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LIGHT-STIMULATED ACCUMULATION OF LEUCOANTHOCYANIDINS AND OTHER FLAVONOIDS IN BUCKWHEAT SEEDLINGS

Introduction

In an earlier series of light studies conducted in this laboratory, rather great quantitative differences were established between the light-dependent accumulation of anthocyanins, rutin, and glycoflavones synthesized in buckwheat seedlings (Hallop, Margna, 1968, 1969; Халлоп, Маргна, 1970a, b). Concerning dark-grown plants, considerable amounts of the two latter compounds and likewise measurable amounts of anthocyanins are produced in their cotyledons without any light participation, the average content of these substances at an age of 90 hr being approximately equal to 110, 28, and 0.15 micrograms per seedling for glycoflavones, rutin, and anthocyanins, respectively. In hypocotyls not capable of producing glycoflavones, the amount of dark-synthesized rutin is about 9 times lower than in cotyledons, whereas anthocyanins are not formed in the absence of light or are produced in trace amounts only, not measurable by the routine quantitative methods. After an exposure of etiolated seedlings to light, a marked stimulation of anthocyanins and rutin in both organs is usually induced. Providing that 3-days-old etiolated seedlings are employed, and white fluorescent light of a moderate intensity (ca 28,000 erg·cm^{-2}·sec^{-1}) is used for irradiation, an about 6-fold maximal increase in the amount of rutin is observed in hypocotyls under saturating or nearly saturating durations of illumination (ca 24 hr), whereas the content of anthocyanins, during a 48-hr period of continuous illumination, not yet completely saturating for the process of their accumulation, is increased from zero to about 2.5 micrograms of pigments per seedling. In cotyledons, shorter durations of illumination are sufficient (12 — 15 hr for accumulation of anthocyanins, one hour or even less for rutin) to reach the boundary at which there begins a dramatic decrease in the capacity of seedlings to respond positively to further prolongation of light exposures. As a result, only an about 2 — 2.5-fold maximal increase in the content of rutin and a 13-fold increase in the amount of anthocyanins can be achieved in this organ by light treatment.

It follows, therefore, that in cotyledons the range of light-induced stimulation of flavonoid accumulation is much smaller, relatively, than in hypocotyls, while in both cotyledons and hypocotyls the processes related to the formation of rutin are less susceptible to light action than those leading to the accumulation of anthocyanins. The formation of glycoflavones in cotyledons is still less dependent upon the presence of light

during the period of their accumulation and is not significantly stimulated by short exposures rather effective in promoting elevated synthesis of rutin and anthocyanin pigments. A certain increase in the accumulation of these specific compounds occurs only when a prolonged illumination of seedlings is used (Халлоп, Маргна, 1970b; Маргна, Халлоп, 1971).

In connection with that, a question arose as to which may be the comparative effect of light on the accumulation of leucoanthocyanidins, the fourth group of buckwheat flavonoids, which have not been involved in our light studies previously. Various experimental investigations of this laboratory have demonstrated that the leucoanthocyanidin-forming processes in buckwheat seedlings are rather stable against environmental influences, revealing but slight tendencies to change under conditions generally found to be inhibitory or stimulatory for anthocyanin formation (Маргна, Оттер, 1971; Margna et al., 1972, 1973). Concerning light influence, only limited information is yet available on buckwheat seedlings. J. R. Troyer (1964) has shown that light has no effect on the accumulation of leucoanthocyanidins in excised hypocotyls, whereas H. Scherf and M. H. Zenk (1967) have reported that a light-induced augmentation of leucoanthocyanidin formation occurs in the hypocotyls of intact seedlings. The cotyledons were not studied by these investigators.

The present work deals with experiments in intact buckwheat seedlings, involving measurements of both hypocotyls and cotyledons.

Experimental

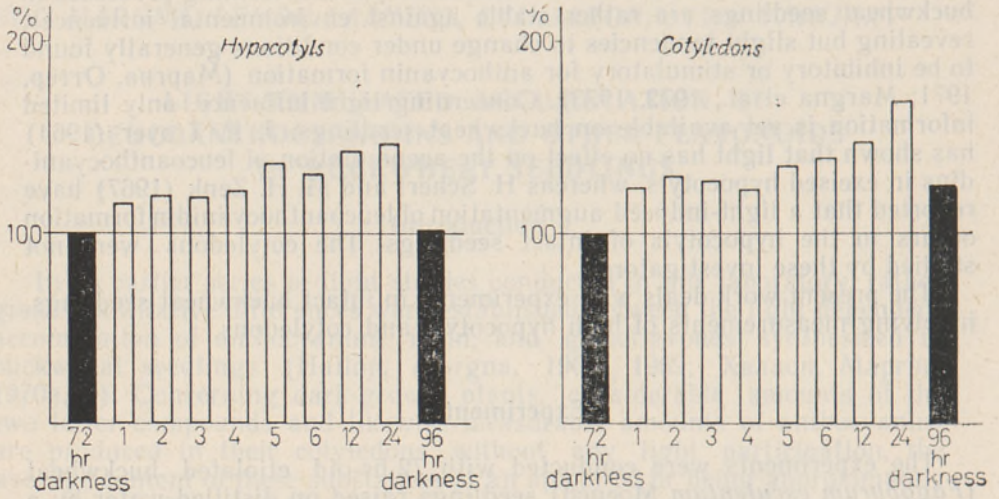
The experiments were conducted with 72-hr-old etiolated buckwheat (*Fagopyrum esculentum* Moench) seedlings raised on distilled water by a standard procedure generally employed in this laboratory (Hallop, Margna, 1968, 1969). The seedlings were exposed to light for various periods and were then returned to darkness until harvesting at the 24th or 48th hr after the onset of the illumination program (at the 96- or 120-hr-age of seedlings, respectively). The plants continuously exposed to light during the whole 24-hr or 48-hr experimental period were assayed immediately after the end of illumination. Two dark controls were run in both series, one of them being assayed at the beginning of the light treatment, and the other — at the end of the experimental period. The light conditions were the same as used by us previously: illumination from white fluorescent tubes, light intensity 28,000 erg·cm^{-2}·sec^{-1}. The temperature was held constant at 25±1 °C in all experiments.

The leucoanthocyanidins were determined by the prescriptions of V. S. Govindarajan and A. G. Mathew (1965) from samples of 150—180 hypocotyls or pairs of cotyledons, respectively. The results were expressed in micrograms per seedling by using for calculations an extinction coefficient 2.7·10^7 cm^2/Mol for cyanidin (Scherf, Zenk, 1967). As no more than about one-third of the total leucoanthocyanidins present in a plant material can be transformed into anthocyanidins by the known quantitative methods for leucoanthocyanidins, including that employed here (Scherf, Zenk, 1967; Govindarajan, Mathew, 1965; Swain, Hillis, 1959), a correction factor 3.0 was used to compute, from the values measured, the actual absolute amounts of leucoanthocyanidins in the plant material.

All experiments were carried out in 3—4 replications, the determination of leucoanthocyanidins in each of them being performed on 5—6 occasions.

Results

The data on seedlings assayed at the 24th hr after the onset of illumination are presented in Fig. As can be seen from the diagram, a certain light-enhanced accumulation of leucoanthocyanidins took place in both seedling organs, but the stimulation, in relative units, was comparatively small, not reaching the level characteristic of rutin and anthocyanin pigments. Thus, the leucoanthocyanidin-forming processes revealed here



The effect of light on the accumulation of leucoanthocyanidins in buckwheat seedlings. Black bars — dark controls, white bars — illuminated seedlings. Numerals below the white bars indicate the duration of illumination (hr) within a total 24-hr experimental period. The 100 per cent line corresponds to an average content of leucoanthocyanidins of about 70 and 130 micrograms per seedling in hypocotyls and cotyledons of 72-hr-old etiolated seedlings, respectively.

almost the same inertness against the modifying factor as it was observed under the influence of temperature (Margna et al., 1973) and some other stress agents (Маргна, Оттер, 1971; Margna et al., 1972). Nevertheless, it is obvious that in hypocotyls an increase in the content of leucoanthocyanidins can be obtained already after rather short (one hr or less) light exposures. By longer durations of illumination, the range of light effect was gradually increased to reach, under conditions of 24-hr continuous illumination, an about 45 per cent maximal stimulation of leucoanthocyanidin accumulation as compared with the content of these compounds in dark controls. During this 24-hr period the level of dark-synthesized leucoanthocyanidins remained practically unchanged (cf. the corresponding data of H. Scherf and M. H. Zenk (1967) for elder seedlings).

In cotyledons, an about 25 per cent dark increase in the amount of leucoanthocyanidins spontaneously occurred during the experimental period, resulting in that the effect of light was less pronounced than in hypocotyls. Significant stimulation of leucoanthocyanidin accumulation was therefore not manifested in these organs until the duration of light exposure of seedlings was prolonged to about 6 hr. In accordance with that, the maximal relative increase in the content of leucoanthocyanidins was much smaller, not exceeding the 32 per cent level in seedlings illuminated continuously.

Identical were the results in seedlings assayed at the 48th hr after the onset of the illumination program, except that in this series the relative increase in the content of leucoanthocyanidins in plants continuously irradiated was somewhat higher than in the preceding ones: about 68 per cent in hypocotyls and about 40 per cent in cotyledons.

Despite the comparatively small relative effect, the absolute light-induced increase in the content of leucoanthocyanidins was rather great. Both seedling organs are capable of synthesizing large amounts of leucoanthocyanidins in complete darkness, their average dark level in 96—120-hr-old etiolated plants being equal to about 70 and 160 micrograms per seedling in hypocotyls and cotyledons, respectively. On that high background, the above comparatively small per cent increases correspond to about 32 micrograms of absolute increase of leucoanthocyanidins in hypocotyls and to about 52 micrograms of leucoanthocyanidins in cotyledons of seedlings continuously illuminated during a 24-hr period,

Absolute light-induced increases in the amount of flavonoids in 5-days-old buckwheat seedlings continuously illuminated during the final 48-hr period of development
(micrograms per seedling)

Flavonoid compound	Hypocotyls	Cotyledons
Anthocyanins	2.5	1.8
Rutin	19	42*
Glycoflavones	—	55*
Leucoanthocyanidins	48	65

* Expected values calculated from the corresponding data for seedlings continuously illuminated for 24 hr.

while in seedlings exposed to continuous light for 48 hr the respective absolute values were still greater. These quantities are much higher than are the absolute increases which, under comparable light conditions, can be recorded in the content of anthocyanins and also of rutin and glycoflavones (Table).

Discussion

The results suggest that the stimulatory action of light in buckwheat seedlings cannot be related specifically to the accumulation of anthocyanins or of any other particular flavonoid derivative only, but represents a process of general significance which covers the whole flavonoid complex of the plant. It strengthens the opinion that the primary light action is localized somewhere outside the range of specific flavonoid pathway of biosynthesis, at some critical metabolic step before the typical C_{15}-flavonoid skeleton is built up. The resulting favourable influence on the level of flavonoids is probably mediated through the light-induced formation of some common substrate material(s) which is equally important for building up all flavonoid compounds. Without attempting to interpret the possible nature of this substrate material, it is obvious that in etiolated seedlings the material is available for flavonoid synthesis in limited amounts only, its dark level being especially low in hypocotyls. This presumably serves as the main endogenous factor which is responsible, first of all, for the comparatively small dark production of flavonoids (up to a complete inability to accumulate anthocyanins without illumination) by

the latter organ, and, secondly, for the much higher light-sensitivity of flavonoid-forming processes in hypocotyls as compared with cotyledons.

In similar terms of substrate availability can be interpreted also the observed quantitative differences between the light-dependent accumulation of various flavonoids in both hypocotyls and cotyledons. All these variations are likely called forth by marked differences in the rate at which the biosynthetic apparatus of separate flavonoids is capable of consuming the postulated common substrate from a common cellular pool. Taking it into consideration, an inference logically ensues that the substrate-consuming rate in question must be the highest in leucoanthocyanidin-forming processes, to some extent smaller in processes leading to the formation of glycoflavones and rutin, and the lowest in processes related to the accumulation of anthocyanin pigments. This provides a satisfactory explanation why the leucoanthocyanidin-forming processes seem to be most saturated with the postulated substrate in darkness and show the smallest relative, and the largest absolute increase of production when extra amounts of substrate materials are supplied by light induction. Similarly it allows to explain why the anthocyanin-forming processes, respectively, occupy the other extreme position, whereas glycoflavones and rutin are intermediate in all these respects.

The situation can be easily understood when one bears in mind the assumed differences in the length and number of separate steps within the terminal part of the biosynthetic pathway of flavonoids from a common key metabolite up to the completion of building of a particular flavonoid derivative, i. e. differences in the biochemical features which supposedly may play an important role in determining the rate at which a common substrate can travel through the whole sequence of reactions in order to yield final products. As there are all probabilities that various flavonoids in this respect can be arranged in the manner "flavanols and flavandiols (resp. catechines and leucoanthocyanidins) — flavones — flavonols — anthocyanins", the members standing left generally having simpler biosynthetic routes than the following ones (Bate-Smith, Lerner, 1954; Harborne, 1962), a strict parallelism with the arrangement of the same compounds with regard to the light-dependence of their accumulation is rather unequivocal.

It is not completely excluded that a certain part of light influence is still somehow mediated through a direct action of light on the enzymic systems immediately functioning within the intimate flavonoid area of biosynthesis. Recent investigations of K. Hahlbrock and his collaborators (Hahlbrock et al., 1971) have shown that some if not all of these enzymes can be activated by light, the resulting rise in their activity frequently paralleling a simultaneous rise in the accumulation of flavonoids. To which extent these enzymic changes may be determining for the general stimulatory influence of light on flavonoid accumulation, remains to be solved in further experiments.

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VALGUSE STIMULEERIV MÕJU LEUKOANTOTSÜANIDIINIDE JA TEISTE FLAVONOIDIDE MOODUSTUMISELE TATRAIDANDEIS

Resümee

Valguse toimel suurenes leukoantotsüanidiinidesisaldus nii tatraidandite hüpokotüülides kui ka idulehtedes. Kuna aga nende ühendite süntees mõlemas mainitud organis kulges märgatava intensiivsusega ka pimedas, siis jäi üldefekt suhteliselt väikeseks. Absoluuthulkades oli leukoantotsüanidiinidesisalduse tõus üsna suur, ületades võrreldavates valgustingimustes esinevad antotsüaanide-, rutiini- ja glükoflavoonidesisalduse maksimaalsed juurdekasvud tunduvalt. Katse tulemuste põhjal järeldatakse, et valguse stimuleeriv toime flavonoidide biosünteesisse realiseerub mingi ühise substraadi indutseeritud moodustumise kaudu, kvantitatiivsed erinevused eri klassidesse kuuluvate flavonoidide moodustumises sõltuvalt valgusest aga tulenevad selle substraadi kasutamise kiirusest eri derivaatide biosünteesil.

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СТИМУЛИРУЮЩЕЕ ВЛИЯНИЕ СВЕТА НА НАКОПЛЕНИЕ ЛЕЙКОАНТОЦИАНИДИНОВ И ДРУГИХ ФЛАВОНОИДОВ В ПРОРОСТКАХ ГРЕЧИХИ

Резюме

Воздействие светом на проростки гречихи приводило к накоплению лейкоантоцианидинов как в гипокотилях, так и в семядольных листочках, но так как значительные количества этих флавоноидов синтезируются и в полной темноте, то общий стимулирующий эффект остался относительно низким. Абсолютные же приросты в содержании лейкоантоцианидинов были в обоих органах весьма большими и значительно превышали соответствующие приросты в содержании антоцианов, рутина и гликофлавонов. Предполагается, что действие света на формирование флавоноидов связано со светиндуцированным накоплением какого-то общего для них предшественника, а количественные различия в конечном эффекте света по отдельным флавоноидам обусловлены различиями в скорости использования этого субстрата на путях биосинтеза отдельных производных.

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