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EFFECT OF EXOGENOUS FEEDING ON C-GLYCOSYLFLAVONE ACCUMULATION IN BARLEY SEEDLINGS

Investigations of recent years have demonstrated that C-glycosylflavones, a group of flavonoids twenty years ago considered as rare constituents of plants, are in fact numerous and of quite common occurrence in the plant kingdom. While phytochemical studies are in progress, information concerning quantitative aspects of C-glycosylflavone metabolism is scanty. Their light-stimulated accumulation in sorghum (Stafford, 1965), duckweed (McClure, 1968), buckwheat (Халлоп, Маргна, 1970; Маргна, Халлоп, 1971), barley (McClure, Wilson, 1970; Carlin, McClure, 1973) and oats (Weissenböck, Effertz, 1974) has been established. Buckwheat researches have revealed the temperature dependence of C-glycosylflavone formation (Margna et al., 1973) and have shown that their accumulation can be modified by gamma-irradiation (Margna, Vainjärv, 1976), boron deficiency (Абышева, 1972) and exogenous sugar, azote and phenylalanine feeding (Маргна, 1970; Margna et al., 1974a, b; Margna, 1977a). Although various treatments generally bring about qualitatively identical changes at the level of all C-glycosylflavones present, individual C-glycosylflavone derivatives usually exhibit characteristic differences in the amplitude of their responses to the factors investigated. So the accumulation of orientin in duckweed (McClure, 1968) showed relatively greater light dependence than that of vitexin, although the absolute values of light-stimulated increase of vitexin exceeded that of orientin. Similarly, in oats the compounds which are minor components in etiolated primary leaves, turned out to be the most light-inducible ones (Weissenböck, Effertz, 1974). Investigations into buckwheat seedlings (Margna, 1977a) showed that while the concentration of apigenin-type (trihydroxy) derivatives in their cotyledons was normally about twice as high as that of the related luteolinic (tetrahydroxy) compounds, labelled phenylalanine incorporated preferably into the tetrahydroxy compounds. The responses of different C-glycosylflavone derivatives to exogenous factors apparently need further investigation by quantitative methods.

The present works deals with the same quantitative aspects of flavonoid accumulation as analyzed by their manifestation in barley (*Hordeum vulgare* L.) seedlings under conditions of feeding exogenous nutritives.

Experimental

The experiments were carried out with barley seedlings ('Kharkov' variety). Spectral and chromatographic investigations of C-glycosylflavones of that variety demonstrated their identity with those present in Atlas 46 barley — saponarin, lutonarin and lutonarin 3-methyl ether (Seikel,

Geissman, 1957; Seikel, Bushnell, 1959; Seikel et al., 1962). The seedlings were raised under laboratory conditions on filter paper moistened with tap water. The first primary leaves were excised from 80 h-old etiolated seedlings, placed on filter paper moistened with distilled water (control) or water solutions of the test substances (1 per cent glucose, 0.1 per cent NH_4NO_3 , 10^{-2} M L-phenylalanine) and incubated for 40 h in light (white fluorescent tubes, $28,000 \text{ erg}\cdot\text{cm}^{-2}\cdot\text{sec}^{-1}$) at 25°C . Material was assayed immediately after the light treatment. C-glycosylflavones were extracted with 80 per cent ethanol, concentrated and chromatographed (FN-11 paper, ascending chromatography in a mixture of isoamyl alcohol-petrol ether-acetic acid-water, 3:1:3:3, organic phase). C-glycosylflavones were detected in u. v. light, the spots were cut from the paper and eluted with 48 per cent ethanol. The amount of C-glycosylflavones was determined spectrophotometrically by their absorbance at 340 nm (saponarin, $\epsilon=1.750\cdot 10^4$), 348 and 353 nm (lutanarin 3'-methyl ether and lutanarin, respectively, $\epsilon=1.885\cdot 10^4$). The content of separate derivatives was expressed in micrograms per seedling. Quantitative values have been corrected for loss during processing.

Results

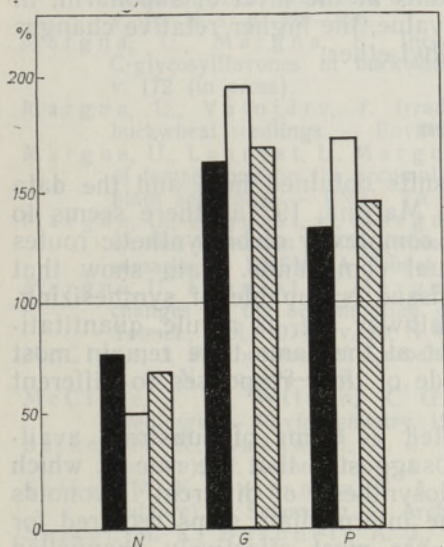


Fig. 1. The effect of various nutritives on the accumulation of saponarin (black bars), lutanarin (white bars), and lutanarin 3'-methyl ether (shaded bars) in isolated primary leaves of barley. N — NH_4NO_3 , G — glucose, P — phenylalanine. The 100 per cent line represents water control.

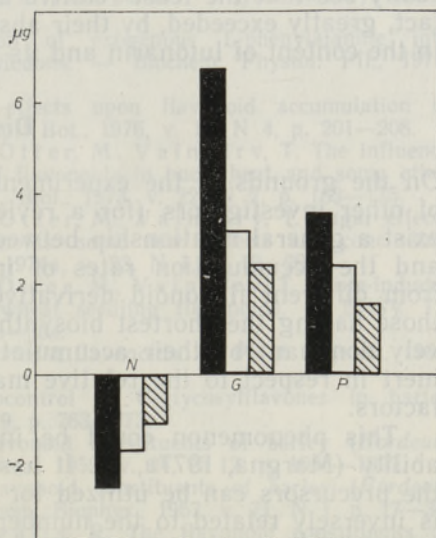


Fig. 2. Absolute changes in the content of barley C-glycosylflavones in isolated primary leaves fed with azote (N), glucose (G), and phenylalanine (P). Black bars — saponarin, white bars — lutanarin, shaded bars — lutanarin 3'-methyl ether. The 0-line corresponds to 10.8 µg of saponarin, 3.3 µg of lutanarin and 3.5 µg of lutanarin 3'-methyl ether per seedling.

The results are presented in Figs 1 and 2. As can be seen from the diagrams, the direction of the metabolic shifts in barley coincides with those obtained in buckwheat experiments of our laboratory earlier: the feeding of exogenous azote was clearly inhibitory for C-glycosylflavone accumulation whereas glucose and phenylalanine induced a sharp rise of their

amount (Fig. 1). Barley C-glycosylflavones, however, showed a greater range of responses under all conditions. Thus, the drop in their content, brought about by azote feeding, amounted from 23 (saponarin) up to 50 (lutonarin) per cent, while in buckwheat the total C-glycosylflavone content of isolated cotyledons, under the same conditions, revealed only an average 12 per cent decrease (Margna et al., 1974b). In excised buckwheat cotyledons glucose showed a tendency to be stimulatory for C-glycosylflavone accumulation which, however, in some cases only was large enough to result in a statistically significant effect (Margna et al., 1974a). In the glucose-treated first primary leaves of barley the induced increase of C-glycosylflavone content was almost twofold. The effect of phenylalanine on barley flavonoids made up between 33 (saponarin) and 72 (lutonarin) per cent, while in buckwheat its stimulatory action on C-glycosylflavone formation proved to be significant in a few cases only (Margna, Margna, 1978).

In spite of the fact that all of the three compounds reacted to the treatments in a similar manner, it is evident that in relative units the formation of the apigeninic derivative, saponarin, was less sensitive under the experimental conditions than the two luteolinic compounds. However, the reverse situation was true regarding absolute changes in their contents (Fig. 2). When comparing these changes with each other one can easily see that the least relative alterations at the level of saponarin, in fact, greatly exceeded, by their absolute value, the higher relative changes in the content of lutonarin and its 3'-methyl ether.

Discussion

On the grounds of the experimental results obtained here and the data of other investigators (for a review see Margna, 1977a) there seems to exist a general relationship between the complexity of biosynthetic routes and the accumulation rates of individual compounds. Data show that from different flavonoid derivatives a tissue is capable of synthesizing, those having the shortest biosynthetic pathway are, as a rule, quantitatively dominant by their accumulation but at the same time remain most inert in respect to the relative magnitude of their responses to different factors.

This phenomenon could be interpreted in terms of substrate availability (Margna, 1977a, b). It has been suggested that the rate at which the precursors can be utilized for the biosynthesis of different flavonoids is inversely related to the number of the intermediate steps required for their building, so that the precursors are most effectively channelled through simpler pathways. Since the latter become more saturated with the substrates, this must result in a relatively lower sensitivity of these processes to enhanced or reduced substrate supply.

The exact number of individual steps along the separate pathways leading to different flavonoid derivatives is at present unknown. It is evident, however, that incorporation of an additional hydroxy group requires at least one more step in the biosynthetic pathway. Barley contains one 4',5-dihydroxy C-glycosylflavone (saponarin) and two 3',4',5-trihydroxy ones (lutonarin and its 3'-methyl ether) which apparently are synthesized independently and do not interconvert as shown for different C-glycosylflavones in the *Lemnaceae* (Wallace et al., 1969; Wallace, 1975). Considering these aspects, preferential accumulation of saponarin as the simplest C-glycosylflavone derivative might be expected to occur under all conditions. Further, saponarin-forming processes as those which

are relatively most saturated with substrates should show the smallest relative responses to different external influences. At the same time the formation of the two minor C-glycosylflavones — trihydroxy derivatives lutonarin and its methyl ether — should be less saturated with common precursors and show a greater response even to relatively small absolute changes in their pool size. The present results are consistent with these expectations.

It must be noted that the reported shifts in barley flavonoid content are considered by the author as net changes in their accumulation without taking into account their possible turnover or degradation which could equally have been affected. To determine which routes are actually responsible for these changes, special investigations with the aid of labelled substrates are necessary.

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EKSOGEENSE TOITMISE MÕJU GLÜKOFLAVOONIDE AKUMULATSIOONILE ODRAIDANDITES

Resüme

Töös on uuritud ammooniumnitraadi, glükoosi ja fenüülalaniini mõju glükoflavoonide moodustumisele odraidandite isoleeritud primaarlehtedes. Tulemused näitavad, et ammooniumnitraat pidurdas tugevasti glükoflavoonide saponariini, lutonariini ja lutonariini 3'-metüülestri kogunemist, seevastu glükoos ja fenüülalaniin põhjustasid nende ühendite hulga suurenemise kuni kahekordseks. Ilmnes korrelatsioon üksikute glükoflavoonide sisalduse ja struktuuri (nende biosünteesiteede suhtelise pikkuse) vahel. Lihtsaima ehitusega glükoflavooni, saponariini, absoluuthulga muutused olid suuremad kui nihked teiste, enam substituueeritud ühendite sisalduses, kusjuures ka normaalingimustes on saponariin odraidandites domineeriv flavonoid. Muutuste suhtelise ulatuse osas ilmnes vastupidine tendents: saponariini absoluuthulga tugevad kõikumised jäid suhteliselt väiksemaks kui nihked luteoliini derivaatide sisalduses. Võib oletada, et täheldatud kvantitatiivsed seaduspärasused on seletatavad erinevustega ühiste lähtesubstraatide jaotumises eri ühendite biosünteesiteede vahel.

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Лембе ЛААНЕСТ

ВЛИЯНИЕ ЭКЗОГЕННОГО ПИТАНИЯ НА НАКОПЛЕНИЕ С-ГЛИКОЗИЛФЛАВОНОВ В ПРОРОСТКАХ ЯЧМЕНЯ

Резюме

Обработка изолированных первичных листьев проростков ячменя азотнокислым аммонием приводит к значительному ингибированию накопления в них С-гликозилфлавонов — сапонарина, лутонарина и его 3'-метилового эфира. Под действием глюкозы и фенилаланина накопление этих соединений, наоборот, сильно стимулируется. Обнаружена корреляция между содержанием отдельных С-гликозилфлавоновых производных и сложностью их структуры (относительной длиной путей их биосинтеза). Под влиянием внешних воздействий изменения в абсолютном количестве самого простого С-гликозилфлавона — сапонарина, доминирующего в проростках и в нормальных условиях, превышают изменения в содержании более субституированных соединений. В то же время в относительном плане значительные колебания абсолютного содержания сапонарина, выраженного в процентах, уступают изменениям в количестве лутеолиновых производных. Можно предположить, что обнаруженные количественные закономерности объясняются особенностями субстратной обеспеченности соответствующих биосинтетических путей общими предшественниками.

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