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BIOSYNTHESIS OF ANTHOCYANINS: A PROCESS NOT SPECIFICALLY CONTROLLED BY LIGHT

Stimulatory action of light on the formation of anthocyanins and other flavonoids is well documented by numerous data but little is known about the mechanisms actually operating in plant cells under light conditions. For the synthesis of anthocyanins, in contrast to other flavonoids, illumination often seems to be an obligatory prerequisite. It is widely believed, therefore, that two different mechanisms are involved and that the biosynthetic pathway related to the formation of anthocyanins is in some way specifically controlled by light. An unprejudiced analysis of various observations and experimental data show, however, that the apparent difference in the light dependence between the formation of anthocyanins and other flavonoids can well be explained in purely quantitative terms without any need of postulating the existence of a special light-sensitive system for governing the anthocyanin pathway. Convincing evidence is accumulating that stimulatory light effects on flavonoid accumulation are not due to a direct action of light on flavonoid enzymes but probably arise from the increased availability and production of initial substrates (Pecket, Bassim, 1974a, b; Bassim, Pecket, 1975; Amrhein, Holländer, 1981; Margna, Vainjärv, 1983; Тохвер, Ыннепалу, 1982; Маргна et al., 1983).

In the present paper we intended to provide an up-to-date survey of available data on the accumulation of anthocyanins in plant tissues not exposed to light. Observations and experimental facts on that matter, continuously becoming more numerous and more diverse, constitute a substantial body of information that does not agree with the widespread idea of the distinctive character of light dependence of anthocyanin biosynthesis. Thus, reviewing these data, we pursued the principal objective to demonstrate that i) many if not the majority of anthocyanin-synthesizing tissues are capable of accomplishing that capacity in complete darkness as it is generally observed in the case of synthesizing flavonols, flavones and any other flavonoid compounds, and ii) great variations in the stimulatory effect of light between various anthocyanin-producing plants, on the one hand, and between the accumulation of anthocyanins and other flavonoids, on the other, are actually all of quantitative nature, with the primary light action located at points not obviously related to the particular enzymic apparatus participating in the biosynthesis of anthocyanins.

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It must be noted, first of all, that plants and tissues capable of synthesizing anthocyanins in complete darkness are not nearly rare but, in point of fact, numerous. As can be seen from Table 1, so far at least 14 different kinds of seedlings, 4 tissue cultures, two kinds of fruits, assimilatory organs of several aquatics, leaves of a terrestrial plant, root tissues

Plants and tissues capable of synthesizing anthocyanins without action of light

Plant	Organ, tissue	References
Red cabbage (*Brassica oleracea* f. *rubra*)	Seedlings	Paech, Eberhardt, 1952; Eberhardt, 1954 ;Kandeler, 1960; and others
Brassica juncea	Seedlings	Kandeler, 1960
Turnip (*Brassica rapa*)	Excised cotyledons	Grill, Vince, 1964, 1969
Maize (*Zea mays*)	Endosperm tissue culture, shoots, aleurone tissue	Straus, 1959; Lawanson et al., 1975; McCormick, 1978
Buckwheat (*Fagopyrum esculentum*)	Cotyledons	Margna et al., 1973; Маргна и др., 1983; and others
Mustard (*Sinapis alba*)	Seedlings	Kandeler, 1960; Schopfer, 1967; Whitelam, Johnson, 1981
Rye (*Secale cereale*)	Coleoptiles	Станко, Закман, 1964; Булах, Гродзинский, 1970
Impatiens balsamina	Hypocotyl segments*, detached petals	Arnold, Alston, 1961; Klein, Hagen, 1961
Sorghum vulgare	First internodes of shoots	Stafford, 1965, 1966
Radish (*Raphanus sativus*)	Seedlings	Bellini, Martelli, 1973
Bean (*Phaseolus vulgaris*)	Seedlings	Withrow et al., 1953
Mung bean (*Vigna radiata*/ *Phaseolus aureus*)	Cotyledons	Dumortier, Vendrig, 1978
Sunflower (*Helianthus annuus*)	Excised cotyledons	Servettaz et al., 1975
Vitis vinifera, 'Saperavi'	Shoot tissue culture*, fruit skin	Slabecka-Szweykowska, 1952; Дурмишидзе, 1955
Strawberry (*Fragaria grandiflora*)	Fruits	Hyodo, 1971
Saxifraga crassifolia	Leaf segments*	Paech, Eberhardt, 1952; Eberhardt, 1954; Eberhardt, Haupt, 1959
Hydrilla verticillata	Leaves*	Cited by Thimann, Edmondson, 1949
Spirodela oligorrhiza	Fronds	Thimann, Radner, 1955, 1958; Elliott, 1977
Hydrocharis morsus ranae	Turions*	Pogorzelska, 1965
Balsaminaceae, Saxifragaceae, Melastomaceae, Compositae and *Crassulaceae* spp. (and several other plants)	Root tips	Blank, 1958; Weber, 1954; Thakur, Nozzolillo, 1978
Crocus, Hyacinthus, Iris, Tulipa spp.	Petals	Cited by Blank, 1958
Petunia hybrida, mutant W18*	Petals	Kho et al., 1975, 1977
Matthiola incana mutant lines D5*, D6*, 18*	Petals	Forkmann, 1977
'Baccara' roses	Petals	Biran, Halevy, 1974
Carrot (*Daucus carota*)	Callus culture (strains R1*, R2, and R3); cell suspension culture	Alfermann et al., 1975; Ozeki, Komamine, 1981
Haplopappus gracilis	Cell suspension culture	Wellmann et al., 1976

* Start to synthesize anthocyanins in the dark when treated with sugars, growth regulators (see Table 2) or flavonoid precursors (see Text).

of a great variety of plants from the families *Crassulaceae*, *Saxifragaceae*, *Compositae*, *Balsaminaceae*, and *Melastomaceae*, and petals of many flower plants have been reported to produce visible amounts of anthocyanins without any light action.

In most cases the amount of anthocyanins synthesized in unilluminated tissues is very low quantitatively. However, in *Phaseolus* cotyledons, cress and red cabbage seedlings, several strains of carrot tissue cultures, ripening grapes and strawberries, and also in a variety of red and blue-coloured petals the production of anthocyanins in dark-developed material is quite considerable, being only 2—4 times lower than is characteristic of these tissues when illuminated normally. Petals of 'Baccara' roses developed from buds shaded at a stage when the buds were still completely unpigmented were reported to show even no difference in their anthocyanin pigmentation as compared with petals developed under normal light conditions (Biran, Halevy, 1974).

Of course, since no systematic investigation has been carried out on that particular aspect of anthocyanin formation, the list of plants presented in Table 1 is largely casual, in part unproportionately reflecting observations made primarily on a variety of popular model objects (seedlings). Therefore the list cannot be regarded as an illustrative set of data adequately representative of all the anthocyanin-synthesizing plants. Nevertheless, it covers quite a wide range of various plants and their organs. For that reason there are enough grounds to presuppose that the whole phenomenon is, in fact, a general one and that any of the cell types which genetically possess the ability of synthesizing anthocyanins can actually produce some amounts of these pigments also in unexposed material, provided that secondary limitations not directly related to the enzymes of anthocyanin biosynthesis do not totally block the realization of that ability. However, there may be cases when the level of anthocyanin synthesis in the dark remains below the limit necessary for allowing the presence of these pigments to be recorded either visually or instrumentally. Calculations made by us on buckwheat seedlings showed, for example, that the minimal amount of anthocyanins still detectable in that material is equal to about 0.02 micrograms or to approximately 10^{13} (!) molecules of anthocyanins per seedling.

In any case, the apparent incapability of a certain anthocyanin-synthesizing tissue to accumulate these pigments in the absence of light need not necessarily mean that the enzymic apparatus responsible for that biosynthesis remains functionally incompetent in the dark. In isolated petals of a white-flowered mutant (W18) of *Petunia hybrida* not capable of synthesizing anthocyanins due to a genetical block excluding transformation of the common flavanone precursor of flavonoids into dihydroflavones, anthocyanins readily started to synthesize when the petals were fed with exogenous dihydroquercetine. The process proved to be independent of light, and in several experiments even showed a somewhat higher rate of anthocyanin synthesis in the dark than under constant illumination (Kho et al., 1975, 1977). In similar experiments with three acyanic lines (D5, D6, and 18) of *Matthiola incana* having the genetical block specifically located at the condensation reaction of p-coumarate with three acetate-malonate moieties, the formation of marked amounts of anthocyanins could be easily initiated by introducing, into petals, exogenous chalcones, a flavanone (naringenin), or dihydroflavonols. As in the case of *Petunia*, also in these *Matthiola* mutants no difference with regard to the intensity of anthocyanin synthesis could be observed between the petals incubated with precursors in complete darkness or under normal daylight conditions (Forkmann, 1977).

These two examples (see also McCormick, 1978) clearly show that

even in those tissues which normally never produce anthocyanins a highly active enzymic apparatus for synthesizing these flavonoids may be present with the enzymes of the apparatus not requiring a pretreatment with light in order to become catalytically potent. Moreover, under a sufficient supply of suitable precursors from outside, illumination may not even exert a stimulatory action on the formation of anthocyanins and the whole process may thus remain completely unmodified by light.

There are many other data which do not agree with the view that a specific light-sensitive step is involved in the biosynthesis of anthocyanin-type flavonoid structures. The idea meets strong objections, for example, in the fact that the action of light on the accumulation of different anthocyanin derivatives in one and the same tissue, on the accumulation of anthocyanins in different organs of one and the same plant, etc., often manifests itself in a different fashion. For instance, in the first internodes of young sorghum shoots the formation of cyanidin-type anthocyanins can be observed only in light, whereas the formation of apigeninidin and luteolinidin anthocyanins differing from cyanidin in lacking a hydroxyl group at the C_3 position of the heterocyclic ring occurs also in unilluminated seedlings (Stafford, 1965, 1966). While small amounts of a pelargonidin derivative in maize seedlings seem to accumulate without any participation of light, the accumulation of a cyanidin glycoside, another anthocyanin typical of that plant, can be detected only when the leaf-sheaths rise above soil level and become exposed to light (Lawanson et al., 1975). Hypocotyls of etiolated buckwheat seedlings and of several other seedling species do not normally show visible anthocyanins, but in cotyledons of the same seedlings measurable or at least trace amounts of these flavonoids are almost always synthesized in the dark (Margna et al., 1973; Dumortier, Vendrig, 1978). If one supposes that in any of such cases differential light effects are related to regulatory mechanisms functioning at the enzymic level and the effects are mediated by a light-induced qualitative change in the catalytic potency of some specific enzyme, then one ought to admit the existence, in the biosynthetic chain of anthocyanins, of several light-sensitive enzymes with specific functions of these enzymes in every particular case being different, depending on the biological properties of the plant species or tissue. Additionally one should postulate that in different parts of one and the same plant the mechanisms involved in the light control of anthocyanin accumulation may be essentially different.

Since the biosynthesis of flavonoids is a highly unified metabolic process with no known deviation from the general sequence of reactions in any of the plant tissues so far studied, such diversity of basic mechanisms for the control of a particular branch of that pathway does not seem to be very likely. It is much more probable that the light-effects on the formation of anthocyanins are, in fact, of secondary nature and arise from some light-induced changes in the intracellular conditions which are able to influence the accumulation of these flavonoids independent of the actual catalytic potency of the relevant enzymes. Such changes may involve an increase in the supply of endogenous substrate materials necessary for building anthocyanins (and other flavonoids/phenolics), a removal of some restraint in the cell structural organization which impedes intracellular transport of substrate molecules to the site of anthocyanin (flavonoid) biosynthesis, and the like.

The apparent «incapability» of plants to synthesize anthocyanins without light influence as a phenomenon which can be only quantitative by its nature is clearly evidenced by the well-known fact that in apples, pears, and several other kinds of fruits the red pigmentation usually develops on the sunny side of the fruits, only, whereas their opposite side

as well as the fruit parts screened from direct sunlight by an occasional leaf often remain green with no traces of anthocyanins. The light dependence of anthocyanin accumulation is revealed in a similar differential manner also in the leaves of several ornamental plants. For example, in the red forms of *Berberis vulgaris* abundant anthocyanin is characteristically present only in the leaves of the outer parts of the foliage, whereas in the shaded leaves growing in the inner parts of the bushes and developing under light deficiency, anthocyanins are lacking (Семкина, 1971). Likewise in Caucasian ivy (*Hėdera caucasigena* Pojark.) anthocyanins accumulate only in well-illuminated leaves, with no red pigmentation occurring in the shaded ones (Джапаридзе, 1973). Since in those and other similar cases the unpigmented leaves or fruits do not develop in complete darkness but still receive some illumination from light of lowered intensities, there should remain no doubt that zero levels of anthocyanin accumulation in such situations must have a quantitative rather than a qualitative basis with respect to the mechanism of light action on that process.

Most substantial arguments against the existence of a specific mechanism controlling the accumulation of anthocyanins via certain light-governed enzymes in their biosynthetic pathway are derived from observations on the induction or stimulation of that biosynthesis in dark-grown plant material by introducing, into tissues, various nutritives or by treating the plants with different physiologically active compounds having no nutritional value. As can be seen from Table 2 summarizing the data available on that topic, an inducing or stimulatory action of light can be more or less effectively substituted by feeding sugars, amino acids, ATP, vitamins, immediate biosynthetic precursors of flavonoids; by subjecting plants to a treatment with antibiotics, analogs of nucleic acids, a variety of membrane active compounds; by supplying growth medium of tissue cultures with 2,4-D or other growth regulators. Additional compounds and treatments of similar activity may be probably found in further experiments (see, for example, Kang, Burg, 1973) but even that list shows clearly enough that the formation of anthocyanins can be easily evoked or promoted in the absence of light with using a great variety of different chemical agents including not only normal components of cell metabolism (sugars, L-phenylalanine), but also metabolic inhibitors (antibiotics), regulatory compounds (2,4-D), and chemicals which are able to modify the physico-chemical properties of cell structural elements (kinetin, EDTA). No doubt that in any of these particular cases primary mechanisms operating must be different. In spite of that, all these treatments obviously lead to a common final change in the intracellular conditions which is favourable for anthocyanin formation and thus plays the role of a determinant directly responsible for the increase in the accumulation of these compounds. There are all probabilities that the final stimulus is related to an improvement of conditions contributing to an endogenous supply with initial substrates necessary for building anthocyanins (and other flavonoids).

These data thus clearly indicate that the absence or the low level of anthocyanin biosynthesis in the dark does not result from a functional inability of the corresponding enzymic apparatus to complete that process without light action but is probably conditioned by limitations occurring in unexposed tissues in the supply and in the availability of substrate materials.

In this respect particular attention should be given to the data obtained in experiments with using kinetin, n-propanol, dimethylsulfoxide, and several other compounds which are known to increase the permeability of cell membranes. Resulting from treatments with such membrane-active

Table 2

Substitution of light action on the formation of anthocyanins by subjecting plants to treatments with chemical agents

Acting compound, range of concentrations	Plant, organ, tissue	Range of enhancement of anthocyanin accumulation in the dark	Range of approximate percentage level as compared with untreated illuminated material	References
1	2	3	4	5
Sucrose (1—12%)	*Spirodela oligorrhiza* fronds, *Saxifraga crassifolia* leaf segments*, *Impatiens balsamina* hypocotyl segments*, *Hydrilla verticulata* leaves, *Vitis vinifera* shoot tissue culture*; red cabbage, cress, and mustard* seedlings	1.5—2.4	15—50	Thimann, Edmondson, 1949; Eddy, Mapson, 1951; Paech, Eberhardt, 1952; Slabecka-Szweykowska, 1952; Eberhardt, 1954; Thimann, Radner, 1958; Kandeler, 1960; Arnold, Alston, 1961; Havelange et al., 1967
Glucose (1—4%)	*Impatiens balsamina* hypocotyl segments*, red cabbage and cress seedlings, turnip excised cotyledons	1.4—4.5	15—150	Eddy, Mapson, 1951; Paech, Eberhardt, 1952; Arnold, Alston, 1961; Grill, Vince, 1969
Fructose, sorbose, galactose, arabinose (1%)	Cress seedlings	1.4—4.5	45—50	Eddy, Mapson, 1951
Na-acetate (10^{-3}—10^{-2}M)	Red cabbage seedlings, turnip excised cotyledons	1.3—2.6	30—40	Kandeler, 1960; Grill, Vince, 1969
L-phenylalanine (10^{-3}—10^{-2}M)	Red cabbage seedlings, turnip and buckwheat excised cotyledons	1.1—2.6	20—40	Kandeler, 1960; Grill, Vince, 1969; Margna, Vainjärv, 1983; Маргна et al., 1983
Dioxyphenylalanine (10^{-4}—10^{-3}M)	Red cabbage seedlings	1.1—1.5	30—40	Kandeler, 1960
ATP (10^{-3}—10^{-1}M)	Red cabbage and mustard* seedlings	1.2—1.7	30—45	Kandeler, 1960; Havelange et al., 1967

Table 2 (continued)

1	2	3	4	5
Ascorbic acid, Na-ascorbate (10^{-5}—10^{-2}M)	Red cabbage and mustard seedlings	1.2—2.0	30—45	Kandeler, 1960; Schopfer, 1967
Riboflavine (10^{-5}—10^{-4}M)	*Spirodela oligorrhiza* fronds	1.5—2.4	20—35	Thimann, Radner, 1958; Elliott, 1977
Indole acetic acid (10^{-6}—10^{-3}M)	Red cabbage seedlings, carrot callus culture (strain R1)*	In red cabbage and *Hydrocharis*: small promotive effect; in carrot: abundant anthocyanins when growth regulators present		Paech, Eberhardt, 1952; Alfermann, Reinhard, 1971; Pecket, Bassim, 1974b; Alfermann et al., 1975
2,4-Dichlorphenoxyacetic acid (10^{-6}—10^{-5}M)	*Hydrocharis morsus ranae* turions*, carrot callus culture (strain R1)*			Pogorzelska, 1965; Alfermann, Reinhard, 1971; Alfermann et al., 1975
1-Naphthalene acetic acid, 4-chlorphenoxyacetic acid, indole butyric acid (10^{-6}—10^{-4}M)	Carrot callus culture (strain R1)*			Alfermann, Reinhard, 1971; Alfermann et al., 1975
Cinnamic acid (200 mg/l)	Red cabbage seedlings	1.2	80	Pecket, Bassim, 1974 a, b
Streptomycin (100—200 µg/ml)	Red cabbage seedlings	1.5—1.7	40—100	Mancinelli et al., 1975
Actinomycin (10 µg/ml)	*Sorghum* first internodes	1.4—1.6	115—185	Stafford, 1966
Puromycin ($5 \cdot 10^{-4}$M)	*Sorghum* first internodes	2.3—3.4	55—440	Stafford, 1966
Azaguanine (10^{-3}M)	*Sorghum* first internodes	1.4—2.7	10—340	Stafford, 1966
Thiouracil ($3 \cdot 10^{-5}$M)	*Spirodela oligorrhiza* fronds	1.4—3.8	20—25	Thimann, Radner, 1955
Benzyladenine (10^{-6}—10^{-4}M)	Sunflower excised cotyledons, *Spirodela oligorrhiza* fronds	1.7—17.8	210—300	Servettaz et al., 1975; Elliott, 1977
Zeatin (10^{-8}—10^{-7}M)	Carrot cell suspension culture	2—5	—	Ozeki, Komamine, 1981
Kinetin (10^{-6}M — saturated solutions)	Red cabbage seedlings, sunflower excised cotyledons, buckwheat cotyledons, carrot cell suspension culture	1.5—9.0	60—110	Pecket, Bassim, 1974b; Bassim, Pecket, 1975; Servettaz et al., 1975; Ozeki, Komamine, 1981; Margna, Vainjärv, 1983
Kinetin in combination with L-phenylalanine (10^{-2}M)	Buckwheat excised cotyledons	22	150	Margna, Vainjärv, 1983

Table 2 (continued)

1	2	3	4	5
n-Propanol (0.025—1%)	Red cabbage and mustard seedlings	1.2—3.5	30—145	Pecket, Bassim, 1974a; Bassim, Pecket, 1975; Mancinelli et al., 1975; Whitelam, Johnson, 1981
Kinetin and n-propanol in combination with shikimic acid (20 mg/l)	Red cabbage seedlings	1.5—1.7	105—110	Pecket, Bassim, 1974a, b
Kinetin and n-propanol in combination with cinnamic acid (200 mg/l)	Red cabbage seedlings	1.6—1.9	105—130	Pecket, Bassim, 1974a, b
Ethylenediaminetetraacetic acid (EDTA) ($5 \cdot 10^{-4}$—10^{-3}M)	Red cabbage seedlings, *Spirodela oligorrhiza* fronds	1.2—2.2	95—300	Bassim, Pecket, 1975; Elliott, 1977
Dimethylsulfoxide (DMSO) (0.05—4.5%)	Red cabbage seedlings, mung bean cotyledons	1.1—2.4	25—115	Pecket, Bassim, 1974a; Mancinelli et al., 1975; Dumortier, Vendrig, 1978
NN-dimethylformamide (DMF) (0.4—12%)	Mung bean cotyledons	1.5—2.6	50—115	Dumortier, Vendrig, 1978, 1982
Tween-20 (0.025—0.2%)	Red cabbage seedlings	1.2—1.3	25—30	Mancinelli et al., 1975
Acetylcholine (10^{-4}M)	Red cabbage seedlings	1.3	—	Pecket, Bassim, 1974
Ethanol (0.1%)	Red cabbage seedlings	1.2	30	Mancinelli et al., 1975

* Reported not to synthesize measurable anthocyanins in the dark when not treated with the corresponding chemicals.

compounds, considerable stimulation of anthocyanin formation was obtained in dark-grown red cabbage, sunflower, mung bean, and mustard seedlings (Pecket, Bassim, 1974a,b; Bassim, Pecket, 1975; Servettaz et al., 1975; Dumortier, Vendrig, 1978, 1982; Whitelam, Johnson, 1981), and also in *Spirodela* fronds (Elliott, 1977). Exogenous shikimic and cinnamic acids introduced after a treatment with kinetin or n-propanol promoted an intense accumulation of anthocyanins in red cabbage seedlings, while both of them remained without effect when fed to untreated material (Pecket, Bassim, 1974a,b).

In the experiments with buckwheat seedlings carried out in this laboratory (Margna, Vainjärv, 1983) the stimulatory effect of kinetin proved to be especially drastic. When intact seedlings were kept in contact with a solution of kinetin for 5—15 min, an up to 7-fold increase in the accumulation of anthocyanins was observed in their cotyledons during subsequent 40 h incubation of the seedlings in the dark. In excised buckwheat cotyledons an improvement of conditions for transmembrane transport caused by kinetin led to a rise in the formation of anthocyanins in the dark equal to about 50—60 per cent of the level of anthocyanin accumulation characteristic of excised cotyledons under continuous illumination. When exogenous L-phenylalanine was supplied after the treatment, the accumulation of anthocyanins in the dark attained practically the same high level as was observed in the illuminated cotyledons fed with L-phenylalanine. In a further experiment with using ^{14}C-labelled L-phenylalanine, parallel to a marked increase in the absolute content of anthocyanins in kinetin-treated cotyledons, a substantial rise in the radioactivity of these flavonoids occurred with both of these effects manifesting themselves also at the level of rutin biosynthesis and, to a lesser extent, in the accumulation of C-glycosylflavones. The activity of phenylalanine ammonia-lyase, the key enzyme of flavonoid biosynthesis, showed no comparable change in the treated material (Margna, Vainjärv, 1983; see also Pecket, Bassim, 1974b).

These data lend unequivocal support to the assumption of the nonenzymatic nature of light action on the accumulation of anthocyanins, leaving no doubt that those are the supply and the availability of initial substrate (L-phenylalanine) rather than the activity of enzymes which are the main intracellular factors limiting biosynthesis of these compounds in the dark.

In making that general conclusion a question still arises why it is namely the formation of anthocyanins that usually shows an extremely striking quantitative dependence on the action of light with up to a practically zero accumulation of these compounds in the dark in many cases, while the other flavonoids, when present, can well be synthesized in complete darkness and often show only a relatively slight increase in their accumulation rate after a stimulatory exposure to light. The likely reasons are: i) L-phenylalanine as the common precursor of polyphenols is normally never produced in plant cells in the amounts which may become saturating for the enzymic apparatus involved in flavonoid biosynthesis (Margna, 1977a); ii) biosynthetic pathways of different flavonoids are not in an equal position with regard to their ability to compete for their common precursors. Due to these two circumstances, the substrate materials available from a common source tend to follow an uneven distribution in favour of simpler pathways. An inevitable consequence is that anthocyanins as the most complex flavonoids usually show the lowest accumulation rate as compared with the other flavonoids (flavones, flavonols) simultaneously synthesized by the same tissue (Harborne, 1962; Margna, 1977b) but they exhibit the highest responsiveness to the influence of external factors (which in most cases probably exert their action through a change in the intracellular level of the substrate supply). Taking that

into consideration it can be easily explained why in a tissue with a low initial level of anthocyanins illumination typically brings about manifold stimulation of their accumulation, while at a markedly reduced substrate supply characteristic of intracellular conditions in the dark that very tissue may practically lose the ability to synthesize anthocyanins or, more exactly, is able to produce these flavonoids only in a very low amount which remains insufficient to be recorded by a student.

It must be pointed out, however, that the high efficiency of light action with respect to the accumulation of anthocyanins is, in fact, merely a seeming phenomenon which reveals itself in that manner solely because of the very low intensity of biosynthesis of these flavonoids at limited precursor availability in the dark. As a marked effect it can thus be interpreted at a relative basis only, and only on the condition that the range of simultaneous changes in the accumulation of other flavonoids or in the accumulation of flavonoid compounds in total is disregarded. When in a tissue flavonoids of several classes are synthesized at the same time, it is the increase in the content of simpler compounds (flavonols, flavones etc.) but not of anthocyanins which usually accounts for the greater part of the total absolute increase in the content of flavonoids caused by the stimulatory action of light, although the relative light effects within these flavonoids (due to the higher initial levels in their quantity) may remain comparatively small or even seem to be insignificant. For example, buckwheat seedling cotyledons showed a 13-fold relative increase in the accumulation of anthocyanins when the seedlings were held under continuous illumination during a 48-hr period. However, that large relative increase actually constituted only a little more than 1 per cent of the absolute increase in the total amount of flavonoids (including also rutin, C-glycosylflavones, and leucoanthocyanins), i. e. was of no practical importance in respect of the overall effect of light on the formation of flavonoids in that material (Margna et al., 1973; Margna, 1977b). Similar by its absolute magnitude was the share of anthocyanins within the total increase in the accumulation of flavonoids observed in buckwheat cotyledons after kinetin treatment (Margna, Vainjärv, 1983).

A comment must be added regarding the possible role of changes in the enzymic activity taking place under light action. Light is generally known to be an effective modifier of the activity of flavonoid enzymes including the initial one, phenylalanine ammonia-lyase (Zucker, 1972; Camm, Towers, 1973), and several others functioning at later stages of the flavonoid pathway (Hahlbrock et al., 1971; Hahlbrock, 1972). Enough reports can be referred to, in which light-induced changes in the activity of these enzymes have been shown to be in a correlation with concurrent changes in the accumulation of flavonoids (see the surveys by Camm, Towers, 1973; Hahlbrock, Grisebach, 1975). Although the enzymes specifically related to the formation of anthocyanin-type structures have not yet been discovered in plants, one may well suppose that such enzymes will display a similar behaviour. Therefore it is tempting to suggest, as has often been done by earlier authors, that the stimulation of anthocyanin accumulation caused by light results, at least partly, from a light-promoted rise in the activity of the corresponding enzymes. That possibility cannot be fully ruled out. It must be emphasized, however, that a temporary rise in the catalytic activity seems to be quite a general response of various enzymes to the action of stress factors. It remains highly questionable therefore whether such a rise has any real functional meaning at all. Furthermore, the occurrence of even marked changes in the activity of a particular enzyme does not necessarily imply that these changes are rate-limiting in respect to the related reactions. It has been recently shown by one of the authors, for example, that the activity of phenylalanine

ammonia-lyase in plants is usually many times higher than is obligatory for satisfying requirements for building flavonoids (polyphenols) and that considerable increase in the formation of those compounds can occur even at lowered activities of the enzyme (Margna, 1977a). Since only flavonoids reaching completion but not the intermediates of their biosynthesis are normally accumulating in plant cells, a similar high catalytic potency seems to be a fundamental characteristic of all flavonoid enzymes.

In conclusion, caution is obviously necessary when attempting to relate the light effects on the formation of anthocyanins and other flavonoids to a light-induced increase in the activity of the enzymes directly involved in that process. Evidence presented in this paper clearly shows that changes arising in the accumulation of anthocyanins in response to light action or to its absence can well be interpreted as quantitative effects originating from changes at the level of substrate supply and availability.

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ANTOTSÜAANIDE BIOSÜNTEES KUI PROTSESS, MILLE SÕLTUVUS VALGUSEST EI OLENE SPETSIIFILISEST KONTROLLMEHHANISMIST

Artiklis on kriitiliselt analüüsitud valguse toimemehhanisme. On antud ülevaade: 1) taimedest ja kudedest, mis on võimelised sünteesima antotsüaane ka pimedas; 2) keemilistest ühenditest, millega mõjutamine võib osaliselt või täielikult asendada valguse stimuleerivat toimet ning 3) teistest faktidest, mis ei ole kooskõlas laialt levinud arvamusega, nagu oleks antotsüaanide moodustumine spetsiifilisel viisil sõltuv valgusest. On näidatud, et suured erinevused mitmesuguste taimede ja kudede võimes sünteesida antotsüaane, samuti erinevused valguse toimes antotsüaanide ja teiste flavonoidide biosünteesile on tegelikult vaid kvantitatiivse iseloomuga ega tulene spetsiifiliste valgustundlike ensüümide olemasolust antotsüaanide biosünteesiahelas. Valguse stimuleeriva toime põhjuseks nende ühendite moodustumisel ei ole muutused selles protsessis vahetult osalevate ensüümide olekus, vaid ensüümide parem varustatus L-fenüülalaniini kui vajaliku lähtesubstraadiga.

Удо МАРГНА, Лембе ЛААНЕСТ

ОБРАЗОВАНИЕ АНТОЦИАНОВ КАК ПРОЦЕСС, ДЕЙСТВИЕ СВЕТА НА КОТОРЫЙ НЕ ЗАВИСИТ ОТ ФУНКЦИОНИРОВАНИЯ СПЕЦИФИЧЕСКОГО КОНТРОЛЬНОГО МЕХАНИЗМА

В статье рассмотрены механизмы, отвечающие за действие света на образование антоцианов. Представлены сводные данные о растениях и тканях, обладающих способностью синтезировать антоцианы без предварительного освещения, о химических веществах, обработка которыми может частично или полностью заменять стимулирующее действие света, и о других фактах, не согласующихся с широко распространенным мнением о том, что образование антоцианов специфически зависит от действия света. На основании проведенного анализа показано, что как различия в способности разных растений и тканей синтезировать антоцианы, так и различия в светозависимости образования антоцианов и других флавоноидов имеют чисто количественный характер и не связаны с существованием специфических светочувствительных звеньев в цепи биосинтеза антоцианов. Причиной стимулирующего действия света на образование этих соединений являются светиндуцированные изменения, в результате которых улучшаются снабжение клеток освещенных растений фенилаланином как исходным субстратом и его доступность ферментному аппарату биосинтеза флавоноидов.