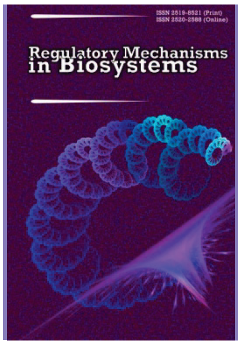

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Genetic variation in four maturity genes and photoperiod insensitivity effects on the yield components and on the growth duration periods of soybean

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Soybean (*Glycine max* (L.) Merr.) is a typical short-day and thermophilic crop. Absence of or low sensitivity to photoperiod is necessary for short-day crops to adapt to high latitudes. Photoperiod insensitivity in soybeans is controlled by two genetic systems and involves three important maturity genes: *E1*, a repressor for two soybean orthologs of Arabidopsis FLOWERING LOCUS T, and *E3* and *E4*, which are phytochrome A genes. The aim of this work was to investigate the role of four maturity genes (*E1* through *E4*) on the yield components, seed quality and on phasic development of near isogenic by E genes lines of soybean: short-day (SD) lines with genotype *e1E2E3E4e5E7*, *e1E2E3e4e5E7*, *E1e2e3E4e5E7* and photoperiodic insensitive (PPI) lines with genotype *e1e2E3E4e5E7*, *e1e2e3E4e5E7* under a long photoperiod (the natural day length of 50 latitude) conditions and short day conditions. The results of the study showed that soybean development processes under conditions of different day lengths depend on the dominant/recessive state of the main maturity genes. In addition, the response to the photoperiod depends on certain combinations of genes. SD lines began flowering on average 16.9% later under the conditions of a natural long photoperiod. Dominant alleles of genes *E1* and *E3* extended the pre- and post-flowering phases under conditions of exposure to long and short photoperiods. The dominant allele of the *E1* gene delayed the onset of flowering by an average of 26.9%, and the period of full maturity by 39.8% compared to the recessive *e1*. The dominant allele of the *E3* gene, compared to the recessive *e3*, lengthened the transition to flowering by an average of 16.1%, and the period of full ripeness by 27.1%. The dominant allele of the *E2* gene lengthened the duration of the vegetative phase by 20% under the conditions of a long photoperiod. No significant influence of the dominant *E4* allele on the duration of the vegetative and generative phases of soybean development was found in our study. PPI lines begin flowering under the conditions of a long and short photoperiod at the same time, but the phases of flowering and full seed maturity in the line with genotype *e1e2e3E4e5E7* occurred earlier, due to the loss of the photoperiod sensitivity of the *E3* gene. PPI line with genotype *e1e2e3E4e5E7* proved to be the most insensitive line to the effect of different photoperiod durations among the studied lines. It was shown that the dominant alleles of *E1–E4* maturity genes reduced the parameters of seed weight per plant and the weight of 1000 seeds under the conditions of a natural long photoperiod in comparison with recessive alleles of these genes. The maximum weight of seeds per plant and the weight of 1000 seeds were recorded in the PPI line with genotype *e1e2e3E4e5E7*. It should be noted that the dominant alleles *E1* and *E3* increased yield under conditions of a short photoperiod. Maturity genes had different effects on the biochemical composition of seeds. It was shown that soybean lines with dominant *E1*, *E2* and *E4* genes showed a higher content of starch and a lower content of total nitrogen and oil in seeds under natural photoperiod conditions compared to lines with recessive alleles of these genes. The dominant *E3* allele reduced the oil content and did not affect the starch and total nitrogen content of seeds under long day conditions compared to the recessive *e3* allele. The analysis of the effect of photoperiod on the timing of phenophases, yield structure indicators and biochemical composition of seeds in soybean plants with different sensitivity to photoperiod showed that the PPI line with the genotype *e1e2e3E4e5E7* was the most adapted to the natural conditions of 50 degrees latitude. The PPI line with the genotype *e1e2e3E4e5E7* was characterized by the shortest phases of days from sowing to flowering and full maturity. As a result, this line had the shortest growing season without reducing the yield and seed quality. Clearly, photoperiod had strong effects on all stages of plant reproduction and often acted indirectly, as shown by delayed responses expressed in later phases of development. The obtained results can be useful for the selection of soybean cultivars adapted to the climatic conditions of cultivation of Kharkiv region.

Keywords: *Glycine max* (L.) Merr.; isogenic lines; days from sowing to flowering; days from flowering to maturity; short-day and photoperiodic insensitive lines.

Introduction

Photoperiod sensitivity is an important trait that enables crops to adapt to diverse latitudinal environments. In particular, absence of or low sensitivity to photoperiod is necessary for short-day crops, such as soybean, to adapt to high latitudes (Xu et al., 2013). Photoperiod and temperature, affect flowering and the development of soybeans, especially, affect the adaptability of soybean varieties to geographic regions, which limits the extension of varieties to broad areas (Song et al., 2016; Wang et al., 2022). The natural (long) photoperiod extends the germination-flowering period, and the reaction of soybean varieties to the photoperiod before flowering is the basis for their distribution into different maturity groups. A long photoperiod also increases the duration of post-flowering phases. The fact that soybean yield is mainly determined during post-flowering phases highlights the importance of post-flowering photoperiodic response in the complex process of determining soybean yield (Nico et al., 2015; Ort et al., 2022). In experiments with indeterminate soybeans of the middle maturity group, the extended photoperiod increases the duration of reproductive phases, the number of nodes, and the number of pods formed on nodes (Kantolic et al., 2013).

Twelve genes, which influence time from planting to first flower, have been identified. These loci were characterized at the phenotypic and genotypic levels in soybean *Glycine max* (L.) Merr. to date: *E1* and *E2*, *E3*, *E4*, *E5*, *E6*, *E7*, *E8*, *E9*, *E10*, *E11* and *J* (Zhang et al., 2020). *E1* is a unique transcription factor in leguminous plants and its expression is strongly repressed in SD, whereas it is up-regulated under LD conditions (Xia et al., 2012). *E2* has been identified as a homologue (GmGIa) of the Arabidopsis GIGANTE (GI) gene (Watanabe et al., 2011). GI is a circadian clock-controlled gene that acts upstream of CONSTANS (CO) and FT in *Arabidopsis*. There was not much difference in the effects of the *E2* allele on flowering time at two different geographic locations (43 °N and 36 °N, in Japan), indicating that *E2* may associate with the geographic adaptation of soybean (Tsubokura et al., 2014). *E3* and *E4* have been identified as phytochrome A genes (Langewisch et al., 2017; Kumawat et al., 2019; Lui et al., 2022). Dominant alleles *E1*, *E2*, *E3*, *E4*, *E5*, *E7* and *E8* delay the transition to the flowering phase, and dominant alleles of genes *E6*, *E9* and *J* prolong it in response to a natural (long) photoperiod (Kumawat et al., 2019; Kumagai et al., 2020). Two phytochrome A genes, namely *E3* and *E4*, regulate the response of pod filling to photoperiod duration (Xu et al., 2013; Liu et al., 2022). This result underscored the importance of the *E* genes in the adaptation of soybean lines to specific climates and highlighted the requirement for a thorough understanding of their function in controlling flowering (Cober et al., 2014; Jiang et al., 2014; Liu et al., 2017). Seed development is an important process for plant propagation, as well as for determining the yield of grain crops (Ali et al., 2022). It has been shown that assimilate supply is a key factor controlling flower and pod development, and assimilate supply during the initial stage of seed development affects the subsequent potential growth rate of the seed during the filling phase. In response to a low supply of assimilates, plants regulate the number of flowers/beans and the ratio of beans/seeds (Ali et al., 2022). Photoperiod affects many aspects of soybean growth and development, such as bean setting, seeding, shoot and root growth, stress responses, rates of transition to flowering, seed maturation, yield, quantity and quality of seeds.

The main components of soybean seeds are protein (37%), carbohydrates (34%), oil (18%), 13% moisture and ash (5%). Most of the carbohydrates in soybeans are insoluble polysaccharides, including pectin, cellulose, hemicellulose, and starch (Poeta et al., 2014). Soluble carbohydrates are represented by monosaccharides (glucose and fructose), disaccharides (sucrose) and oligosaccharides (raffinose and stachyose). Soybean seeds contain 9–12% of total soluble carbohydrates, including 4–5% sucrose, 1–2% raffinose, and 3.5–4.5% stachyose (Bueno et al., 2018). Insoluble polysaccharides include 20–30% non-starch polysaccharides and 1% starch (Dhungana et al., 2017). Multiple factors affect soybean seed composition and yield but environmental conditions are known to modify protein and oil concentrations by roughly 20%, these factors dominating the variation in soybean seed composition (Poeta et al., 2014; Saryoko et al., 2017; Assefa et al., 2019). Assefa et al., have shown that the maturity group differences in seed composition were not significant, but a declining trend in oil and an increase in protein concentrations with increasing soybean maturity group were observed in southern latitudes (Assefa et al., 2019). Depending on the photoperiodic reaction of the plant and the length of the daylight hours, the processes of accumulation and outflow of metabolites differ. Greater accumulation and more complete outflow of assimilation products at a favourable day length causes a greater influx of them to growth points, accelerating the sequential formation and growth of vegetative organs and the transition to the formation of generative organs, which is expressed in earlier flowering of plants. In conditions of unfavourable day length, insufficient flow of assimilation products to growth points with a lower intensity of their accumulation and outflow leads to later formation of generative organs (Hider & Zhmurko, 2020). It has also been shown that in the field, the final weight of the seed can reach 68% to 85% of its potential, depending on environmental conditions (Chiluwal et al., 2021). The starch content of the seed is a decisive factor affecting the quality of soybean food products. The variation of starch content in seeds affects the composition of the main components of soybean oil and protein (Piper & Boote, 1999).

In view of the above, the purpose of our research was to study the effect of soybean photoperiod insensitivity on flowering time, seed composition, yield components and the length of growth periods in the conditions of Kharkiv region (50°00′21″ N 36°13′45″ E), Ukraine, of spring sowing under a natural (long) photoperiod (16 hours) and an artificial short day (9 hours). Here, attention has been focused on photoperiodic effects on the duration of all phases of development for near isogenic by *E* genes lines of soybean (*Glycine max* (L.) Merr.): of the cultivar “Clark” with different combinations of alleles.

The current findings improve our understanding of the influence of genetic diversity in pre-flowering photoperiod insensitivity and mechanisms of post-flowering photoperiod the formation of the yield of high-quality soybean seeds. From a practical point of view, the research will provide an opportunity to understand which combination of alleles of *E* genes will give a high and stable soybean yield in the conditions of Kharkiv region.

Materials and methods

The objects of the study were near isogenic by *E* genes lines of soybean (*Glycine max* (L.) Merr.): of the cultivar “Clark” with different combinations of alleles. In the dominant state, these genes cause a short-day response, and in the recessive state – photoperiodic insensitive. The studies used the short-day (SD) the cultivar “Clark” with genotype *e1E2E3E4e5E7*, lines L63-3016 with genotype *e1E2E3e4e5E7* and L80-5879 – *E1e2e3E4e5E7*, and photoperiodic insensitive (PPI) lines L63-3117 with genotype *e1e2E3E4e5E7*, L71-920 – *e1e2e3E4e5E7*, which were provided by the National Center for Plant Genetic Resources of Ukraine.

Scheme of the study. Field experiments were conducted from May to November at the research fields of the Department of Physiology and Biochemistry of Plants and Microorganisms of V. N. Karazin Kharkiv National University during the growing season in 2018–2019 and 2021. The seeds were sowed in the soil at the beginning of May. Plants were grown on plots of 1 m^2 in triplicate for each variant of the experiment. Until the phase of the third true leaf, plants were grown under natural long day (about 16 hours at the latitude of Kharkov – 50º N), then half of the plants were exposed to short day (9 hours) by covering with a light-proof material from 5:00 p.m. to 9:00 a.m. Exposure to a short photoperiod was carried out for 14 days. After this the plants were grown again under long day conditions until the end of the growing season. The second part of the plants during the entire growing season were grown under natural day conditions. The research scheme is presented in the diagram (Fig. 1).

Determination of morphometric parameters of soybeans. We defined the following phenophases: R1 the beginning of flowering (the date when 50% of the plants had at least one flower in the node of the main stem), R3 the beginning of the formation of beans (the date when 50% of the plants had at least one bean 5 mm long on one of the four upper nodes on the main stem with fully developed leaves), R5 onset of seed set (the date when 50% of the plants had at least one 10 mm long pod on one of the four uppermost nodes on the main stem with fully developed leaves), R7 onset of ripening (the date when 50 % of plants had at least one mature bean on the main stem) and R8 full maturity (date when plants had mature beans on the main shoot) (Kumagai et al., 2020).

Variants of the experiment: group I – SD line *e1E2E3E4e5E7* under long (16 h) photoperiod, group II – SD line *e1E2E3e4e5E7* under long (16 h) photoperiod, group III – SD line *E1e2e3E4e5E7* under long (16 h) photoperiod, IV – PPI line *e1e2E3E4e5E7* under long (16 h) photoperiod, group V – PPI line *e1e2e3E4e5E7* under long (16 h) photoperiod, group VI – SD line *e1E2E3E4e5E7* under short (9 h) photoperiod, group VII – SD line *e1E2E3e4e5E7* under short (9 h) photoperiod, group VIII – SD line *E1e2e3E4e5E7* under short (9 h) photoperiod, group IX – PPI line *e1e2E3E4e5E7* under short (9 h) photoperiod, group X – PPI line *e1e2e3E4e5E7* under short (9 h) photoperiod.

The indicators of the production process were the number of beans, the number and weight of grains per plant, and the weight of 1,000 grains, which were determined at the stage of full seed maturity.

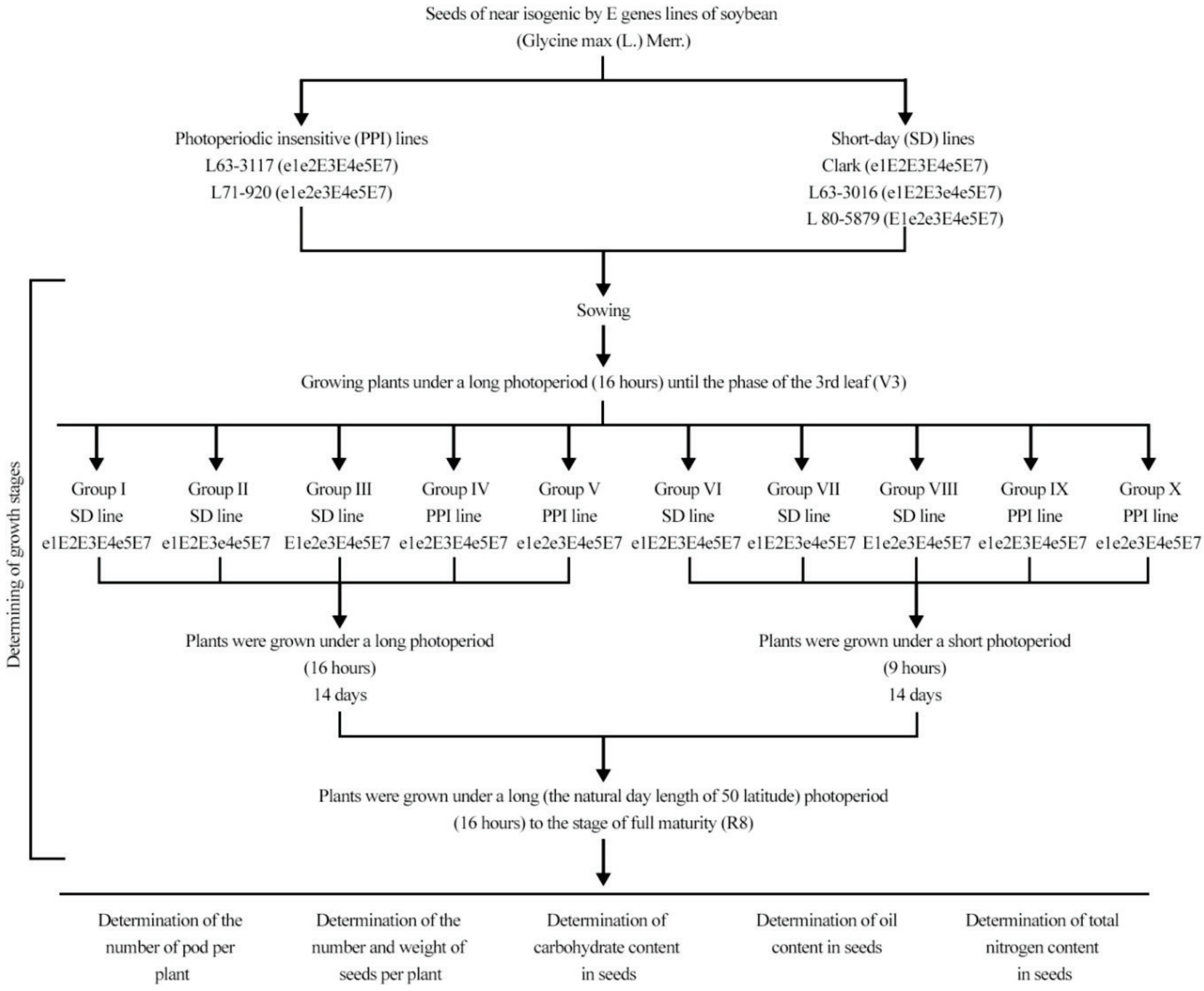

Fig. 1. The scheme of the research

Determination of carbohydrates, total nitrogen and oil. Determination of reducing sugars in soybean seeds was carried out according to the micromethod of Shvetsov-Lukyanenko for the reduction of potassium ferricyanide in an alkaline medium with iron oxide (Yermakov et al., 1987). Starch content in seeds determined by the Yastrembovich and Kalinin method (Yermakov et al., 1987). Determination of total nitrogen was performed by the Kjeldahl method (Yermakov et al., 1987). Determination of oil was carried out by the weight of the fat-free residue in the Rushkovsky modification (Yermakov et al., 1987).

The data were statistically analysed using Statistica 7.0 software (StatSoft Inc., 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

It was established that determination of the duration of the seedling-flowering period in soybean lines isogenic for *E* genes showed that the state of the dominant/recessive loci in the genotype of isogenic lines determines the rate of transition of plants to flowering (Table 1).

Indicators of the timing of the transition to flowering were used to confirm the photoperiodic response of plants to the duration of the photoperiod. Thus, the lines that had a sufficient difference between the time of transition from germination to flowering are classified as short-day lines, namely the cultivar Clark – the difference was equal to 9.2 days, line L63-3016 – 11.1 days, and line L80-5879 – 8.5 days. Under the short-day conditions of cultivation, these short-day lines went to flowering earlier than under the long day conditions. The indicators of the relationship to the natural (long) photoperiod were the highest in the lines L80-5879 and the cultivar Clark and were equal to 85.5% and 83.6%, they were slightly lower in the line L63-3016 – 80.0% from indicators under the natural (long) photoperiod. The lines in which the difference in the time of transition to flowering under the conditions of long and short photoperiod was less than two days were attributed to plants with a photoperiodically neutral reaction. The lines L63-3117 and L71-920 were classified as such lines, the difference in the time of transition to flowering was 1.1 and 1.0 day. The presence of the dominant *E3* allele in the line L63-3117 was manifested in the lengthening of the transition to flowering in plants both under short and long photoperiods.

Under natural long photoperiod conditions, the short-day soybean lines started flowering almost simultaneously at 55 days from germination. Under artificial short photoperiod conditions, the short-day soybean lines started flowering almost simultaneously at 44–47 days from germination. Thus, under the conditions of a short photoperiod, short-day soybean lines moved to the generative phase of development earlier in comparison with the natural long photoperiod. For all SD lines, the days to flowering were all significantly different from 9 to 16 h. With the lengthening of the daylength from 9 to 16 h, the average days from emergence to flowering of soybean cultivars from different SD lines were prolonged at different levels (Table 1). Plants of the PPI line L63-3117 entered the generative phase at day 43 under the short day conditions, and at day 44 under the conditions of the natural long photoperiod. Plants of the PPI line L71-920 started flowering under long and short photoperiods at the same time (36–37 days).

Lines with dominant *E1* or *E2E3* alleles (lines with the genotype *e1E2E3E4e5E7*, *e1E2E3e4e5E7*, *E1e2e3E4e5E7*) started flowering significantly later than lines with recessive alleles of these genes (lines with genotype *e1e2E3E4e5E7*, *e1e2e3E4e5E7*). According to the degree of sensitivity to the length of the photoperiod, the investigated soybean lines can be divided as follows *e1E2E3e4* ≥ *e1E2E3E4* > *E1e2e3E4* > *e1e2E3E4* > *e1e2e3E4* (all the investigated lines have the same *e5E7* alleles).

Table 1
The effect of photoperiod duration on the seedling-flowering period of near isogenic by *E* genes lines of soybean (*Glycine max* (L.) Merr.), 2018–2019, 2021, field experiment (n = 3 x 30, x ± SD)

Isolines of soybean, genotype	Photoperiod, h	Days from sowing to flowering	The difference, day	Percentage from a 16-hour day, %	Photoperiodic reaction
Clark – *e1E2E3E4e5E7*	16	55.2 ± 1.2^{ace}	9.2	83.6	short-day (SD) line
	9	46.4 ± 2.0^{bdfh}			
L63-3016 line – *e1E2E3e4e5E7*	16	55.6 ± 1.2^{cae}	11.1	80.0	short-day (SD) line
	9	44.5 ± 2.1^{dbfh}			
L80-5879 line – *E1e2e3E4e5E7*	16	55.2 ± 0.7^{eac}	8.5	85.5	short-day (SD) line
	9	47.1 ± 0.7^{fbd}			
L63-3117 line –*e1e2E3E4e5E7*	16	44.7 ± 1.7^{gh}	1.1	97.7	photoperiodic insensitive (PPI) line
	9	43.3 ± 2.1^{hgbd}			
L71-920 line –*e1e2e3E4e5E7*	16	37.2 ± 2.2^{ij}	1.0	97.3	photoperiodic insensitive (PPI) line
	9	36.4 ± 1.6^{ji}			

Note: firstly, comparisons were made within the isogenic line of soybean between long (16 h) and short (9 h) photoperiods; secondly, comparisons were made between the isogenic lines of soybean within long (16 h) and short (9 h) photoperiods, means in each column followed by different letters are significantly different one from another on the results of comparison using the Tukey test (P < 0.05) with Bonferroni correction: *a* – SD line *e1E2E3E4e5E7* under long (16 h) photoperiod, *b* – SD line *e1E2E3E4e5E7* under short (9 h) photoperiod, *c* – SD line *e1E2E3e4e5E7* under long (16 h) photoperiod, *d* – SD line *e1E2E3e4e5E7* under short (9 h) photoperiod, *e* – SD line *E1e2e3E4e5E7* under long (16 h) photoperiod, *f* – SD line *E1e2e3E4e5E7* under short (9 h) photoperiod, *g* – PPI line *e1e2E3E4e5E7* under long (16 h) photoperiod, *h* – PPI line *e1e2E3E4e5E7* under short (9 h) photoperiod, *i* – PPI line *e1e2e3E4e5E7* under long (16 h) photoperiod, *j* – PPI line *e1e2e3E4e5E7* under short (9 h) photoperiod.

The reaction of plants to the duration of the photoperiod affected not only the indicators of the period of transition of plants to flowering, but also the duration of other phases of the development of soybean plants. Figure 2 shows that the duration of the photoperiod affects the timing of the transition of soybeans to flowering, in addition, this factor also affected the passage of other phases of soybean development. Thus, plants of short-day lines under the conditions of the natural photoperiod were characterized by a long R1–R3 phase of flowering, in contrast to plants of this group under a short photoperiod. In the photoperiodic insensitive line L63-3117, the duration of the R1–R3 phase was 58% longer under the LD conditions, and in the photoperiodic insensitive line L71-920, the duration of the flowering phase was almost the same in both cases.

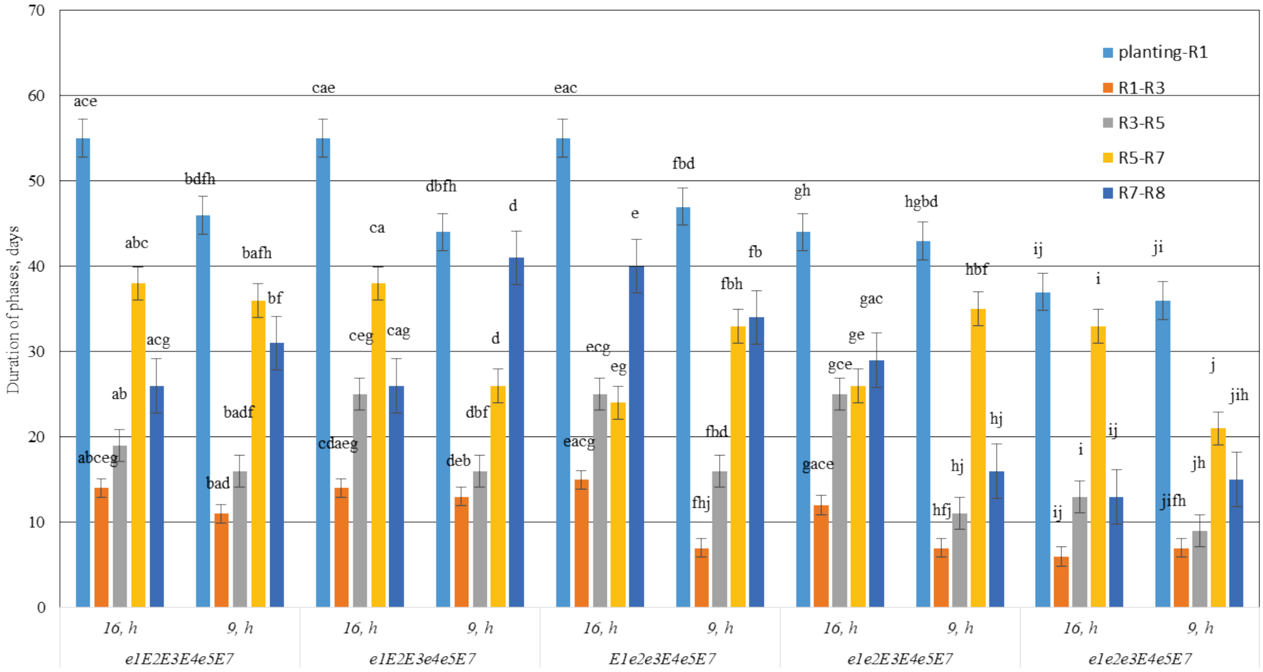

Fig. 2. The influence of photoperiod on the growth duration of near isogenic by *E* genes lines of soybean (*Glycine max* (L.) Merr.), 2018–2019, 2021, field experiment, days, (n = 3 x 30, x ± SD): firstly, comparisons were made within the isogenic line of soybean between long (16 h) and short (9 h) photoperiods; secondly, comparisons were made between the isogenic lines of soybean within long (16 h) and short (9 h) photoperiods, means in each column followed by different letters are significantly different one from another on the results of comparison using the Tukey test (P < 0.05) with Bonferroni correction; see Table 1 for letters labels description; growth stages were recorded according to (Kumagai, 2020); stage indicated R1 – beginning of flowering; R3 – beginning of pod; R5 – beginning seed-filling; R7 – beginning of maturity; R8 – full maturity

The number of days from sowing to the beginning of the R7 phase (beginning of seed maturation) (Fig. 2) in the natural photoperiod varied between 89–132 days, and under short-day conditions between 73–109 days.

Short-day lines were characterized by a longer period of development from sowing to the R7 phase under the conditions of exposure to both natural and short photoperiods compared to photoperiodic insensitive lines, which quickly transitioned to the seed maturation phase. The development period of short-day soybean lines under long-day conditions was 125.5 days for the cultivar "Clark", 131.6 days for the line L63-3016 and 118.8 days for the line L80-5879, and under short photoperiod 109.4 days, 98.8 days and 102.8 days, respectively. The photoperiodic insensitive line L63-3117 transitioned to the R7 phase under natural long photoperiod at 107.1 days, and on a short day at 96.3 days. The photoperiodic insensitive line L71-920 had the shortest period of development to the seed maturation phase among the studied lines and it was 89.4 days under the natural photoperiod and 73.4 days under the short photoperiod.

The R7–R8 period (full ripening of soybean seeds) in the short-day lines under the long natural photoperiod was shorter than under the short photoperiod in the cultivar "Clark" by 25.5 days and the line L63-3016 by

26.2 days, and under conditions of a short photoperiod 31.2 and 41.4 days, respectively. In the short-day line L80-5879, the period of full seed maturation lasted 39.7 days under the long natural photoperiod, and 34.3 days under short photoperiod.

In the photoperiodic insensitive soybean line L63-3117, the R7-R8 period lasted 16.2 days under short photoperiod and 28.6 days under the long natural photoperiod. Plants of the photoperiodic insensitive line L71-920 completed the R7-R8 period under long and short photoperiods almost simultaneously (13.4 and 15.1 days, respectively).

The results of the study of the crop structure of the studied soybean lines showed that the state of *E* genes affects the elements of the structure of the yield in the studied lines (Table 2).

Table 2
The influence of photoperiod duration on the yield component of near isogenic by *E* genes lines of soybean (*Glycine max* (L.) Merr.), 2018–2019, 2021, field experiment (n = 3 x 30, x ± SD)

Isolines of soybean, genotype	Photoperiod, h	Pod number, n/plant	Seed number, n/plant	Seed weight	
				g, per plant	g, per 1000 seed
Clark – *e1E2E3E4e5E7*	16	15.7 ± 0.9^{abgi}	29.1 ± 2.2^{abi}	2.52 ± 0.62^{ac}	67.4 ± 0.9^{a}
	9	16.8 ± 0.3^{badh}	34.5 ± 4.9^{badhj}	6.41 ± 0.85^{bdfh}	170.0 ± 15.4^{bdfhj}
L63-3016 line – *e1E2E3e4e5E7*	16	20.2 ± 0.7^{cd}	36.3 ± 0.1^{cdi}	3.15 ± 0.34^{ca}	87.3 ± 12.2^{ce}
	9	17.3 ± 3.4^{dcbhj}	35.9 ± 5.5^{dcbhj}	5.87 ± 0.23^{dbh}	166.0 ± 19.0^{dbfhj}
L80-5879 line – *E1e2e3E4e5E7*	16	23.2 ± 1.3^{ef}	51.3 ± 3.5^{ef}	4.93 ± 0.37^{eg}	93.3 ± 6.8^{ec}
	9	22.5 ± 1.3^{fe}	47.1 ± 2.8^{fe}	6.51 ± 0.31^{fbh}	141.4 ± 16.7^{fbdhj}
L63-3117 line –*e1e2E3E4e5E7*	16	16.9 ± 0.4^{ghai}	38.9 ± 0.1^{gi}	5.08 ± 0.42^{ghei}	126.5 ± 9.7^{g}
	9	16.8 ± 0.9^{hgbdj}	35.5 ± 2.8^{hbdj}	5.94 ± 0.31^{hgbdf}	164.3 ± 7.8^{hbdfj}
L71-920 line –*e1e2e3E4e5E7*	16	16.1 ± 2.7^{ijag}	35.5 ± 4.2^{iacg}	6.06 ± 0.65^{ig}	176.8 ± 2.7^{i}
	9	14.4 ± 1.8^{jidh}	32.0 ± 7.2^{jbdh}	4.52 ± 0.54^{j}	145.3 ± 17.8^{jbdfh}

Note: firstly, comparisons were made within the isogenic line of soybean between long (16 h) and short (9 h) photoperiods; secondly, comparisons were made between the isogenic lines of soybean within long (16 h) and short (9 h) photoperiods, means in each column followed by different letters are not significantly different one from another on the results of comparison using the Tukey test ($P < 0.05$) with Bonferroni correction: see Table 1 for letters labels description.

Short-day lines showed a different response to the length of the photoperiod depending on the state of the *E4* gene and the combination of *E1* or *E2* and *E3*. It is shown that *E1* causes high sensitivity to photoperiod, in contrast to *E2* and *E3*. However, both *E2* and *E3* together induce sensitivity to photoperiod duration comparable to that induced by *E1* alone. The number of beans and the number of grains was slightly higher in the long photoperiod in all investigated lines, except the cultivar "Clark". We can assume that such a reaction is caused by the dominant state of the *E4* gene. Analysing the indicators of the mass of grains per plant showed that the mass of grains was higher in short-day lines under short-day conditions, and in photoperiodic insensitive lines, the reaction was different. PPI line L63-3117 had slightly higher values under short day conditions, and PPI line L71-920 under long day conditions. The mass of 1000 grains in short-day lines was higher in variants of the experiment with exposure to a short photoperiod during 14 days, in photoperiodic insensitive lines under long-day conditions.

Figure 3 shows that the seeds of soybean lines isogenic for *E* genes differed in size and degree of ripeness under conditions of exposure to different durations of the photoperiod. Thus, the seeds of SD lines had a greater mass and were more mature under the conditions of a short photoperiod, the seeds of PPI lines were fully ripe under the conditions of both natural (long) and short photoperiod.

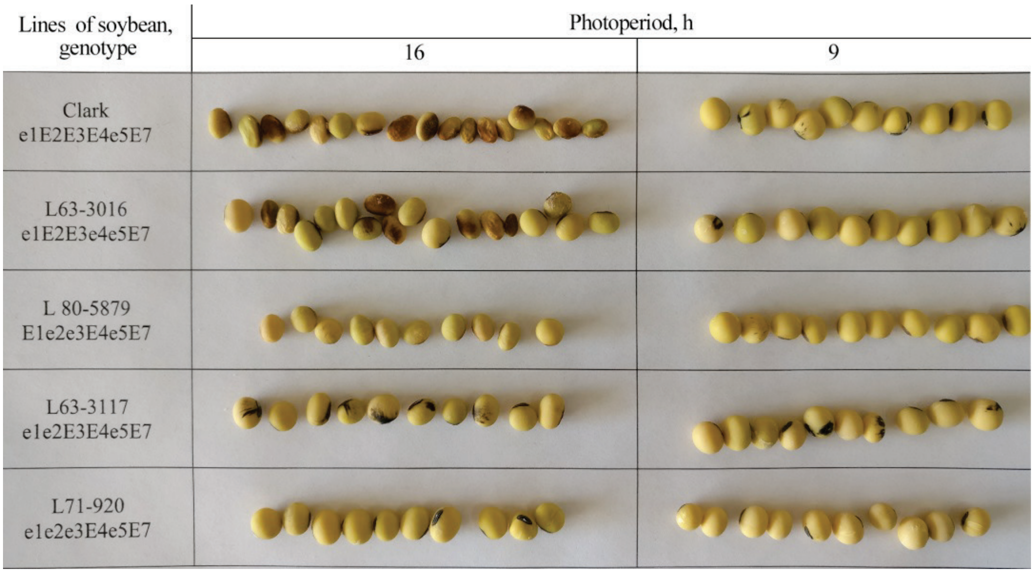

Fig. 3. Seeds of near isogenic by *E* genes lines of soybean (*Glycine max* (L.) Merr.) under different durations of the photoperiod

Carbohydrate content in soybean seeds is influenced by such factors as genotype, growing conditions, and the interaction of these factors. The results of the study of the effect of photoperiod on the content of carbohydrates in the seeds of soybean lines isogenic for *E* genes are presented in Table 3.

In SD lines L63-3016 and L80-5879, the content of soluble sugars in seeds under natural photoperiod conditions was higher than under short photoperiod conditions. The seeds of the cultivar "Clark" and photoperiodic insensitive lines L63-3117 and L71-920 did not have significant differences in the content of soluble sugars in the seeds under long and short photoperiods. Starch content in seeds is a significant factor determining oil and protein content in soybean seeds. Thus, all studied soybean lines, with the exception of the photoperiodic insensitive line L71-920, were characterized by a higher starch content under long-day conditions than under short-day conditions. The starch content of the short-day line L80-5879 was higher than that of the short-day the cultivar "Clark" and L63-3016. The starch content of the photoperiodic insensitive line with the genotype L71-920 under the conditions of a short photoperiod was slightly higher than under the conditions of a long photoperiod in the seeds.

Table 3
Effect of photoperiod duration on carbohydrate content in seeds of near isogenic by *E* genes lines of soybean (*Glycine max* (L.) Merr.), 2018–2019, 2021, field experiment, mg/g of dry weight (n = 9, x ± SD)

Isolines of soybean, genotype	Photoperiod, h	Soluble sugars content, mg/g dry weight	Total starch content, mg/g of dry weight	Total N content, mg/g of dry weight
Clark – *e1E2E3E4e5E7*	16	33.8 ± 1.7^{abcegi}	94.9 ± 2.4^{a}	126.6 ± 7.9^{a}
	9	32.6 ± 1.6^{badfhj}	85.2 ± 1.1^{b}	145.4 ± 1.3^{bh}
L63-3016 line – *e1E2E3e4e5E7*	16	36.6 ± 1.8^{cdae}	88.7 ± 2.7^{cgi}	163.5 ± 1.1^{cdgi}
	9	31.4 ± 1.6^{dcbfhj}	81.4 ± 0.8^{dh}	159.8 ± 2.7^{dc}
L80-5879 line – *E1e2e3E4e5E7*	16	35.6 ± 1.8^{efacgi}	106.6 ± 1.3^{e}	156.4 ± 3.4^{e}
	9	31.4 ± 1.5^{febdhj}	90.0 ± 3.1^{fj}	149.3 ± 1.2^{f}
L63-3117 line –*e1e2E3E4e5E7*	16	32.2 ± 1.7^{ghaei}	88.1 ± 1.1^{gci}	169.5 ± 6.9^{gci}
	9	34.7 ± 1.8^{hgbdfj}	80.8 ± 0.9^{hd}	153.1 ± 7.4^{hb}
L71-920 line –*e1e2e3E4e5E7*	16	32.2 ± 1.6^{ijaeg}	89.5 ± 2.8^{ijcg}	164.6 ± 1.1^{icg}
	9	31.4 ± 1.5^{jibdfh}	92.4 ± 3.5^{jif}	169.1 ± 1.7^{j}

Note: firstly, comparisons were made within the isogenic line of soybean between long (16 h) and short (9 h) photoperiods; secondly, comparisons were made between the isogenic lines of soybean within long (16 h) and short (9 h) photoperiods, means in each column followed by different letters are significantly different from one another on the results of comparison using the Tukey test (P < 0.05) with Bonferroni correction: see Table 1 for letters labels description.

The results of the study of the effect of the photoperiod on the oil content in the seeds of soybean lines isogenic for *E* genes (Fig. 4) showed that both the duration of the photoperiod and the genotype of the lines influenced this indicator. In short-day lines, the difference between the indicators under the long and short photoperiod was more significant than the differences between the lines, that is, the decisive factor in this case is the duration of the photoperiod. For photoperiodically neutral lines, the influence of both genotype and length of daylight is shown, the lines had a different nature of reaction (Fig. 4). In addition, in all studied soybean lines, the oil content in seeds under natural photoperiod conditions was lower than under short day conditions.

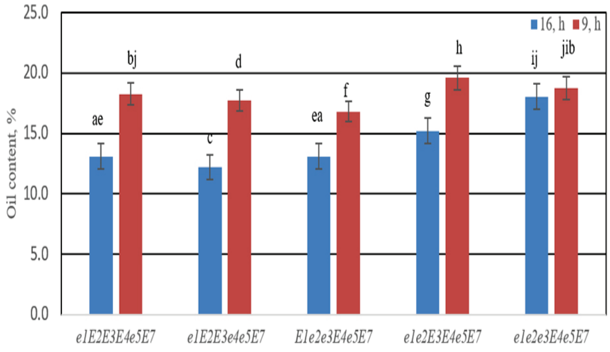

Fig. 4. Effect of photoperiod on oil content in seeds of near isogenic by *E* genes lines of soybean (*Glycine max* (L.) Merr.), 2018–2019, 2021, field experiment (%): firstly, comparisons were made within the isogenic line of soybean between long (16 h) and short (9 h) photoperiods; secondly, comparisons were made between the isogenic lines of soybean within long (16 h) and short (9 h) photoperiods, means in each column followed by different letters are significantly different one from another on the results of comparison using the Tukey test (P < 0.05) with Bonferroni correction: see Table 1 for letters labels description

The oil content in the seeds of the soybean SD lines L80-5879, L63-3016 and the cultivar "Clark" under the long-day conditions was equal to 13.1%, 12.2% and 13.1% and under the short day conditions it was 16.8%, 17.8% and 18.2% respectively, the difference ranged from 3.7% to 5.6%. The greatest difference between the indicators under long and short photoperiods was in the lines with dominant genes *E2E3E4* and *E2E3* and amounted to 5.2% and 5.6%. In the short-day line with dominant genes *E1E4* the difference was smaller. It should be noted that the short-day soybean line L80-5879 was characterized by low oil content in the seeds under long-day conditions compared to other short-day lines.

The oil content in the seeds of the photoperiodic insensitive line L63-3117 under conditions of a long day was 15.2% and under conditions of a short photoperiod it was 19.6% the difference was 4.4%. The content of oil in the seeds of the photoperiodic insensitive line L71-920 under conditions of long and short photoperiods were almost the same 18.1% and 18.8% respectively.

Thus, the duration of the photoperiod has different effects on the oil content in the seeds of photoperiodic insensitive lines, but the genotype, namely the *E4* gene, has a greater influence on the oil content.

Discussion

The difference in photoperiodic response is one of the most important characteristics that affect the adaptation of soybean cultivars. Identification of the critical photoperiod of different genotypes is of particular importance to match their growth process to the photoperiodic conditions of target environments. Impacts of global weather changes demand that agriculture becomes more efficient. To be adapted, the duration has to be sufficient to accumulate adequate photosynthate for boosting yield production, but meanwhile, plants have to mature early enough to allow for maturation before frost and to fit into the overall agronomic scheme of the operation. Moreover, early harvest may be desirable to allow for timely operations associated with planting of a cool-season cover crop or cash crop.

In our study of the effect of soybean photoperiod insensitivity on flowering time, seed composition, yield components and the length of growth periods artificially set photoperiod treatments (16 h natural daylength, and 9 h) were applied. Previously Wu et al. (2019), had shown that the response to the critical photoperiod of the extremely photoperiod-sensitive cultivars was less than 11 h. Due to significant delay of flowering time with the increasing photoperiod from 12 h, we estimated the critical photoperiods of these photoperiod-sensitive lines to be 9 h. In this study, 5 near-isogenic lines derived from cultivar "Clark" were used to analyze the effects of 4 major maturity gene pairs including *E1/e1*, *E2/e2*, *E3/e3* and *E4/e4*.

To determine the effect of each gene on phenology, crop structure and biochemical composition of seeds under conditions of different photoperiod duration, the following groups were formed: SD line *E1e2e3E4e5E7* – PPI line *e1e2e3E4e5E7*, SD line *e1E2E3E4e5E7* – PPI line *e1e2E3E4e5E7*, PPI line *e1e2E3E4e5E7* – PPI line *e1e2e3E4e5E7*, SD line *e1E2E3E4e5E7* – SD line *e1E2E3e4e5E7*.

The results showed that the dominant alleles tend to delay the flowering and maturity time in most of the genotypes studied. It has long been known that the *E1* locus plays a major role in regulating photoperiodic flowering. Xia et al. (2012) delimited the *E1* locus to a single gene (Glyma.06G207800), which encodes a putative transcription factor containing a *B3* domain. Its dysfunctional forms have been intensively selected in high-latitude regions of Asia and North America (Xia et al., 2012; Zhou et al., 2015). In the soybean genome, *E1* has two highly similar homologs (Glyma.04G156400 and Glyma.04G143300, designated *E1La* and *E1Lb*, respectively). The three genes are severely repressed under short-day conditions and induced, with rhythmic expression patterns, under long-day conditions (Xia et al., 2012; Xu et al., 2015; Zhai et al., 2015; Cao et al., 2017). These data coincide with our results that the dominant alleles *E1* delayed the transition to flowering under long-day conditions by an average of 14.5% compared to periods under short-day photoperiod (Table 1). In addition, the line with the dominant *E1* gene extended all phases of soybean development compared to the line with the recessive allele of this gene under conditions of exposure to both photoperiods. It also significantly extends the duration of the stages of flowering and pod formation under natural long-day conditions (Fig. 2). As a result of the increase in the duration of these phases, the soybean line with the dominant allele *E1* increased the number of beans per plant and the number of seeds per plant (Table 2). On the other hand, with shorter duration of R5–R7 phases, the soybean line with the dominant allele *E1* showed decreased weight of seeds per plant, the weight of 1,000 seeds and yield in general under long-day condition (Table 2).

The biochemical composition of the seed quality also changed significantly. The line with the dominant allele *E1* showed a significant increase in the content of starch in the seeds, but a significant decrease in the content of total nitrogen and oil in the seeds under long and short day conditions compared to the line with the recessive allele of this gene (Table 3, Fig. 4).

Soybean lines with genotypes *e1E2E3E4e5E7* – *e1e2E3E4e5E7* differed in the dominant/recessive state of the *E2* gene. Dong et al. showed that soybean accessions carrying an *e2* allele flowered earlier than those with an *E2* allele under long day conditions. In addition, they found that *E2* delayed the flowering time of soybeans by enhancing the effect of the *E1* gene (Dong et al., 2022). Wang et al. showed that in regions with high latitudes, single mutants of *e2* showed earlier flowering and high grain yield (Wang et al., 2022). In our work, we obtained similar results. The line with the dominant allele delayed the transition to the flowering phase by 20.0% under long day conditions (Table 1).

In studies of the effect of maturity genes *E2* and *E3* in soybean cultivar "Enrei" carried out in Fukuyama, Japan (34°30′ N, 133°23′ E) on yields and yield formation, it was shown that *E2* extended the period from sowing to R1, as well as the period from R1 to R7 (Kawasaki et al., 2018). These data coincide with the results of our study (Fig. 2). In the short-day line with the genotype *e1E2E3E4e5E7*, the extending of the flowering (R1–R3) and seed filling (R7–R8) phases was observed under the conditions of a long photoperiod. Under conditions of a short day, all phases of development were longer compared to the line with the recessive allele *e2*. The soybean line with the dominant *E2* allele showed a decrease in the number of seeds per plant, seed weight per plant, 1000-seed weight, and overall yield under long-day conditions compared to the line with the recessive *e2* allele (Table 2). No influence of the dominant *E2* gene on the content of soluble sugars in seeds was found (Table 3). The line with the dominant allele *E2* showed an increase in the starch content and a decrease in the oil content in the seeds under conditions of long and short photoperiod in comparison with the line with the recessive allele *e2* (Fig. 4). The dominant allele *E2* reduced the content of total nitrogen under long day conditions (Table 3).

Photoperiodic insensitive soybean lines with genotypes *e1e2E3E4e5E7* and *e1e2e3E4e5E7* differed in the dominant/recessive state of the *E3* gene. That why we assumed that differences in the response of photoperiodic insensitive lines to the duration of the photoperiod are related to the manifestation of this gene. Kawasaki et al. (2018) showed that *E3* prolonged the period from emergence to R1 and did not affect the passage of the R1–R7 phase. In addition, *E3* extended the reproductive period in the presence of recessive *e2* and shortened reproductive periods in the presence of dominant *E2* (Kawasaki et al., 2018). In our study, we obtained similar differences in terms of the passage of phenophases (Table 1). The dominant *E3* allele also extended the transition to flowering by 15.9% under long day conditions compared to short photoperiod. In addition, the dominant *E3* allele significantly extended the R1–R8 phases under long-day conditions and the flowering initiation phase, and R3–R7 under short-day conditions (Fig. 2). It has been shown that the *E3* and *E4* loci can affect reproductive development after flowering by increasing the duration of pod filling, as well as the number of nodes and pods (Xu et al., 2013). In our study, the PPI soybean line with a dominant *E3* gene showed an increase in all yield structure indicators under conditions of a short photoperiod compared to the PPI line with a recessive *e3* allele. Under the conditions of a long photoperiod, the *E3* gene reduced the parameters of seed mass per plant and 1000 seeds in comparison with the effect of *e3* (Table 2). The *E3* gene also affected the biochemical composition of seeds. PPI line with genotype *e1e2E3E4e5E7* showed a decreased content of oil in seeds under long photoperiod conditions (Fig. 4). There was a decreased content of starch and nitrogen under short photoperiod conditions compared to the line with genotype *e1e2e3E4e5E7* (Table 3). In our opinion, this is evidence that the action of the dominant *E3* allele occurred more under the conditions of a short photoperiod than under conditions of a long photoperiod.

SD lines with genotypes *e1E2E3E4e5E7* and *e1E2E3e4e5E7* differed in the dominant/recessive state of the *E4* gene. The lines had similar times of the transition period to flowering (Table 1). Under the conditions of a long photoperiod, the line with genotype *e1E2E3E4e5E7* showed a shortening of the R3–R5 (beginning of pod) phase, while under the conditions of a short photoperiod, the dominant allele *E4* prolonged the R5–R7 phase (beginning of seed filling) and shortened the seed maturity phase (R7–R8, Fig. 2). Xu et al. (2013) suggested that two Phytochrome A genes, *E3* and *E4*, regulate the response of pod filling to photoperiod (Abugalieva et al., 2016). In our experiment, the line with the dominant *E4* gene showed a decrease in the number of beans, seeds and weight of 1000 seeds under long day conditions (Table 2). The *E4* gene also affected the biochemical composition of seeds. Our study showed that a soybean line with a dominant *E4* allele had higher oil and starch content and lower total nitrogen content in seeds under long and short photoperiod conditions (Table 3, Fig. 4). The development of early-ripeness (short season) soybeans for different regions of Ukraine requires effective use of early maturity genes (Zharikova et al., 2019).

In studies of the interaction of major flowering genes and quantitative trait loci (QTLs) under conditions of different photoperiods, it has been shown that major flowering genes and QTLs often interact with each other to determine time to flowering. In addition, each of the *E2* and *E3* alleles positively interacts with the *E1* allele to increase photoperiod sensitivity. The effect of the *E1* allele has been found to be similar to the effect of the *E2* and *E3* alleles together (Harada et al., 2012; Watanabe et al., 2012). In our study, we compared the effects of the *E1* allele and the combined effect of the *E2E3* alleles. The results of the study showed that the lines started flowering almost simultaneously, but the differences appeared in the periods after flowering (Fig. 2). The line with the genotype *E1e2e3E4e5E7* under long day conditions prolonged the phases of the beginning of pod (R3–R5) and full maturity (R7–R8, Fig. 2). With the increase in the duration of these phases, the indicators of the yield structure under long day conditions increased (Table 3). An increase in the content of starch and total nitrogen under the influence of long and short photoperiods was also observed (Table 3). On the other hand, the effect of exposure to different photoperiod durations showed some similar answers in these lines. Thus, under the conditions of a long photoperiod, an increase in the weight of seeds per plant and 1000 seeds was observed (Table 2). The indicators of the number of beans and seeds did not significantly depend on the duration of the photoperiod (Table 2). In addition, under conditions of a long photoperiod, the content of starch in seeds increased (Table 3) but under a short day conditions the content of oil in seeds increased (Fig. 4).

Conclusion

Our results show that the main genes of sensitivity to the length of daylight had different degrees of manifestation in the climatic growing conditions of the Kharkiv region. The three year observations of the 5 mutant near isogenic by *E* genes lines of soybean obtained under field conditions of the Kharkiv region of Ukraine permit us to make the following conclusion: dominant genes *E1*, *E2*, *E3* extended the period of transition of soybean plants to flowering, and the duration of phases after flowering. The dominant allele *E4* did not show a significant effect on the duration of flowering and the duration of phases after flowering. The dominant allele of the *E1* gene showed a similar effect to the combined effect of the *E2* and *E3* alleles in terms of transition to flowering. However, it should be noted that the line with the dominant *E1* allele somewhat shortened the phases of flowering and beginning of pod under the conditions of a short photoperiod. Genes of sensitivity to the photoperiod influenced the structure of the crop. In general, it can be said that the dominant alleles *E2*, *E3* and *E4* reduced the parameters of the weight of seeds per plant and the weight of 1000 seeds under the influence of a natural long day. The studied genes had different effects on the biochemical composition of soybean seeds. Thus, lines with dominant alleles *E1*, *E2*, *E4* showed an increase in starch content and a decrease in total nitrogen content in seeds under conditions of exposure to a long photoperiod. Lines with dominant alleles *E1*, *E2*, *E3* showed a decrease in oil content in seeds under long day condition. The analysis of the effect of early maturity genes on the timing of phenophases, yield structure indicators and biochemical composition of seeds in soybean plants under different photoperiod showed that the PPI line with the genotype *e1e2e3E4e5E7* was the most adapted to the natural conditions of 50 degrees of latitude. That line with the genotype *e1e2e3E4e5E7* was characterized by the shortest phases of days from sowing to flowering and full maturity. As a result, this line had the shortest growing season without reducing the yield and seed quality. These findings demonstrate again that under Ukrainian growing conditions the *E* genes studied can be useful tools for breeding of soybean cultivars with programmed terms of development in order to widen its cultivation range as much as possible. Furthermore, when searching for new high-yielding foreign cultivars, one should pay attention to their photoperiodic response and, taking into account the results of our research, give preference to photoperiodically insensitive ones, that is, with a recessive state of *E* genes. Short-day cultivars during the growing season will show a powerful growth of many pods per plant, but seeds will not have time to fully germinate and ripen, which will lead to a loss of yield.

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The authors declare no conflict of interest.

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