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LIGHT DEPENDENCE OF THE SHIKIMIC ACID PATHWAY IN BUCKWHEAT SEEDLINGS

Light-induced changes in the functioning of the shikimic acid pathway in glyphosate-treated buckwheat seedlings showed that hypocotyls and cotyledons differ by the light dependence of the pathway. In isolated hypocotyls, a 6—10-hr light treatment appeared to be sufficient to bring about maximum stimulation of the process. Light dependence of the pathway in isolated cotyledons was much more pronounced, and in the range of light exposures from 16 to 40 hrs the degree of photoactivation of the process was linear to the duration of illumination. A clear-cut preillumination effect on the functioning of the shikimate pathway in cotyledons indicated that in this seedling organ light action on the pathway could be effected through two different mechanisms. Characteristics of light dependence of the shikimic acid pathway in seedlings of different age suggest that the highest responsiveness of the pathway to illumination as well as the highest general capacity for shikimate formation may occur at different age in hypocotyls and cotyledons.

It is generally known that light stimulates synthesis of phenylalanine — the common precursor of phenolic compounds — and other shikimate derived amino acids in chloroplasts of photosynthesizing tissues (for a review see Schmidt et al., 1987). However, there is evidence for light dependence of the shikimic acid pathway also in etiolated tissues. Thus, light was shown to stimulate the flow of carbon through the shikimic acid pathway in etiolated buckwheat hypocotyls (Holländer et al., 1979; Amrhein et al., 1980), and the elevated rate of synthesis of phenylpropanoid compounds in the light was supposed to be linked to a light-enhanced activity of the shikimate pathway (Amrhein, Holländer, 1981). Experiments carried out in our laboratory confirmed this suggestion and showed that the flow of phenylalanine for anthocyanin synthesis through the shikimic acid pathway was controlled by the high irradiance reaction (Тохвер, Ыннепалу, 1982; Тохвер, Мядамюрк, 1984) and phytochrome (Тохвер, 1990).

Under normal physiological conditions neither the intermediates of the shikimic acid pathway nor its end products — aromatic amino acids — accumulate in plant cells, and the rate of their synthesis can only be determined by using indirect methods. In most studies on the shikimic acid pathway glyphosate (N-phosphonomethylglycine), a herbicide which inhibits the formation of 3-enolpyruvylshikimate-5-phosphate (Steinrücken, Amrhein, 1980) and results in the accumulation of shikimic acid, is used. In the glyphosate-treated buckwheat hypocotyls the content of shikimic acid in the light was shown to be 5 times as high as that in the dark (Amrhein, Holländer, 1981), whereas in the cotyledons of buckwheat seedlings the rate of shikimate accumulation under illumination was twice as high as it proved to be in the absence of light (Тохвер, Пальм, 1986).

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In order to gain a better insight into the light-controlled role of the shikimic acid pathway in the formation of phenolic compounds, more detailed light studies of the pathway combined with a comparison of their results with corresponding data on flavonoid accumulation are necessary. These studies should involve, among other problems, elucidation of possible age-dependent differences in the light control of the pathway as well as possible differences in the light responses of different parts of seedlings. As one of the feasible approaches in this line, light-induced changes in the functioning of the shikimic acid pathway in glyphosate-treated buckwheat seedlings of different age were examined in the present work.

Material and methods

The experiments were carried out with buckwheat (*Fagopyrum esculentum* Moench cv. Victoria) hypocotyls and cotyledons excised from 60—96-hr-old etiolated seedlings grown in water at 25 °C. The detached material was transferred to Petri dishes onto filter paper moistened with 10 mM glyphosate, and was thereafter incubated either in the dark or in the light (continuous illumination from white fluorescent tubes, fluence rate 28 $W \cdot m^{-2}$, temperature 25 °C). A subsequent incubation in the dark followed in a number of the light series. In some series with preillumination, the excised material was incubated in water prior to its transfer into the solution of glyphosate.

Shikimic acid was extracted according to Tohver and Palm (Тохвер, Пальм, 1986). The content of shikimic acid in the effluent from polyamide columns was determined according to Gaitonde and Gordon (1958) modified by Margna et al. (1989) and expressed in nmols per seedling.

The experiments were run in 4—5 replicate series. In each series, three sets of 25 hypocotyls or pairs of cotyledons were assayed per time of incubation.

Results

Kinetic studies. Tohver and Palm (Тохвер, Пальм, 1986) established that in 72-hr-old buckwheat cotyledons treated with glyphosate (up to 1 mM) the accumulation of shikimic acid started within the first hour after the application of the inhibitor and continued at a more or less constant rate during 24 hrs; in 0.2 mM glyphosate the treated material showed an about 8-fold difference in the production of shikimic acid in the dark and in the light. Considering these data it could be expected that a transfer of illuminated seedlings into the dark would result in a gradual shift of the rate of shikimate production from the initial value to a lower one. To check that supposition, we compared kinetic characteristics of shikimic acid accumulation typical of the glyphosate-treated plant material under continuous illumination with the kinetics of the same process in a similar plant material which was exposed to light during the first 10 hrs of the total 60-hr time period of treatment with the herbicide. Hypocotyls and cotyledons from buckwheat seedlings of two different ages (60 and 80 hrs at the onset of illumination in a 10 mM glyphosate solution) were examined.

The results presented in Fig. 1 show that the rate of shikimate accumulation increases with seedling age whereas the shape of the kinetic curves for 60- and 80-hr-old seedlings is practically similar. However, great differences were observed in the response of cotyledons and hypocotyls.

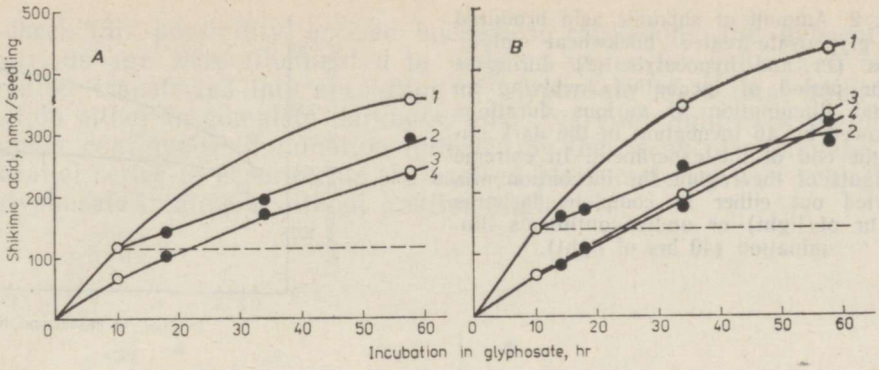

Fig. 1. Kinetics of shikimic acid accumulation in glyphosate-treated buckwheat cotyledons (*1*, *2*) and hypocotyls (*3*, *4*). A half of the plant material was incubated in the continuous light (open symbols), another half (after a preliminary 10-hr period of illumination) — in the dark (closed symbols). Age of seedlings at the time of glyphosate application — 60 hrs (*A*) and 80 hrs (*B*).

In cotyledons, probably due to much higher concentration of glyphosate as compared to the report cited above (Тохвер, Пальм, 1986), the accumulation of shikimic acid in the light was not completely linear in time. However, after the illuminated cotyledons were transferred into the dark, the new established rate of shikimate accumulation could be considered constant from the very beginning of the dark process. A comparison of the amount of shikimic acid produced during the last 48 hrs of the experiment in the light or in the dark (see curves above the hatched line in Fig. 1) showed that in younger seedlings the amount of shikimic acid formed in the dark reached 73% of that of the illuminated plant material, whereas in 80-hr-old seedlings the dark level of shikimic acid was about a half of its amount formed in continuously illuminated cotyledons.

Surprisingly enough, in hypocotyls the accumulation of shikimic acid in the dark did not differ significantly from the same process in the light. A question arose, therefore, whether light had already manifested its effect during the first 10 hrs of illumination so that the subsequent changes in the illumination conditions were already not able to modify the process of formation of shikimic acid or, on the contrary, the 10-hr light exposure remained insufficient to reveal the influence of light on the accumulation of shikimate in hypocotyls. For this purpose, we studied the effect of the duration of illumination on the amount of shikimic acid produced in the glyphosate-treated plant material by the end of a given experimental period.

Production of shikimic acid in the glyphosate-treated buckwheat hypocotyls and cotyledons incubated under combined illumination conditions. Cotyledons and hypocotyls excised from 80-hr etiolated seedlings were illuminated in a glyphosate solution for different periods of time and thereafter transferred to darkness until shikimate assay at the end of the experiment. The duration of the combined light-dark treatment was either 40 or 64 hrs. Control material was excised in dim green light and kept during the whole period of incubation in the dark (0-point on the axis of the duration of illumination in Fig. 2). An additional sample of plant material was incubated in glyphosate under continuous illumination (the last point on the same axis).

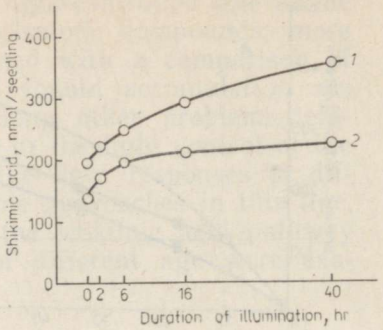

Fig. 2. Amount of shikimic acid produced in glyphosate-treated buckwheat cotyledons (*1*) and hypocotyls (*2*) during a 40-hr period of incubation involving an initial illumination of various durations followed by an incubation in the dark until the end of the experiment. In extreme variants of the regime the incubation was carried out either in complete darkness (0 hr of light) or under continuous illumination (40 hrs of light).

As can be seen in Fig. 2, glyphosate-treated hypocotyls incubated in darkness accumulated about 140 nmols of shikimic acid during the 40-hr experimental period whereas in hypocotyls illuminated continuously the amount of shikimate reached 226 nmols per seedling. A clear-cut stimulatory effect of light — an about 25—50% increase in the production of shikimate as compared with the level of that process in the dark — could be observed in case of the combinations 2 hr light (2L) + 38 hr darkness (38D) and 6L + 34D. Further increase of the duration of illumination had only little additional effect. The light-dark combination 16L + 24D gave already results practically identical to those obtained under continuous illumination. Similar were the characteristics of light-dependent production of shikimate when the duration of the combined light-dark period of incubation of glyphosate-treated hypocotyls was prolonged up to 64 hrs (data not shown). It may be concluded, therefore, that the 10-hr preillumination period chosen for our kinetic experiments was indeed sufficient for maximum stimulation of the shikimic acid pathway in buckwheat hypocotyls and, thus, the process was not inhibited by the subsequent dark incubation.

In cotyledons, however, the stimulatory effect of light on shikimate formation was considerable under all light regimes used (Fig. 2); in the range of light periods from 16 to 64 hrs (data not shown) the degree of light activation of the process was linear to the duration of illumination.

Experiments with preillumination in water prior to glyphosate treatment. In the experiments described in the preceding section, glyphosate was applied to etiolated plant material before illumination, and, hence, the metabolic shift from the etiolated phase of seedling development to a photosynthesizing plant started in the presence of the inhibitor. In consequence, the aromatic amino acid deficiency developing under the influence of glyphosate could probably bring about a marked inhibition of protein biosynthesis in the treated material at the very first stages of the incubation period already, with the result that the formation of the whole enzymic apparatus rather than only a particular, susceptible step of the shikimic acid pathway, was blocked. One may assume that the situation will be different when the seedling material, before starting its treatment with glyphosate, is preilluminated without the presence of the inhibitor. During the period of preillumination the shikimic acid pathway can probably reach the level of functioning, which is, normally, characteristic of illuminated tissues, so that glyphosate when introduced at later stages of incubation could really exert its action mainly through the blockage of 3-enolpyruvylshikimate-5-phosphate synthase, the primary molecular target of the herbicide (Steinrücken, Amrhein, 1980).

To check this possibility, excised buckwheat cotyledons and hypocotyls of various age were illuminated in distilled water for 6 hrs and only thereafter transferred into a solution of glyphosate for a total 40-hr-incubation either in complete darkness, under varying light-dark regimes, or under continuous illumination followed by the assay of shikimate. In a parallel series of experiments the excised plant material was subjected to glyphosate treatment without preillumination.

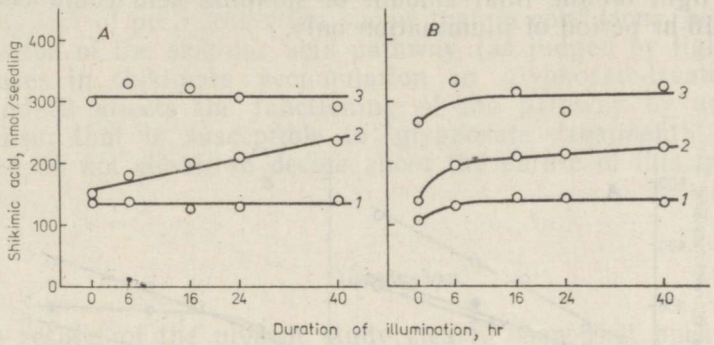

Fig. 3. Amount of shikimic acid produced in glyphosate-treated buckwheat hypocotyls during a 40-hr period of incubation either preceded (*A*) or not (*B*) by a 6-hr preillumination of the material in water. The 40-hr treatment with glyphosate was carried out either in the dark or under continuous illumination (extreme variants of the regime) or involved an initial period of illumination of various durations followed by an incubation in the dark until the end of the experiment. Age of seedlings at the time of glyphosate application — 66 (*1*), 80 (*2*), and 96 (*3*) hrs.

In preilluminated hypocotyls (Fig. 3*A*), in case of 66- and 96-hr-old material, the rate of shikimate accumulation in the glyphosate-containing medium proved to be similar under all incubation regimes tested: there was practically no difference between the final amount of shikimic acid synthesized during a 40-hr incubation in the dark or in the light and at various intermediate light-dark regimes as well. However, in 80-hr seedlings a slight stimulating effect of light, proportional to the duration of illumination, was observed. The rate of dark accumulation of shikimic acid in hypocotyls not preilluminated in water (Fig. 3*B*, the first points of the curves) was considerably lower than in the preilluminated hypocotyls of the same age. A short exposure of hypocotyls to the light, however, raised the level of the functioning of the shikimic acid pathway to its normal maximum also in that material, so that beginning with the illumination regime 6L + 34D the two sets of hypocotyls (preilluminated as well as not preilluminated in water) started to accumulate equal and stable amounts of shikimate. These data indicate that in hypocotyls a distinct stimulatory effect of light on the shikimic acid pathway needs rather short duration of illumination to become apparent, and that the level of light saturation of that process can be achieved already within the first 6 hrs of illumination, if not earlier. The absolute effect of light in hypocotyls illuminated during 6 hrs was identical both in the presence and absence of the inhibitor, the biosynthetic capacity of the pathway depending solely on the age of the seedlings.

In buckwheat cotyledons the effect of light on shikimic acid accumulation had a different character. A supplementary light treatment in glyphosate subsequent to preillumination in water caused in that material a further increase in the accumulation of shikimic acid during a 40-hr period of incubation. In all age groups examined, the stimulation was practically proportional to the duration of supplementary illumination, being greatest in 80-hr-old cotyledons (Fig. 4*A*). Shikimate accumulation in a parallel set of cotyledons not subjected to preillumination in water (Fig. 4*B*) showed a similar dependence on the duration of illumination. However, in 96-hr-old cotyledons increasing stimulatory effect of light on the final amount of shikimic acid could be observed within a 16-hr period of illumination only.

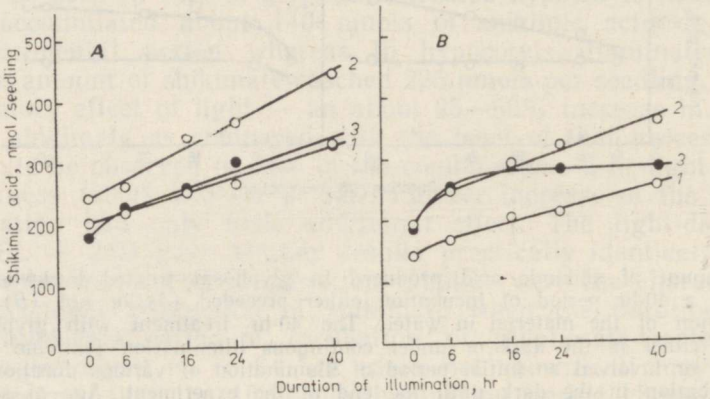

Fig. 4. Amount of shikimic acid produced in glyphosate-treated buckwheat cotyledons during a 40-hr period of incubation either preceded (*A*) or not (*B*) by a 6-hr preillumination in water. Treatment conditions as in Fig. 3.

A closer examination of the curves in Fig. 4*A* and 4*B* reveals that prolongation of the light period by 1 hr (within the total 40-hr period of incubation) resulted in a 3—4- or 5—6-nmol increase (in 66- or 80-hr age group, respectively) in the shikimate production per pair of cotyledons both in preilluminated and not preilluminated material. Thus, the degree of activation of the shikimic acid pathway by light seems to be virtually the same in both cases. However, the initial point of the curves expressing the level of dark accumulation of shikimic acid starting from the moment of glyphosate application was in preilluminated cotyledons much higher than in the cotyledons not exposed to a similar preceding light treatment in water. It means that light effects resulting from various light-dark regimes during the basic 40-hr period of incubation of plant material in glyphosate actually started to develop from different initial levels of activity of the shikimic acid pathway in these two sets of cotyledons. The effects, nevertheless, remained equal by their absolute range. For that reason in preilluminated cotyledons, due to an elevated initial level of activity of the pathway evoked by that preceding light treatment, the total production of shikimate during the 40-hr period of incubation in glyphosate proved to be much greater than it was in cotyledons not preilluminated.

The raised initial activity of the pathway in preilluminated cotyledons established in 66- and 80-hr-old tissues was not observed in 96-hr cotyledons (cf. 0-points of curves 3 in Fig. 4*A* and 4*B*), although in this plant material the stimulatory effect of light on the shikimic acid pathway, similar to the other cases, increased with the duration of illumination during the whole 40-hr period of glyphosate treatment. Hence, in spite of the fact that the general synthesizing capacity of the pathway in 96-hr-old cotyledons remained lower than it was in 80-hr-old tissues, under prolonged light exposures the absolute amount of shikimic acid produced during 40 hrs in preilluminated cotyledons was greater than that in the cotyledons not preilluminated before glyphosate treatment.

The effect of preillumination shows that in cotyledons, besides a photoactivation of the shikimic acid pathway (as judged by light-dependent differences in shikimate accumulation in glyphosate-treated tissues), light indeed affects the functioning of the pathway by an additional mechanism that is susceptible to glyphosate treatment. The results obtained do not enable to decide about the nature of this mechanism.

Discussion

The results of the present study clearly show that buckwheat hypocotyls and cotyledons differ by the light dependence of the shikimic acid pathway in these organs. In hypocotyls, the effect of light on the functioning of the pathway is rather limited. A 6—10-hr light treatment seems to be sufficient to bring about maximum stimulation of the process. This is demonstrated by the identity of kinetic curves of shikimate accumulation in the dark and in the light after a 10-hr exposure of hypocotyls to light (Fig. 1), by the lack of additional light effects in case of prolonged illumination (Fig. 2), and by the fact that the stimulatory effect of a short preillumination in water on the shikimate formation was not increased by a further illumination of hypocotyls during their subsequent treatment with glyphosate (Fig. 3A).

The light dependence of the shikimic acid pathway in cotyledons was much more pronounced. It became evident from the immediate decline of kinetic curves of shikimate accumulation after the illuminated cotyledons were transferred into darkness (Fig. 1) and from the continuous increase in the total production of shikimate in glyphosate-treated cotyledons under prolonged illumination during that treatment (Fig. 2 and 4). Besides that, the clear-cut preillumination effect on the functioning of the shikimic acid pathway in cotyledons indicated that in this organ photostimulation of the pathway could be effected through two different mechanisms.

The more distinct light dependence of the shikimic acid pathway in buckwheat cotyledons might perhaps be explained by the more important role of the plastidic compartment in that seedling organ as compared with its role in hypocotyls. Firstly, the presence and functioning of photosynthetic apparatus in cotyledons creates in their cells favourable conditions for plastidic protein synthesis, enabling, among other proteins, enhanced formation of the shikimic acid pathway enzymes. Secondly, photosynthesis supplies the shikimate pathway with substrates and energetic factors. Thirdly, illumination increases permeability of cell membranes facilitating intracellular transport of these molecules. In detached buckwheat hypocotyls where storage materials are practically absent, the chloroplasts are rather faintly developed and, hence, the photosynthesizing capability is negligible, the light-induced changes in membrane permeability cannot contribute much to enhancing the pre-

cursor supply for the shikimic acid pathway. A possible direct influence of illumination on the activity of enzymes of that pathway could not lead to a significant stimulation of the pathway under such circumstances.

A comparison of the present results with the earlier data of our laboratory on the photocontrol of the formation of various buckwheat flavonoids (Hallop, Margna, 1968, 1969; Халлоп, Маргна, 1970a, 1970b; Margna et al., 1973) allows to conclude that the organ-dependent light responses of the two metabolic processes — shikimate production and biosynthesis of flavonoids — are rather similar. Calculations made on the basis of these studies show that in hypocotyls, a 12-hr light exposure is sufficient to bring about maximum light stimulation of the accumulation of rutin and leucoanthocyanidins which together form 98% of the total sum of flavonoids in that seedling organ. In cotyledons, the amount of flavonoids produced in response to light treatments showed practically linear dependence on the duration of the illumination period. This similarity of responses indicates that light dependence of the two processes may be interrelated, and that at least a part of the changes in the accumulation of flavonoids induced by light may directly arise from the preceding light-induced changes in the production of their primary precursors shikimate and L-phenylalanine.

The same interrelations may also play a role in age-dependent differences of the two processes. It is generally known that flavonoid formation depends on the age of seedlings. The highest accumulation rate of flavonoids and other secondary metabolites and maximum activity of the enzymes of phenylpropanoid metabolism have been established in young differentiating tissues (references in Wiermann, 1981). Information about the age dependence of the shikimic acid pathway is scanty (see Schmidt et al., 1987). In a recent investigation with barley leaves (Тохвер, Пальм, 1991) it was observed that with increasing leaf age the activity of the pathway decreased. Considering these results and the fact that in buckwheat hypocotyls the capacity for anthocyanin formation shows the highest level at the age of 6—7 days (Karstens, 1939; Mohr, van Nes, 1963), the present experiments were designed in the hope that the results could also shed some light on the possible age dependence of the functioning of the shikimic acid pathway in buckwheat seedlings. However, the results obtained proved to be not too informative in this respect. In hypocotyls the activity of the pathway, as judged by the absolute amount of shikimic acid synthesized in glyphosate-treated tissues during 40 hrs, clearly increased with the seedling age (Fig. 3), whereas in glyphosate-treated cotyledons the highest rate of shikimate accumulation was found to occur in 80-hr-old seedlings (Fig. 4, see also Fig. 1). The preillumination effect on dark accumulation of shikimic acid in cotyledons was, however, greatest in the youngest seedlings. Hence, it is not excluded that in buckwheat seedlings the highest responsiveness of the shikimic acid pathway to illumination as well as the highest general capacity of the pathway for producing shikimate occur at different age in hypocotyls and cotyledons. Due to that also the possible age-dependent interrelations between the shikimic acid pathway and the formation of phenolics may not be followed so simply but need more sophisticated experimental approaches.

In further investigations in this field it is important to focus attention on the clarification of the role of ontogenetic factors in development of the synthesizing potential of the shikimic acid pathway during the normal greening process of etiolated seedlings. Parallel to the production of aromatic amino acids via the shikimate pathway, their consumption in protein and phenolic synthesis should be necessarily studied to establish control mechanisms operating in these metabolic areas.

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REFERENCES

Amrhein, N., Holländer, H., 1981. Light promotes the production of shikimic acid in buckwheat. — Naturwiss., **68**, 43.

Amrhein, N., Deus, B., Gehrke, P., Steinrücken, H. C., 1980. The site of the inhibition of the shikimate pathway by glyphosate. II. Interference of glyphosate with chorismate formation *in vivo* and *in vitro*. — Plant Physiol., **66**, 830—834.

Gaitonde, M. K., Gordon, M. W., 1958. A microchemical method for the detection and determination of shikimic acid. — J. Biol. Chem., **230**, 1043—1050.

Hallop, L., Margna, U., 1968. Antotsüaani moodustumise kineetika tatraidandite hüpokotüülides, olenevalt indutseeriva valgusperioodi kestusest ja valguse intensiivsusest. — Eesti NSV TA Toim., Biol., **17**, 154—163.

Hallop, L., Margna, U., 1969. Rutiini moodustumise kineetika tatraidandite hüpokotüülides olenevalt valgustusest. — Eesti NSV TA Toim., Biol., **18**, 184—195.

Holländer, H., Kiltz, H.-H., Amrhein, N., 1979. Interference of L-α-aminooxy-β-phenylpropionic acid with phenylalanine metabolism in buckwheat. — Z. Naturforsch., **34c**, 1162—1173.

Karstens, W. K. H., 1939. Anthocyanin and anthocyanin formation in seedlings of *Fagopyrum esculentum* Moench. — Recueil Trav. bot. néerl., **36**, 85—179.

Margna, U., Laanest, L., Margna, E., Vainjärv, T., 1973. Light-stimulated accumulation of leucoanthocyanidins and other flavonoids in buckwheat seedlings. — Proc. Acad. Sci. ESSR. Biol., **22**, 226—232.

Margna, U., Margna, E., Vainjärv, T., 1989. Influence of nitrogen nutrition on the utilization of L-phenylalanine for building flavonoids in buckwheat seedling tissues. — J. Plant Physiol., **134**, 697—702.

Mohr, H., van Nes, E., 1963. Der Einfluß sichtbarer Strahlung auf die Flavonoid-Synthese und Morphogenese der Buchweizen-Keimlinge (*Fagopyrum esculentum* Moench). I. Synthese von Anthocyan. — Z. Botanik, **51**, 1—16.

Schmidt, C. L., Gross, C., Hennig, H., Homeyer, U., Fiedler, E., Schultz, G., 1987. The introductory enzymes of shikimate pathway in spinach (*Spinacia oleracea* L.): general features of enzymes and possible mode of regulation of aromatic compound synthesis in eucaryotic cells. — Plant Physiol. (Life Sci. Adv.), **6**, 35—42.

Steinrücken, H. C., Amrhein, N., 1980. The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimic acid-3-phosphate synthase. — Biochem. Biophys. Res. Comm., **94**, 1207—1212.

Wiermann, R., 1981. Secondary plant products and cell and tissue differentiation. — The Biochemistry of Plants, Vol. 7, Acad. Press, Inc., 85—116.

Тохвер А. К., 1990. Влияние интенсивности освещения и качества света на накопление шикимовой кислоты в проростках гречихи под воздействием глифозата. — Физиол. растений, **37**, 712—717.

Тохвер А. К., Мядамюрк У. В., 1984. Взаимодействие фитохрома и синего света в фоторегуляции образования антоцианов в проростках гречихи. — Физиол. растений, **31**, 1071—1076.

Тохвер А. К., Пальм Э. В., 1986. Светозависимость ингибирующего действия глифозата на шикиматный путь у семядольных листьев проростков гречихи. — Физиол. растений, **33**, 972—978.

Тохвер А. К., Пальм Э. В. 1991. Интенсивность функционирования шикиматного пути и накопление фенольных соединений в листьях ячменя разного возраста. — Физиол. растений, **38**, 485—491.

Тохвер А., Ыннепалу У., 1982. О роли шикиматного пути в фотостимуляции образования антоцианов в проростках гречихи. — Изв. АН ЭССР. Биол., **31**, 213—218.

Халлоп Л., Маргна У., 1970a. Влияние света на образование гликофлавонов в проростках гречихи. — Изв. АН ЭССР. Биол., **19**, 167—171.

Халлоп Л., Маргна У., 1970b. О светозависимости образования антоцианов и рутина в семядольных листочках проростков гречихи. — Изв. АН ЭССР. Биол., **19**, 17—24.

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Lembe LAANEST, Tiiu VAINJÄRV

ŠIKIMAATSE TEE VALGUSTUNDLIKKUS TATRAIDANDITES

Šikimaatse tee aktiivsuse uurimiseks blokeeriti šikimaadi edasine kasutamine glüfosaadiga ja jälgiti sellest tulenevat šikimihappe kogunemist tatraidandite isoleeritud idulehtedes ja iduvartes. Leiti, et valgustatud idulehtede paigutamisel pimedusse langeb šikimaatse tee aktiivsus järsult; erinevus 48 tunni jooksul pimedas ja valges moodustunud šikimihappe hulga vahel oli kuni kahekordne idandite vanusest sõltuvalt. Iduvartes valgustusrežiimi muutmisest tingitud olulisi erinevusi ei leitud. Tehti kindlaks, et selles organis avaldab valgus šikimaatsele teele maksimaalset aktiveerivat toimet juba esimese 6—10 tunni jooksul, seejärel šikimihappe edasine sünteesikiirus ei sõltu valgustustingimustest. Idulehtedes aga toimub protsessi pidev valgusstimulatsioon ka valgustusaja pikendamisel 64 tunnini. Šikimaatse tee valgustundlikkuse erinev iseloom iduvartes ja idulehtedes langeb kokku varasemates töödes ilmnenud iseärasustega flavonoidide moodustumise osas analoogilise katsekorralduse puhul. Eelvalgustuskatsete tulemused lubavad järeldada, et idulehtedes stimuleerib valgus šikimaatset teed lisaks otsesele ensümaatilise aktiivsuse tõstmisele veel mingi teise toimemehhanismi kaudu. Šikimaatse tee valgustundlikkuse erinevused eri vanusega idandites lubavad oletada, et šikimihappe sünteesi maksimaalne potentsiaal kujuneb idandite eri organites eri aegadel.

Лембе ЛААНЕСТ, Тийу ВАЙНЯРВ

СВЕТОЗАВИСИМОСТЬ ШИКИМАТНОГО ПУТИ В ПРОРОСТКАХ ГРЕЧИХИ

Для оценки активности шикиматного пути в проростках гречихи изучали накопление шикимовой кислоты в изолированных семядольных листьях и гипокотилях гречихи, обработанных глифосатом. Обнаружили, что после перенесения освещенных семядольных листьев в темноту интенсивность функционирования шикиматного пути резко падает; количества шикимовой кислоты, синтезированной за 48 ч в темноте и на свету, различались до двух раз. В гипокотилях изменение режима освещения практически не влияло на кинетику накопления шикимовой кислоты. Установили, что в этом органе максимальное возможное влияние света на функционирование шикиматного пути осуществляется уже в течение первых 6—10 ч освещения, а дальнейшая модификация режима освещения на скорость образования шикимовой кислоты дополнительного влияния уже не оказывает. В семядольных листьях не обнаруживали ослабления стимулирующего действия света даже при 64-часовом освещении. Неодинаковый характер светозависимости шикиматного пути в семядольных листьях и гипокотилях совпадает с установленными ранее особенностями светозависимости накопления флавоноидов в аналогичных условиях. Результаты экспериментов с предварительным освещением проростков в воде без присутствия глифосата указывают на то, что в семядольных листьях свет не только активирует энзимы шикиматного пути, но стимулирует этот путь и через какой-то другой механизм. На основе различий светозависимости шикиматного пути в проростках разного возраста можно предполагать, что максимальный потенциал функционирования его формируется в разных органах проростков в разное время.