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PROTEIN CONTENT IN LEAVES OF *GLYCINE MAX* (L.) MERR ISOGENIC LINES UNDER BACTERIZATION AND DIFFERENT PHOTOPERIOD DURATION

ВМІСТ БІЛКУ В ЛИСТКАХ ІЗОГЕННИХ ЛІНІЙ *GLYCINE MAX* (L.) MERR. ЗА УМОВИ БАКТЕРИЗАЦІЇ ТА РІЗНОЇ ТРИВАЛОСТІ ФОТОПЕРІОДУ

Hlushach D. V.
PhD student at the Plant and Microorganisms Physiology and Biochemistry Department, V. N. Karazin Kharkiv National University

Avksentieva O. O.
Candidate of Biological Sciences, Associate Professor at the Plant and Microorganisms Physiology and Biochemistry Department, V. N. Karazin Kharkiv National University Kharkiv, Ukraine

Глушач Д. В.
аспірант кафедри фізіології та біохімії рослин і мікроорганізмів, Харківський національний університет імені В. Н. Каразіна

Авксентьєва О. О.
кандидат біологічних наук, доцент кафедри фізіології та біохімії рослин і мікроорганізмів, Харківський національний університет імені В. Н. Каразіна м. Харків, Україна

One of the most important adaptive properties of plants is sensitivity to photoperiod. It is known that it determines the plants distribution by growing zones, the vegetative stage duration and, as a result, the productivity and crop quality [1, p. 3]. In soybean (*Glycine max* (L.) Merr.) sensitivity to photoperiod is determined by E-series genes that influence on growth and development indirectly through changes of plants phytohormonal and metabolic status. Thus, it determine the growth, development rates and soybean productivity [2, pp. 1–2].

Also, interactions between plants and bacteria play an important role in the regulation of plant growth and development [3, pp. 1-5]. The most active influence is observed when soybean interact with symbiotic diazotrophs, which are capable of nitrogen fixation, thereby regulating nitrogen nutrition and protein metabolism [3, pp. 5–12; 4, pp. 128–135]. In addition, bacteria are able to regulate the plants metabolic status, by providing it with phosphates, potassium, iron, as well as by excretion phytohormones into the environment – auxins, abscisins, gibberellins and other growth-regulating compounds [3, pp. 13–14; 5, pp. 160–175].

So, the aim of the work is to analyze the influence of symbiotic diazotrophs on the protein content in the leaves of soybean lines isogenic by photoperiodic sensitivity control genes cultivated in field conditions under different duration photoperiod (9 and 16 hours).

We used near-isogenic lines (NILs) of soybean by photoperiodic response control genes, which differ from each other only by the state of separate loci: the short-day lines are Clark and L 80-5879 (genotype, respectively, *e1E2E3E4e5E7* and *E1e2E3E4e5E7*); the photoperiod insensitive lines are L 63-3117 and L 71-920 (genotype, respectively, *e1e2E3E4e5E7* and *e1e2E3E4e5E7*). The plants were bacterized with a specific symbiotic diazotroph of soybean – strain *Bradyrhizobium japonicum 634b*.

The sterilized seeds were treated with *Bradyrhizobium japonicum 634b* and cultivated in the experimental plot of the Plant and microorganisms physiology and biochemistry Department of V.N. Karazin Kharkiv National University. In the control variant, the seeds were treated with distilled water. After germination and until the formation of the third true leaf, all plants were grown under a long natural day (16 hours at the latitude of Kharkiv – 50° N). At the third true leaf (V3) stage, half of the plants were exposed to a short 9h photoperiod, and the other half continued to be grown under a long 16h photoperiod. The short photoperiod was artificially created by darkening the plants with opaque black films from 5:00 pm to 9:00 am for a fortnight. Formed 2 and 3 leaves were selected during the vegetation phase V3 (before exposure to short day) and V5 (after exposure). Protein from the fixed material was extracted using a phosphate-salt buffer (pH=7.4) and its content was determined by Bradford. The obtained data check on normality test and analyzed using ANOVA (two- and three-factor) with the calculation of the factor strength of genotype, bacterization, length of day and their interactions.

The protein content in the leaves of the short-day variety Clark in the variant with bacterization in the V3 phase is significantly higher than in the variant without treatment (table 1). In the V5 phase, we observe a significant increase of the protein content in all experimental variants in comparison with the V3 phase. Compared to the control, we observe a significant increase of the protein content in the variant with treatment with *Bradyrhizobium japonicum 634b* both on the long and on the short photoperiod. Moreover, during a long day, this increase is significant compared to a short day.

Table 1

Protein content in leaves of soybean isogenic lines by genes photoperiod sensitivity control under bacterization and different day length

Protein content in leaves, mg/g dry matter						
Isogenic lines	Development stage V3		Development stage V5			
			Short day (9 hours)		Long day (16 hours)	
	Without treatment	Inoculation	Without treatment	Inoculation	Without treatment	Inoculation
Clark	60,06±6,17	74,30±4,42*	86,90±2,58	105,22±2,38**	85,93±3,95	126,44±6,22***
L 80-5879	74,77±8,75	65,79±11,87	79,06±2,18	91,05±5,57**	86,46±5,30	88,21±4,79
L 63-3117	62,15±12,10	87,19±6,94*	105,29±4,24	101,37±5,22	115,94±1,93	122,05±1,58***
L 71-920	77,53±8,69	83,30±8,03	91,14±3,76	110,33±4,00**	116,54±8,58	106,37±9,16

* – the differences with the control (without treatment) are significant at the $p<0,05$

** – the differences with the control (without treatment) are significant at the $p<0,05$

*** – the differences with the control (without treatment) are significant at the $p<0,05$

In the short-day isoline L 80-5879 significant changes in protein content were not observed in the V3 phase. A significant increase is observed in the V5 phase. But the difference between the treated plants and the control is observed only in a short day.

In the photoperiod insensitive isoline L 63-3117, we observed a significant increase of protein in the leaves of bacterized plants compared to the control in the V3 phase. In the V5 phase, we observe a significant increase of the protein content in the leaves of control and bacterized plants grown on short and long days. But during the long day, there is a significant difference between bacterized and control plants. Also, it is worth noting that in both options, the protein content is higher on the long day than on the short day.

In the leaves of the L 71-920 isoline, which is photoperiod neutral, differences between the variants were not observed in the V3 phase. An increase of protein in the leaves of bacterized and control plants was observed in the V5 phase under short and long days, but there are significant differences between the variants in plants grown under short days.

Thus, according to the obtained data, it can be concluded that both the genotype, and bacterization, and the day length can influence on the protein content in the soybeans leaves. Indeed, according to our calculations, the greatest effect of the factor on the protein content in the V5 phase has the genotype of the line (39.6%), bacterization (12.5%) and day length (10.7%) – a total of 62.8%. And only 27.7% (in total) of the protein content is determined: by the interaction of genotype and bacterization – 14.1%; by the interaction of genotype, bacterization and day length – 11.0%; by interaction of genotype and day length – 2.6%. It is worth noting that, according to the calculations, the interaction of day length and bacterization does not have a significant effect on protein content. This can be explained by the absence of a direct effect of day length on the physiological and biochemical features of *Bradyrhizobium japonicum 634b*, through which they could affect protein metabolism.

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