). Nevertheless, there are currently no proven mechanisms linking the *E2* gene to symbiotic nodule formation. The dominant state of the *E1* gene is present in the L80-5879 line. It is worth noting that *E1* and *E2* genes are functionally similar – both are repressors of *GmFT2a*. Since different results were obtained for the L80-5879 line (with the *E1* allele) and the Clark variety (with the *E2* allele), these effects cannot be associated with the repressed state of *GmFT2a*. Therefore, it is hypothesized that there are independent repression pathways of *GmFT2a* by *E1* and *E2* genes, with links that may intersect with other links in the gene network. Recent studies have shown that *E1* is also a repressor of *MADS-box* genes, including *GmMDE17* (Glyma.17G081200) and *GmMDE05* (Glyma.05G018800) (Zhai et al., 2022). Using the Phytozome database, it was determined that the expression of these genes occurs in symbiotic conditions, both in leaves and roots, as well as in symbiotic nodules. Considering that *E1* may regulate the expression of genes determining leaf development at early stages (Li et al., 2021) and the proved interactions between *E1* and *GmMDE17* and *GmMDE05*, it can be assumed that the *E1* gene's action under bacterial treatment affects biomass accumulation rate and morpho-anatomical changes, although the mechanisms of this influence are currently unknown. The L63-3117 line has a dominant state of the phytochrome *A3* gene (*E3* is *GmPHYA3*) together with its homolog, the *E4* gene encoding phytochrome *A2* (*GmPHYA2*). According to the literature, this gene combination determines reduced sensitivity to photoperiod in this line (Xu et al., 2013). When analyzing these genes using the Phytozome database, the expression of *E3* and *E4* genes in leaves, roots under symbiotic conditions, and in symbiotic nodules was identified, which may impact assimilation processes. Some scientists, starting from the 20th century, noted the influence of phytochromes and the ratio of red to far-red light on the formation of nodules (Lie, 1971; Suzuki et al., 2011). However, detailed mechanisms of such a hypothetical influence are still unknown today.

Conclusion

The research was conducted under the climatic conditions of the Kharkiv region, Ukraine (50° north latitude) with the influence of natural long-day conditions (16 hours). Under these conditions, the impact of *B. japonicum* 634b inoculation on biomass accumulation speed and changes in the assimilation apparatus was observed. The identified influence depends on the allelic state of the *E1-E3* genes in the genotype of lines. In other words, based on the obtained data, we assume that the soybean reaction depends on the interaction of photoperiod sensitivity genes and rhizobia. Thus, the recessive state of all mentioned genes, along with the presence of the *E3* gene (in the recessive state of all others), is associated with almost zero biomass accumulation in plants interacting with *B. japonicum* 634b during the V3-V5 developmental phases. This indicates that by the V3 stage, these plants have accumulated sufficient biomass to transition to the generative phase of development. These lines are related to neutral-day plants. The Clark variety with the *E2* gene in the dominant state also reduces the biomass accumulation rate during inoculation in the studied developmental phases. However, this reduction is not as strong as in neutral-day lines. Inoculation of the line L80-5879 with the dominant state of the *E1* gene does not lead to a decrease in biomass accumulation rate during the V3-V5 development period. Different gene states also show the impact of inoculation on changes in the assimilation apparatus, which can affect the level of assimilation processes. Thus, inoculation in the V3 phase leads to a significant decrease of LAR in all lines, except for

the line in which all genes are in a recessive state, where no significant effect is observed. In the V5 development phase, inoculation leads to a decrease in LAR only in lines with dominant states of the *E1* (L80-5879) and *E3* (L63-3117) genes, while in others, no significant changes are observed. The decrease in the intensity of assimilation processes during inoculation is also indicated by differences in the amount of chlorophyll *a* and *b*. In neutral-day lines, inoculation leads to a significant reduction in photosynthetic pigments during the V3 and V5 development phases.

Therefore, similar studies are crucial in breeding work to find high-yielding soy varieties for cultivation in specific regions of the world. It is necessary to consider their genotype, which determines sensitivity to photoperiod and the plant's reaction to the application of bacterial fertilizers based on different allelic states of the *E*-series genes.

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The authors declare no conflict of interest.

References

Chen, Z., Tao, X., Khan, A., Tan, D. K. Y., & Luo, H. (2018). Biomass accumulation, photosynthetic traits and root development of cotton as affected by irrigation and nitrogen-fertilization. Frontiers in Plant Science, 9, 173.

Esteban, R., Barrutia, O., Artetxe, U., Fernández-Marín, B., Hernández, A., & García-Plazaola, J. I. (2014). Internal and external factors affecting photosynthetic pigment composition in plants: A meta-analytical approach. New Phytologist, 206(1), 268–280.

Fagorzi, C., Bacci, G., Huang, R., Cangioli, L., Checcucci, A., Fini, M., Perrin, E., Natali, C., diCenzo, G. C., & Mengoni, A. (2021). Nonadditive transcriptomic signatures of genotype-by-genotype interactions during the initiation of plant-*Rhizobium* symbiosis. MSystems, 6(1), e00974-20.

Hlushach, D., Zhmurko, V., & Avksentieva, O. (2023). Vplyv henotypu ta bakteryzatsiji na rist, rozvytok ta vmist rozchynnykh vuhlevodiv u izohennykh za E-henamy liniji soji kulturnoji [Influence of genotype and bacterization on growth, development, and soluble carbohydrate content in soybean E-genes isogenic lines]. The Journal of V. N. Karazin Kharkiv National University, Biology, 40, 59–70 (in Ukrainian).

Hu, Y., Chen, Y., Yang, X., Deng, L., & Lu, X. (2023). Enhancing soybean yield: The synergy of sulfur and rhizobia inoculation. Plants, 12(22), 3911.

Hunt, R. (2017). Growth analysis, individual plants. In: Thomas, B., Murray, B. G., & Murphy, D. J. (Eds.). Encyclopedia of applied plant sciences. 2nd ed. Academic Press, Oxford. Pp. 421–429.

Keller, B., Zimmermann, L., Rascher, U., Matsubara, S., Steier, A., & Muller, O. (2021). Toward predicting photosynthetic efficiency and biomass gain in crop genotypes over a field season. Plant Physiology, 188(1), 301–317.

Kong, F., Nan, H., Cao, D., Li, Y., Wu, F., Wang, J., Lu, S., Yuan, X., Cober, E. R., Abe, J., & Liu, B. (2014). A new dominant gene E9 conditions early flowering and maturity in soybean. Crop Science, 54(6), 2529–2535.

Kozar, S. F., Skoryk, V. V., Usmanova, T. O., & Yevtushenko, T. A. (2012). Vplyv stabilizatoriv na rist, zhyttiezdatnist i funktsionalnu aktyvnist *Bradyrhizobium japonicum* [The influence of stabilizators on the growth, viability and functional activity of *Bradyrhizobium japonicum*]. Agricultural Microbiology, 15, 58–70 (in Ukrainian).

Lamont, B. B., Williams, M. R., & He, T. (2023). Relative growth rate (RGR) and other confounded variables: Mathematical problems and biological solutions. Annals of Botany, 131(4), 555–568.

Li, X., Schmid, B., Wang, F., & Paine, C. E. T. (2016). Net assimilation rate determines the growth rates of 14 species of subtropical forest trees. PLoS One, 11(3), e0150644.

Li, Y., Hou, Z., Li, W., Li, H., Lu, S., Gan, Z., Du, H., Li, T., Zhang, Y., Kong, F., Cheng, Y., He, M., Ma, L., Liao, C., Li, Y., Dong, L., Liu, B., & Cheng, Q. (2021). The legume-specific transcription factor E1 controls leaf morphology in soybean. BMC Plant Biology, 21(1), 531.

Lichtenthaler, H. K., & Wellburn, A. R. (1983). Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. Biochemical Society Transactions, 11(5), 591–592.

Lie, T. A. (1971). Nodulation of rooted leaves in leguminous plants. Plant and Soil, 34(3), 663–673.

Liu, P.-C., Peacock, W. J., Wang, L., Furbank, R., Larkum, A., & Dennis, E. S. (2020). Leaf growth in early development is key to biomass heterosis in *Arabidopsis*. Journal of Experimental Botany, 71(8), 2439–2450.

Melnykova, N. M., & Kots, S. Y. (2019). Vplyv stabilizatoriv na rist, zhyttiezdatnist' i funktsional'nu aktyvnist' *Bradyrhizobium japonicum* 634b [Effect of goat's rhizobia on the formation and functioning of the soybean – *Bradyrhizobium japonicum* 634b symbiosis]. Agricultural Microbiology, 29, 29–36 (in Ukrainian).

Mishra, P., & Panigrahi, K. C. (2015). GIGANTEA – an emerging story. Frontiers in Plant Science, 6, 8.

Osnato, M., Cota, I., Nebhnani, P., Cereijo, U., & Pelaz, S. (2022). Photoperiod control of plant growth: Flowering time genes beyond flowering. Frontiers in Plant Science, 12, 805635.

Pagano, M. C., & Miransari, M. (2016). The importance of soybean production worldwide. In: Miransari, M. (Ed.). Abiotic and biotic stresses in soybean production. Academic Press, Oxford. Pp. 1–26.

Rani, R., Raza, G., Ashfaq, H., Rizwan, M., Shimelis, H., Tung, M. H., & Arif, M. (2023). Analysis of genotype × environment interactions for agronomic traits of soybean (*Glycine max* [L.] Merr.) using association mapping. Frontiers in Genetics, 13, 1090994.

Roeber, V. M., Schmülling, T., & Cortleven, A. (2022). The photoperiod: Handling and causing stress in plants. Frontiers in Plant Science, 12, 781988.

Singh, A. (2022). GIGANTEA regulates lateral root formation by modulating auxin signaling in *Arabidopsis thaliana*. Plant Signaling and Behavior, 17(1), 2096780.

Sun, H., Jia, Z., Cao, D., Jiang, B., Wu, C., Hou, W., Liu, Y., Fei, Z., Zhao, D., & Han, T. (2011). GmFT2a, a soybean homolog of Flowering locus T, is involved in flowering transition and maintenance. PLoS One, 6(12), e29238.

Suzuki, A., Suriyagoda, L., Shigeyama, T., Tominaga, A., Sasaki, M., Hiratsuka, Y., Yoshinaga, A., Arima, S., Agarie, S., Sakai, T., Inada, S., Jikumaru, Y., Kamiya, Y., Uchiumi, T., Abe, M., Hashiguchi, M., Akashi, R., Sato, S., Kaneko, T., Tabata, S., & Hirsch, A. M. (2011). *Lotus japonicus* nodulation is photomorphogenetically controlled by sensing the red/far red (R/FR) ratio through jasmonic acid (JA) signaling. Proceedings of the National Academy of Sciences, 108(40), 16837–16842.

Taniguchi, T., Murayama, N., Ario, N., Nakagawa, A. C. S., Tanaka, S., Tomoita, Y., Hasegawa, M., Hamaoka, N., Iwaya-Inoue, M., & Ishibashi, Y. (2020). Photoperiod sensing of leaf regulates pod setting in soybean (*Glycine max* (L.) Merr.). Plant Production Science, 23(3), 360–365.

Tasma, I. M., & Shoemaker, R. C. (2003). Mapping flowering time gene homologs in soybean and their association with maturity (E) loci. Crop Science, 43(1), 319–328.

Tripathi, S., Hoang, Q. T. N., Han, Y. J., & Kim, J. I. (2019). Regulation of photomorphogenic development by plant phytochromes. International Journal of Molecular Sciences, 20(24), 6165.

Tsubokura, Y., Watanabe, S., Xia, Z., Kanamori, H., Yamagata, H., Kaga, A., Katayose, Y., Abe, J., Ishimoto, M., & Harada, K. (2013). Natural variation in the genes responsible for maturity loci E1, E2, E3 and E4 in soybean. Annals of Botany, 113(3), 429–441.

Waese, J., Fan, J., Pasha, A., Yu, H., Fucile, G., Shi, R., Cumming, M., Kelley, L. A., Sternberg, M. J., Krishnakumar, V., Ferlanti, E., Miller, J., Town, C., Stuerzlinger, W., & Provart, N. J. (2017). ePlant: Visualizing and exploring multiple levels of data for hypothesis generation in plant biology. The Plant Cell, 29(8), 1806–1821.

Wang, T., Guo, J., Peng, Y., Lyu, X., Liu, B., Sun, S., & Wang, X. (2021). Light-induced mobile factors from shoots regulate rhizobium-triggered soybean root nodulation. Science, 374(6563), 65–71.

Wang, W., Cao, Y., Sheng, K., Chen, J., Zhu, S., & Zhao, T. (2023). GhFP positively regulates chlorophyll content and seedling biomass in upland cotton. Industrial Crops and Products, 204, 117388.

Wang, Y., Yang, W., Zuo, Y., Zhu, L., Hastwell, A. H., Chen, L., Tian, Y., Su, C., Ferguson, B. J., & Li, X. (2019). GmYUC2a mediates auxin biosynthesis during root development and nodulation in soybean. Journal of Experimental Botany, 70(12), 3165–3176.

Wani, S. P., Gopalakrishnan, S. (2019). Plant growth-promoting microbes for sustainable agriculture. In: Sayyed, R., Reddy, M., & Antonius, S. (Eds.). Plant growth promoting rhizobacteria (PGPR): Prospects for sustainable agriculture. Springer, Singapore. Pp. 19–45.

Weraduwage, S. M., Chen, J., Anozie, F. C., Morales, A., Weise, S. E., & Sharkey, T. D. (2015). The relationship between leaf area growth and biomass accumulation in *Arabidopsis thaliana*. Frontiers in Plant Science, 6, 167.

Xia, Z., Watanabe, S., Yamada, T., Tsubokura, Y., Nakashima, H., Zhai, H., Anai, T., Sato, S., Yamazaki, T., Lü, S., Wu, H., Tabata, S., & Harada, K. (2012). Positional cloning and characterization reveal the molecular basis for soybean maturity locus E1 that regulates photoperiodic flowering. Proceedings of the National Academy of Sciences, 109(32), e2155-e2164.

Xu, M., Xu, Z., Liu, B., Kong, F., Tsubokura, Y., Watanabe, S., Xia, Z., Harada, K., Kanazawa, A., Yamada, T., & Abe, J. (2013). Genetic variation in four maturity genes affects photoperiod insensitivity and PHYA-regulated post-flowering responses of soybean. BMC Plant Biology, 13(1), 91.

Yun, J., Wang, C., Zhang, F., Chen, L., Sun, Z., Cai, Y., Luo, Y., Liao, J., Wang, Y., Cha, Y., Zhang, X., Ren, Y., Wu, J., Hasegawa, P. M., Tian, C., Su, H., Fergu-

son, B. J., Gresshoff, P. M., Hou, W., Han, T., & Li, X. (2023). A nitrogen fixing symbiosis-specific pathway required for legume flowering. Science Advances, 9(2), eade1150.
Zhai, H., Wan, Z., Jiao, S., Zhou, J., Xu, K., Nan, H., Liu, Y., Xiong, S., Fan, R., Zhu, J., Jiang, W., Pang, T., Luo, X., Wu, H., Yang, G., Bai, X., Kong, F., & Xia, Z. (2022). GmMDE genes bridge the maturity gene E1 and florigens in photoperiodic regulation of flowering in soybean. Plant Physiology, 189(2), 1021–1036.
Zhang, X., Wu, T., Wen, H., Song, W., Xu, C., Han, T., Sun, S., & Wu, C. (2021). Allelic variation of soybean maturity genes E1–E4 in the Huang-Huai-Hai River Valley and the Northwest China. Agriculture, 11(6), 478.
Zhao, C., Takeshima, R., Zhu, J., Xu, M., Sato, M., Watanabe, S., Kanazawa, A., Liu, B., Kong, F., Yamada, T., & Abe, J. (2016). A recessive allele for delayed flowering at the soybean maturity locus E9 is a leaky allele of FT2a, a Flowering locus T ortholog. BMC Plant Biology, 16(1), 20.
Zhao, X., Cao, D., Huang, Z., Wang, J., Lu, S., Xu, Y., Liu, B., Kong, F., & Yuan, X. (2015). Dual functions of GmTOE4a in the regulation of photoperiod-mediated flowering and plant morphology in soybean. Plant Molecular Biology, 88(4–5), 343–355.
Zheng, N., Guo, Y., Wang, S., Zhang, H., Wang, L., Gao, Y., Xu, M., Wang, W., Liu, W., & Yang, W. (2023). Identification of E1-E4 allele combinations and ecological adaptability of soybean varieties from different geographical origins in China. Frontiers in Plant Science, 14, 1222755.
Zimmer, G., Miller, M. J., Steketee, C. J., Jackson, S. A., de Tunes, L. V. M., & Li, Z. (2021). Genetic control and allele variation among soybean maturity groups 000 through IX. The Plant Genome, 14(3), e20146.