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THE DEPENDENCE OF LEUCOANTHOCYANIDIN ACCUMULATION UPON METABOLIC SHIFTS CAUSED BY EXTERNALLY INTRODUCED NUTRITIVE FACTORS

In an earlier paper of this laboratory (Margna, Otter, 1971) it has been demonstrated that in buckwheat seedlings the responsiveness of leucoanthocyanidin forming processes to external influences is much weaker than that of the processes leading to the formation of anthocyanins. Particularly, when intact seedlings were fed with glucose, phenylalanine, and ammonium nitrate considerable changes in the accumulation of anthocyanins in hypocotyls and cotyledons were obtained, while the content of leucoanthocyanidins remained practically unchanged in both organs. Some quantitative shifts in the accumulation of this group of compounds could be detected only when excised organs were used.

One reason for this situation may be that in those experiments the amount of leucoanthocyanidins was recorded as a sum of both soluble and insoluble fractions of these compounds in seedling material. No experimental data are known about the behaviour of tissue-bound leucoanthocyanidins under alternating environmental conditions, but it seems quite likely that this fraction is metabolically much less mobile than are the soluble forms. Therefore it is not excluded that the unresponsiveness of the leucoanthocyanidin-forming system stated previously accounts merely for a masking interference of the presence of inert tissue-bound leucoanthocyanidins, but is not a typical character of the whole system. In view of this possibility, an additional experimental study was undertaken to elucidate, in that case, the influence of the same metabolically active compounds on the formation of the soluble forms of leucoanthocyanidins, only.

The experiments were carried out with buckwheat (*Fagopyrum esculentum* Moench) seedlings raised at 25 °C by a procedure generally employed in this laboratory (Margna, Otter, 1968). When intact material was used, the seedlings were grown in darkness for the first 56 h, then they received 16 h of light (illumination from white fluorescent tubes, light intensity 28000 erg·cm^{-2}·sec^{-1}) and after that were returned to darkness for an additional 24 h; test substances dissolved in distilled water were introduced to growth medium of seedlings before the onset of the illumination program. In experiments with isolated organs the hypocotyls and cotyledons needed were excised from 80 h-old etiolated seedlings; the isolated material was then floated for 3—5 min in test-solutions followed by a 40 h incubation (16 h light+24 h darkness) on filter paper moistened with the same solutions used for floating.

The concentrations of the test-substances in growth-medium of intact seedlings as well as in solutions used for floating were chosen as follows: glucose — 1 per cent; phenylalanine — 10^{-2} M; NH_4NO_3 — 0.1 per cent.

The quantitative determination of leucoanthocyanidins was performed as described by V. S. Govindarajan and A. G. Mathew (1965), from samples of 150—180 hypocotyls or

pairs of cotyledons, respectively. The results were expressed as arbitrary units by the scale of optical density (O. D.×1000) per seedling.

All data presented in this paper are averages of three separate experiments the determination of leucoanthocyanidins in each of them being performed in 8—10 replications.

The effect of glucose, phenylalanine, and ammonium nitrate on the accumulation of soluble leucoanthocyanidins in buckwheat seedlings

(* significant difference at $P<0.05$)

Type of experiments	Content of leucoanthocyanidins, units/seedling	Change, %
Intact seedlings		
Cotyledons:		
Control	380	—
Glucose	395	3.9
Phenylalanine	402	5.8
NH_4NO_3	356	—6.3
Hypocotyls:		
Control	207	—
Glucose	214	3.4
Phenylalanine	259	25.1*
NH_4NO_3	201	—2.9
Isolated organs		
Cotyledons:		
Control	500	—
Glucose	584	16.8*
Phenylalanine	560	12.0*
NH_4NO_3	433	—13.4*
Hypocotyls:		
Control	280	—
Glucose	325	16.1*
Phenylalanine	328	17.1*
NH_4NO_3	260	—7.1*

The results (Table) are in complete accordance with those obtained earlier (Margna, Otter, 1971). They clearly show that in intact seedlings the leucoanthocyanidin-forming processes represent a system of biosynthetic reactions which is rather stable against moderate changes in environmental conditions and can only slightly be influenced by the shifts taking place in cellular pools of different metabolites.

An introduction of phenylalanine only was effective enough to give rise to a marked change in the accumulation of leucoanthocyanidins in hypocotyls (but not in cotyledons), whereas in all other cases the changes caused by treatments remained too small to reach a statistically significant level.

In excised organs all three substances tested induced clearcut and typical, for separate substances, responses in both hypocotyls and cotyledons, phenylalanine and glycose acting as stimulators and ammonium nitrate — as an inhibitor of leucoanthocyanidin accumulation. As shown earlier (Margna, Otter, 1971), analogous, under the same conditions, is the effect of these substances also on the accumulation of anthocyanins.

Parallel changes in the accumulation of anthocyanins and leucoanthocyanidins in isolated organs confirm the absence of differences of principle between the responses of the two biosynthetic systems to external influences, indicating that the relative inertness of the leucoanthocyanidin-forming processes in intact seedlings must be quantitative in its nature.

The main reason of such an inertness may be the differences in the complexity of biochemical reactions involved in the building of leucoanthocyanidins and anthocyanins, and the resulting differences in the supply and degree of saturation of these processes with metabolites. According to present knowledge, the whole group of flavonoids is built up from common precursors by a common biochemical mechanism, the biosynthetic pathways of separate flavonoids differing from each other by some terminal steps, only. The details of these differences are yet unknown, but enough information is available to regard the less oxidized forms of flavonoids, such as flavanols (= catechins) and flavandiols (= leucoanthocyanidins), the most simple and primitive representatives of flavo-

noid compounds, whereas anthocyanins belong to the most complex derivatives of flavonoid nature, their biosynthetic pathway being a number of steps longer than that of the simpler ones (Bate-Smith, Lerner, 1954; Harborne, 1962). It may be assumed, therefore, that the catalytic system responsible for the leucoanthocyanidin formation occupies a more favourable position for utilization of common precursors from an internal metabolic pool than the system responsible for the formation of anthocyanins. As a result, the leucoanthocyanidin forming system, in principle, must be less susceptible to possible changes in other metabolic areas of the cell — a situation which was actually observed in intact seedlings. It seems very likely that in intact seedlings the amount of precursor metabolites available for flavonoid biosynthesis is sufficiently large to secure an almost maximal functioning of the enzymic apparatus responsible for leucoanthocyanidin formation, and, consequently, the latter may be too saturated to respond actively to any of the changes taking place in the seedling metabolism after external introduction of metabolically active compounds. In excised organs the catalytic capacity of that apparatus is evidently increased (see Table), and therefore it becomes more susceptible to substrate changes.

As to the nature of metabolites, the supply of which is presumed to be critical for manifestation of the above quantitative differences in the accumulation of leucoanthocyanidins and anthocyanins in intact seedlings, phenyalalanine seems to be one of the most likely compounds of such an importance. This opinion is in agreement with the key-position of phenylalanine in the biosynthesis of flavonoids (Neish, 1964), and is supported by a rather considerable stimulatory action of feeding that compound on the formation of leucoanthocyanidins (and anthocyanins) in excised organs. A decrease in the accumulation of both flavonoids in excised material after treatment with ammonium nitrate likewise speaks in favour of that possibility. Under these conditions an activation of protein biosynthesis takes place in seedling cells (Otter, Margna, 1967), resulting in an increased flow of phenylalanine (and other aminoacids) into these processes must occur. Accordingly, the portion of the phenylalanine available, from a common pool, for flavonoid synthesis has presumably decreased, thus leading to a diminished accumulation of those compounds.

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LEUKOANTOTSÜANIDIINIDE MOODUSTUMISE SÕLTUVUS EKSOGEENSETE TOIMEAINETE MANUSTAMISEL TEKKINUD NIHETEST AINEVAHETUSES

Resümee

Artiklis näidatakse, et intaktsetes tatraidandites on leukoantotsüanidiinide moodustumisele viivad protsessid võrdlemisi vähetundlikud rakusisestes metaboliidifondides tekkivate nihete suhtes ega reageeri praktiliselt niisuguste metaboolselt aktiivsete ühendite nagu glükoosi, fenüülalaniini ja ammooniumnitraadi manustamisele. Isoleeritud hüpokotüülides ja idulehtedes sellist inertsust ei täheldatud ning leukoantotsüanidiinidesisaldus suurenes fenüülalaniini ja glükoosi toimel analoogiliselt antotsüaanidele, lämmastiku toimel aga alanes. Analüüsitakse põhjusi, millest võib oleneda leukoantotsüanidiinide moodustumisega seotud katalüütilise aparaadi inertsus intaktsetes idandites.

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УДО МАРГНА, ТИЙУ ВАЙНЪЯРВ, ЭВИ МАРГНА

ЗАВИСИМОСТЬ НАКОПЛЕНИЯ ЛЕЙКОАНТОЦИАНИДИНОВ ОТ ОБМЕННЫХ СДВИГОВ, ВЫЗВАННЫХ ВВЕДЕНИЕМ ЭКЗОГЕННЫХ ПИТАТЕЛЬНЫХ ВЕЩЕСТВ

Резюме

Показано, что в интактных проростках гречихи процессы образования лейкоантоцианидинов относительно малочувствительны к сдвигам во внутриклеточных фондах метаболитов и практически не реагируют на введение таких метаболически активных веществ как фенилаланин, глюкоза и азотнокислый аммоний. В изолированных гипокотилях и семядольных листочках этого не наблюдалось и накопление лейкоантоцианидинов, аналогично антоцианам, под влиянием фенилаланина и глюкозы возрастало, под влиянием азота — снижалось. Обсуждаются возможные причины инертности лейкоантоцианидин-синтезирующей системы в интактных проростках.

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