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THE ANTHOCYANINS OF *SECALE CEREALE*

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Abstract—Delphinidin-3-rutinoside was identified as the pigment in rye seeds. Cyanidin-3-rutinoside was detected in coleoptiles and cyanidin-3-glucoside in the first leaves.

RESULTS AND DISCUSSION

SEED colour of rye (*Secale cereale* L.—Gramineae) has been described as either green due to blue anthocyanin pigmentation or yellow from absence of anthocyanins. Red anthocyanin pigmentation can be observed in the coleoptiles and the first leaves of seedlings.

Watkins and White[1] have reported that the inheritance of anthocyanins in Prolific rye is controlled by complementary action of genes A and B for the seed colour and A and R for the red coleoptile colour. It has now been found that delphinidin-3-rutinoside (Dp3-Ru) is the anthocyanin in the aleurone layer of blue seed phenotypes; cyanidin-3-rutinoside (Cy3-Ru) is the anthocyanin in the coleoptile and cyanidin-3-glucoside (Cy3-Gl) is present in the first leaves of the red coleoptile phenotypes. This would imply that gene B complements gene A for delphinidin production while R complements A for cyanidin synthesis.

The finding that the delphinidin and cyanidin glycosides are independently inherited is unusual since genes controlling the hydroxylation of cyanidin to delphinidin have been uncovered in many plants. This would suggest that in rye, hydroxylation occurs at an earlier than at the terminal stage. The synthetic pathway here could be similar to that in *Campanula medium* flowers where hydroxycinnamic acids have proven to be good precursors of delphinidin with best incorporation into delphinidin from 3,4,5-trihydroxycinnamic acid.[2] The different glycosides of cyanidin present in the coleoptile and in the leaf would indicate that in the coleoptile an enzyme is present which is capable of coupling rhamnose to glucose. This material may therefore be suitable as a source of this enzyme for further investigation.

EXPERIMENTAL

Plant Material

Seeds of Prolific rye and six genotypes obtained from it were used for identification of the aleurone anthocyanin. Coleoptiles and the first leaves were obtained by germinating the seeds in Petri dishes for 3 days at room temperature. After this period the coleoptiles and the leaves were removed and separated. Since fresh berries were not available, preserved fruit was used as a source of anthocyanins for co-chromatography. Blackberry (*Rubus* sp.) jam was found to be a suitable source of Cy3-Gl and Cy3-Ru.[3] Black currant (*Ribes nigrum*) jelly provided the Dp3-Ru as well as the delphinidin 3-glucoside (Dp3-Gl) and the corresponding cyanidin glycosides.[3] The skins of blueberries (*Vaccinium myritillus*) was the source of the anthocyanidins cyanidin, delphinidin, malvidin, and pentunidin.[4]

[1] R. WATKINS and W. J. WHITE, *Can. J. Genet. Cytol.* **6**, 403 (1964).
[2] M. MEIER and M. H. ZENK, *Z. Pflanzenphysiol.* **53**, 415 (1965).
[3] J. B. HARBORNE and E. HALL, *Phytochem.* **3**, 453 (1964).
[4] H. SUOMALAINEN and A. J. A. KERANEN, *Nature* **191**, 498 (1961).

Extraction

The rye seeds were ground and extracted with 1 per cent HCl in methanol for at least 72 hr at room temperature. Overnight extraction of coleoptiles and leaves with 1 per cent HCl in methanol was sufficient.

Chromatography

The extracts were chromatographed on Whatman No. 1 paper in butanol–acetic acid–water, 4:1:5 (BAW), upper phase; butanol–2 N HCl, 1:1 (BuHCl), upper phase; and acetic acid–HCl–water, 15:3:82 (HAc–HCl). Whatman No. 3 paper was used for heavier loading. The anthocyanin of the green seeds co-chromatographed with the black currant Dp3-Ru, while the anthocyanins of the red coleoptiles and red leaves co-chromatographed with the blackberry Cy3-Ru and Cy3-Gl respectively (Table 1). The anthocyanins of the different genotypes in the rye are presented in Table 2; small amounts of Dp3-Gl and Cy3-Gl were detected in extracts containing their corresponding rutinosides but these probably were degradation products.

TABLE 1. CHROMATOGRAPHIC DATA OF RYE, BLACKBERRY AND BLACK CURRANT ANTHOCYANINS. VALUES IN BRACKETS ARE FROM HARBORNE[5]

Anthocyanin	R_f values (×100) in BAW	BuHCl	HAc–HCl
Cy3-glucoside	32 (38)	20 (25)	29 (26)
Cy3-rutinoside	32 (37)	20 (25)	44 (43)
Dp3-rutinoside	25 (30)	16 (15)	32 (37)

TABLE 2. ANTHOCYANINS OF PROLIFIC RYE AND THE SIX GENOTYPES OBTAINED FROM THE VARIETY

Genotype	Phenotype: Seed	Coleoptile	First leaf
Prolific	Green (Dp3-Ru)	red (Cy3-Ru)	Red (Cy3-Gl)
AABBRR			
AABBrr		Non-red (no anthocyanin)	Non-red (no anthocyanin)
AAbbRR		Cy3-Ru	Cy3-Gl
aaBBRR	Yellow (no anthocyanin)	No anthocyanin	No anthocyanin
aabbRR			
AAbbrr			

Hydrolysis and Anthocyanidin Detection

The rye anthocyanins were hydrolyzed by boiling 30 min in 3 N HCl. The anthocyanidins were extracted with n-amyl alcohol and chromatographed on cellulose as described by Nybom.[6] Delphinidin was identified in rye seed extracts, while extracts of coleoptiles and leaves contained cyanidin. Extracts containing Dp3-Ru and Cy3-Ru were partially hydrolyzed by boiling for 5 min in 3 N HCl to give the respective 3-glucosides. The purified anthocyanin of the rye seed had λ_{max} at 540 nm in MeOH contg. 0·01 per cent HCl.

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[5] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, p. 31, Academic Press, New York (1967).
[6] N. NYBOM, *Physiol. Plantarum* **17**, 157 (1964).