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EFFECT OF Pr AND pr ALLELES ON ANTHOCYANIN BIOSYNTHESIS IN *ZEA MAYS*

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Abstract—Anthocyanins present in the aleurone layer in **Pr** colour genotypes of *Zea mays* are cyanidin 3-glucoside and two unstable acylated derivatives of this pigment. The aleurones of **pr pr** lines are coloured by the corresponding pelargonidin derivatives and, in addition, have traces of cyanidin 3-glucoside; the **pr** allele is thus a hypomorph. No evidence could be found for a *p*-coumaroyl derivative of cyanidin 3-galactoside reported earlier as a pigment of maize seed.

INTRODUCTION

THE GENETIC control of anthocyanin biosynthesis in *Zea mays* L. Gramineae is well established. Pigment production in the aleurone, the outermost layer of the endosperm, is controlled by five complementary genes, distributed upon different chromosomes and symbolized $\mathbf{A_1}$, $\mathbf{A_2}$, $\mathbf{C_1}$, $\mathbf{C_2}$ and **R**. Other genes **Pr**, $\mathbf{Bz_1}$ and $\mathbf{Bz_2}$ are known to exert their control upon the kind of pigment that is synthesized. Each must be present in the homozygous recessive condition to cause a qualitative change in the anthocyanins. Other genes control the aleurone colour pattern (**Bh**) and pigment concentration (**in**). Anthocyanin biosynthesis is not confined to the aleurone; its production in sporophytic tissues like anthers, silks and pericarp is controlled by members of the **R** and $\mathbf{A_1}$ series.[1–3] Yet other genes are known which exert their control upon anthocyanin pigmentation in this plant (for a review, see Refs. 4, 5).

In spite of the abundance of available genetic colour markers, little attention has been so far given to the biochemical study of their gene products, and of their manner of interaction. Furthermore the chemical analysis of the aleurone pigment so far performed has not always led to the same results. Coe[6] identified the purple pigments of the aleurone, in a **Pr** line, as cyanidin glycosides and those of red pigmented aleurone, in a **pr** line, as pelargonidin glycosides. On the other hand, Straus[7] found three anthocyanins in endosperm tissue culture extracts from "Black Mexican Sweet Corn;" two were identified as cyanidin 3-glucoside and pelargonidin 3-glucoside while the third, present only in small amount, was not identified.

[1] L. J. STADLER, *Cold Spring Harbor Symp. Quant. Biol.* **16**, 49 (1951).
[2] J. R. LAUGHNAN, *Genetics* **33**, 488 (1948).
[3] G. R. K. SASTRY, Ph.D. Thesis, University of Wisconsin Library, Madison, Wisconsin (1965).
[4] R. A. EMERSON, G. W. BEADLE and A. C. FRASER, *Cornell Univ. Agri. Expt. Sta. Mem.* **180**, 83 (1935).
[5] R. W. BRIGGS, *J. Heredity* **57**, 35 (1964).
[6] E. H. COE, *Genetics* **40**, 568 (1955).
[7] J. STRAUS, *Plant Physiol. Lancaster* **34**, 536 (1959).

Finally Baraud *et al.*[8] reported that the pigment of a Peruvian dark-seeded variety was cyanidin 3-galactoside acylated with *p*-coumaric acid.

As part of a reinvestigation of flavonoid biosynthesis in maize and in order to resolve the above discrepancies, the anthocyanins produced by two lines carrying the five complementary colour genes and differing in their **Pr** constitution have been analysed. The results are given in the present paper.

RESULTS

In a preliminary chromatographic study,[9] three red–purple pigments were consistently detected in aleurone, roots, coleoptile and anthers of a **Pr** line of maize. A similar analysis of **pr** tissues showed the presence of three orange–red pigments of correspondingly higher R_f values. Since plant and seed pigmentation are conditioned by two components of the **R** locus, these results indicate that the two components produce the same anthocyanins in the different tissues and that **pr** substitution causes a change in the anthocyanins produced, in all the tissues analysed. More detailed analysis has been limited to the aleurone pigment, which can be obtained, rather laboriously, by scraping the surface of seeds, from which the pericarps have been removed, and extracting the powder with methanol–HCl.

The three pigments in **Pr** aleurone tissue were carefully purified by repeated paper chromatography, in order to remove leucoanthocyanins and other phenolic contaminants and were then subjected to analysis by standard procedures.[10] One pigment was readily identified as cyanidin 3-glucoside, by its giving cyanidin and glucose on hydrolysis, by R_f and spectra and by co-chromatography with authentic material. The other two pigments were of higher R_f in BAW and Bu–HCl but both gave cyanidin and glucose on hydrolysis. They were, therefore, assumed to be acylated derivatives, and, in fact, both gave cyanidin 3-glucoside on alkaline treatment. Spectral analysis indicated that the acyl group was not *p*-coumaric or any other hydroxycinnamic acid, but the actual identity of the acylating radical has not yet been determined. The considerable instability of the two acylated glucosides, as compared with known acylated glycosides,[10] and the difficulties of isolation and purification have hindered further studies. The possibility that the two acylated derivatives are chromatographic artifacts caused by contamination of cyanidin 3-glucoside with, e.g. leucoanthocyanin, can be ruled out by their separation under a range of different chromatographic conditions (including TLC on silica gel),[11] by their behaviour on alkaline treatment and by their consistent presence in widely different plant organs (see above).

Similar analysis of the pigments of **pr** aleurone tissue showed the presence of pelargonidin 3-glucoside and two acylated derivatives, presumably of similar structure to the cyanidin derivatives described above. In addition, **pr** lines contained appreciable quantities of cyanidin 3-glucoside, the pigment otherwise characteristic of **Pr** types. Thus, although the dominant **Pr** clearly controls hydroxylation at the 3′-position in anthocyanins, some of the same pigment appears in the allelic forms and **pr** is in this respect a hypomorph. This situation is not a common one in higher plant genetics, but has been noted before in the case of flavonol glycoside production in *Solanum* (Solanaceae).[12]

No trace was detected during the present studies of the *p*-coumaroyl 3-galactoside of

[8] J. BARAUD, L. GENEVOIS and J. P. PONART, *J. Agric. Trop. Bot. Appl.* **11**, 55 (1964).
[9] G. GAVAZZI and S. SPASCIANI, *Maize News Letter* **42**, 116–120 (1968).
[10] J. B. HARBORNE. *Comparative Biochemistry of the Flavonoids*, Academic Press, New York (1967).
[11] D. HESS and C. MEYER, *Z. Naturf.* **176**, 853 (1962).
[12] J. B. HARBORNE, *Biochem. J.* **84**, 100 (1962).

cyanidin reported in seeds of a dark-coloured maize variety by Baraud *et al.*[8] and this pigment thus seems to be absent from any of the known maize genotypes.

EXPERIMENTAL

The two lines used for pigment extraction were homozygous for the following genetic markers. (1) **b pl** A_1 A_2 C_1 C_2 **R Pr**; (2) **b pl** A_1 A_2 C_1 C_2 **R pr**. Both lines, hereafter referred to as **Pr** and **pr** lines, have the same genetic background of an inbred line, commercially known as W22. Pericarps of 100 seeds of each line were peeled off and the pigmented aleurone was removed with a file. The powder so obtained (*ca.* 2 g) was then extracted with 1% HCl in methanol and concentrated. Extracts were separated and purified by chromatography on Whatman No. 3 paper, using BAW (butanol–acetic acid–water, 4:1:5), Bu–HCl (butanol–2 N HCl, 1:1), and varying mixtures of acetic acid and water. The spectral and chromatographic properties of the purified pigments are shown in Table 1. The pigments A2, A3, B2 and B3 were unstable during chromatography and on re-chromatography always gave considerable amounts of the respective 3-glucosides. On acid hydrolysis, A2 and A3 gave cyanidin (identified by spectrum and co-chromatography) and glucose, the sugar being clearly separated on chromatograms from added galactose; B1–B3 gave pelargonidin and glucose.

TABLE 1. CHROMATOGRAPHIC AND SPECTRAL PROPERTIES OF **Pr** AND **pr** ANTHOCYANINS

Pigment*	R_f (×100) in BAW	BuHCl	1% HCl	HOAc–HCl
A1	26	28	9	37
A2	31	44	14	36
A3	37	54	15	53
B1	37	31	12	34
B2	41	43	12	35
B3	42	65	12	34
Cyanidin 3-glucoside	25	26	8	35
Pelargonidin 3-glucoside	35	28	16	39

Pigment	Spectral properties: λ_{max} in MeOH–HCl (nm)	$E440/E_{max}$ (as %)	$E310/E_{max}$ (as %)	$E280/E_{max}$ (as %)
A1	283, 534	26	6	49
A2	284, 532	27	30	95
A3	284, 533	25	29	96
B1	290, 512	45	24	—
B2	292, 514	47	22	—

*A1–3 are cyanidin-based pigments from **Pr** lines; B1–3 are pelargonidin-based pigments from **pr** lines. Insufficient material of B3 was available for spectral studies.

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