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AZOTE-INDUCED CHANGES IN THE ACCUMULATION OF BUCKWHEAT SEEDLING FLAVONOIDS

Feeding exogenous azote is one of the most effective laboratory means which can be employed to evoke quantitative changes in the accumulation of anthocyanins in plant tissues. As a rule, the treatment brings about a considerable decrease in the pigment-synthesizing capacity of the plant material (Slabecka-Szweykowska, 1952; Szweykowska, 1959; Szweykowska et al., 1959; Eberhardt, Haupt, 1959; Faust, 1965; Otter, 1966; Маргна, Оттер, 1968; Маргна, 1971; Lawanson et al., 1972) contrary to the stimulation of anthocyanin formation frequently observed in plants grown at azote deficiency (Blank, 1958). As simultaneously with the lowered pigment accumulation a marked increase in the content of protein nitrogen occurs (Eberhardt, Haupt, 1959; Faust, 1965; Otter, 1966; Оттер, Маргна, 1967), the whole phenomenon may serve as an excellent experimental evidence for the existence, in plants, of balanced (*resp.* competitive) relationships between the processes of protein biosynthesis and those leading to the formation of flavonoid compounds (Otter, 1966; Маргна, 1971). There are all probabilities that these characteristic relationships arise from substrate limitations at the level of phenylalanine (Маргна, 1971) which, beyond its immediate function of being involved in building proteins, plays an important role as a key-metabolite for the formation of a variety of plant phenolics, including flavonoids (Neish, 1964).

From this a deduction automatically ensues that azote-induced changes should be revealed not only in the accumulation of anthocyanins, but must also cover the accumulation of other flavonoid derivatives present in the plant tissue exposed to treatment. Various fertilization data seem to support this point quite satisfactorily (Virtanen, Oland, 1954; Chouteau, Loche, 1965; Loche, 1966; Butler et al., 1967; Rossiter, 1969; Miidla et al., 1970; Процко, Кравец, 1972; Krause, Reznik, 1972; Hilton et al., 1973), yet all of them have been obtained from prolonged field or water-culture experiments while special data from short-term model experiments with laboratory objects are still completely lacking. However, in some of our previous studies with excised buckwheat organs we were able to demonstrate that with leucoanthocyanidins a rather good analogy of responses compared with anthocyanins can be observed in azote-fed material, both of the two flavonoids showing a considerable decrease in their amounts after the completion of the feeding procedure (Маргна, Оттер, 1971; Margna et al., 1972). In this communication an evidence is presented

that a similar decrease also takes place in the content of glycoflavones and flavonols, thus indicating that the inhibitory effect of exogenous azote indeed covers, as expected, the formation of all flavonoid compounds.

Experimental

The majority of experiments was carried out with isolated buckwheat (*Fagopyrum esculentum* Moench) hypocotyls and cotyledons excised from 80-h-old etiolated seedlings raised at 25°C by a standard procedure generally employed in this laboratory (Hallop, Margna, 1968). The excised material was soaked for 3–5 min in a solution of ammonium nitrate followed by a 40 h incubation on filter paper moistened with the same solution. When intact material was used, the azote solution was added into the growth medium at a 56 h age of etiolated seedlings, after which, as with isolated material, a 40 h incubation followed. In both cases the incubation involved an initial 16 h period of illumination with white luminescent light of the intensity of 29000 erg·cm⁻²·sec⁻¹ and a final 24 h period during which the material was maintained in contact with the acting solution in darkness. In a number of series other growth regimes were used (see below), yet in all cases the solution of ammonium nitrate was introduced to the material prior to illumination (or prior to the first light period if repeated illumination was employed), the concentration of the factor in incubation media being equalled to 0.1 per cent generally. If not otherwise stated, the temperature was constantly held at 25° during the whole period of incubation. The content of separate flavonoids was determined by methods described elsewhere (Margna et al., 1973).

Results and discussion

Typical results are presented in Table 1. As can be seen, an incubation of the material in azote solution resulted in a decrease in the accumulation of individual compounds over the whole set of flavonoids of both hypo-

Table 1

The content of flavonoids in isolated buckwheat hypocotyls and cotyledons incubated in water (control) or in a 1 per cent solution of ammonium nitrate (μg/seedling)

Flavonoid compound	Hypocotyls			Cotyledons		
	H ₂ O	N	Decrease, %	H ₂ O	N	Decrease, %
Anthocyanins	3.70	2.75	25.7	5.73	3.15	45.0
Rutin	14.5	11.2	22.8	48.6	32.5	33.1
Leucoanthocyanidins	140	130	7.1	250	216	13.6
Glycoflavones total	—	—	—	150	132	12.0
Including:						
Vitexin	—	—	—	34.3	29.4	14.3
Isovitexin (saponaretin)	—	—	—	61.3	55.9	8.8
Orientin	—	—	—	18.1	15.9	12.2
Iso-orientin (homo-orientin)	—	—	—	36.7	30.9	15.8
Total flavonoids	158	144	8.9	455	384	15.6

cotyls and cotyledons, the relative decrease being greatest in the case of anthocyanins and rutin, representing the more complex (by their biosynthetic routes) yet the less abundant forms of flavonoid derivatives synthesized by these tissues. The decrease was somewhat less pronounced

Table 2

The influence of exogenous azote on the formation
of flavonoids in hypocotyls of intact buckwheat seedlings*
($\mu\text{g}/\text{seedling}$)

Flavonoid compound	H ₂ O	N	Decrease, %
Anthocyanins	3.40	3.13	7.9
Rutin	17.4	12.8	26.4
Leucoanthocyanidins	107	97.0	9.3

* In anthocyanin series — 5-day-old seedlings, in rutin and leucoanthocyanidin series — 4-day-old seedlings; in all cases the seedlings were incubated in azote medium (NH_4NO_3 , 0.1 per cent) during the terminal 40 h period of their development (16 h light + 24 h darkness).

Table 3

The influence of exogenous azote on the accumulation
of anthocyanins in cotyledons of intact buckwheat
seedlings grown at various growth regimes
($\mu\text{g}/\text{seedling}$)

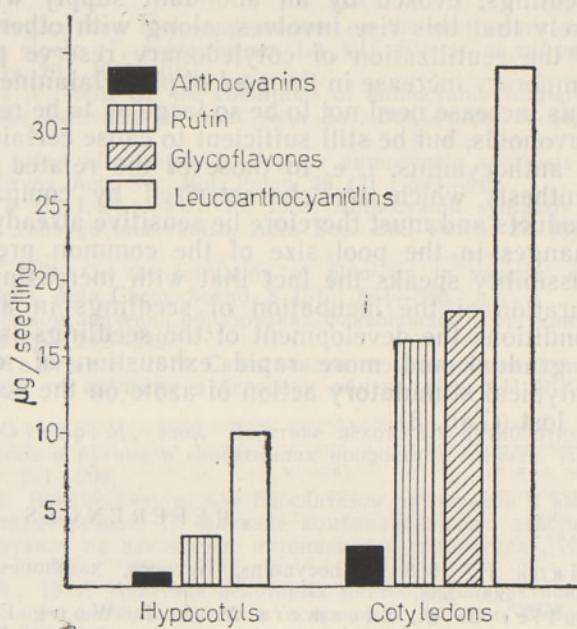
Regime*	H ₂ O	N	Change, %
56D+16L+24D at various t°:			
15°	1.65	2.57	+55.7
25°	1.66	2.22	+33.8
35°	1.22	1.58	+29.5
32D+16L+24D	0.80	1.12	+40.0
56D+16L+24D	1.90	2.96	+55.8
80D+16L+24D	2.14	2.53	+18.2
104D+16L+24D	3.05	3.14	Insignificant
80D+16L+8D+16L	2.94	3.90	+32.6
56D+16L+2 cycles of (8D+16L)	3.24	3.28	Insignificant
32D+16L+3 cycles of (8D+16L)	3.02	2.85	Insignificant

* D — darkness, L — light, numerals — duration in hours. The solution of ammonium nitrate was added into the growth medium before the onset of the illumination program; in series with varying temperatures during postillumination dark period the concentration of the factor in growth medium was equalled to 0.2 per cent, in other series — to 0.1 per cent.

but still significant in the case of simpler forms — glycoflavones and leucoanthocyanidins quantitatively dominating in the material as compared with the amounts of the preceding two groups of flavonoids. In terms of total flavonoids, the treatment brought about a ca 10 per cent decrease in their content in hypocotyls and an about 15 per cent decrease in cotyledons, which in absolute units corresponds to about 85 micrograms of flavonoids in total per seedling. The major part of this absolute decrease, as illustrated by the Figure, is accounted for by the changes in the content of glycoflavones and leucoanthocyanidins.

The results once again emphasize the general resemblance of responses of all flavonoids to external influences, as demonstrated by us in a number of cases earlier (Margna et al., 1969, 1974; Margna et al., 1974; Халлон, Маргна, 1970; Маргна, Халлон, 1971). They lend further support to the idea that the balanced relationships between protein synthesis and flavonoid accumulation are valid not only with respect to the anthocyanins or any other separate group of these compounds but must be considered as a biological phenomenon of overall importance related to the building of compounds of flavonoid structure in general (Маргна, 1971).

It is interesting to note, however, that in intact buckwheat seedlings the effect of azote is somewhat less pronounced. In hypocotyls, for example, the content of anthocyanins, leucoanthocyanidins, as well as of rutin is occasionally decreased quite considerably (Tab. 2, see also Маргна, Оттер, 1968, 1971), yet the inhibitory effect is not manifested steadily under these experimental conditions, and cases of insignificant influence occur frequently (Маргна, Оттер, 1971; Margna et al., 1972). In cotyledons, as ascertained by a number of experimental series, significant changes in the accumulation of leucoanthocyanidins (Маргна, Оттер, 1971; Margna et al., 1972), rutin and glycoflavones can be observed only in rare cases, while the formation of anthocyanidins, contrary to isolated cotyledons, is markedly enhanced rather than inhibited by azote feeding (Tab. 3, see also Маргна, Оттер, 1971).



Absolute decreases in the content of separate groups of flavonoids in isolated buckwheat hypocotyls and cotyledons after a 40 h incubation (16 h light + 24 h darkness) in a 0.1 per cent solution of ammonium nitrate as compared with water controls ($\mu\text{g}/\text{seedling}$).

The lesser efficiency of feeding azote to intact seedlings is most probably conditioned by a somewhat slower rate of the entering of the nutritive into the seedling cells when imbibed by root system, due to which the comparatively short incubation periods used may simply remain insufficient for full development of the inhibitory effect (cf. Krause, Reznik, 1972). The unusual stimulatory action of azote on anthocyanin accumulation in cotyledons, however, indicates that some differences between regulatory mechanisms operating in intact seedlings and their isolated organs may also be involved (Margna et al., 1974). Nevertheless, the stimulation cannot be regarded as an experimental fact critically contradicting the typical inhibitory influence of azote on flavonoid formation. Two possible explanations may be suggested.

First of all, it may be a result of certain balance shifts in the distribution of a common precursor (phenylalanine) between the biosynthetic

pathways of separate flavonoids, a phenomenon observed in intact buckwheat seedlings also under some other experimental conditions (Margna et al., 1973, 1974). As anthocyanins form only less than a one-hundredth part of the total amount of flavonoids synthesized in cotyledons of intact seedlings, a possibility exists that a decrease in the content of major flavonoids, relatively too small to be statistically significant, may still be sufficient for securing an increase in the accumulation of anthocyanins. This may be so even in that case when a slight absolute decrease in the total flavonoids actually occurs (compare, for example, the absolute decreases presented in the Figure).

However, the increased accumulation of anthocyanins may also result from the overall rise in the growth and development processes of young seedlings, evoked by an abundant supply with exogenous azote. It is likely that this rise involves, along with other processes, an acceleration of the reutilization of cotyledonary reserve proteins, due to which a temporary increase in the pool of phenylalanine may occur in the seedlings. This increase need not to be so large as to be reflected in the level of major flavonoids, but be still sufficient to cause certain shifts in the accumulation of anthocyanins, i. e. in those of the related branches of flavonoid biosynthesis which are characterized by comparatively small yields of products and must therefore be sensitive already to relatively small absolute changes in the pool size of the common precursor. In favour of this possibility speaks the fact that with increasing age or with a prolonged duration of the incubation of seedlings in azote media, under which conditions the development of the seedlings is obviously accompanied by a gradual and more rapid exhaustion of cotyledonary reserves, the untypical stimulatory action of azote on the accumulation of anthocyanins is lost (Tab. 3).

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LÄMMASTIKU TOIME FLAVONOIDIDE MOODUSTUMISELE TATRAIDANDEIS

Resümee

Tatraidandite isoleeritud hüpokotüülid ja idulehtede mõjutamine ammoniumnitraadiga põhjustas neis nii antotsüaanide kui ka kõikide teiste flavonoidide sisalduse märgatava languse. Lämmastiku pärssiv efekt oli suhteliselt suurem antotsüaanide ja rutini puhul, kuid tuli selgelt ilmsiks ka lihtsamat biosünteesi teed omavate ning hulga poolset domineerivate derivaatide — glükoflavoonide ja leukoantotsüanidiinide — juures. Eksogeense lämmastiku viimisel intaktseesse idanditesse oli efekt põhuliselt samasugune, kuigi avaldus mõnevõrra nõrgemini, eriti idulehtedes. Tulemused kinnitavad hüpoteesi, mille kohaselt taimedes valkude biosünteesi ning flavonoidide moodustumisele viivate protsesse vahel esinevades balansseeritud vahekorrud, ning kinnitavad järjekordseid, et need vahekorrud haaravad flavonoidide kompleksi tervikuna.

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Удо МАРГНА, Лембе ЛААНЕСТ, Эви МАРГНА, Маргареете ОТТЕР, Тийу ВАЙНЬЯРВ ВЛИЯНИЕ ЭКЗОГЕННОГО АЗОТА НА НАКОПЛЕНИЕ ФЛАВОНОИДОВ В ПРОРОСТКАХ ГРЕЧИХИ

Резюме

Показано, что обработка изолированных органов проростков гречихи азотнокислым аммонием приводит к значительному уменьшению накопления в них как антоцианов, так и всех других flavonoidных производных (рутин, лейкоантосидинов, гликофлавонов). То же самое в принципе наблюдается при введении экзогенного азота в интактные проростки, хотя ингибирующий эффект при этом выражен несколько слабее.

Результаты подтверждают существование в растениях сбалансированных взаимоотношений между биосинтезом белков и процессами формирования flavonoidных соединений.

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- тогда и количество антоцианов в проростках. Установлено, что введение азота в виде азотнокислого аммония приводит к значительному уменьшению накопления в проростках гречихи антоцианов, а также и других производных flavonoidных (рутин, лейкоантосидинов, гликофлавонов). Аналогичное явление наблюдается в интактных проростках, хотя ингибирующий эффект выражается слабее.
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