Phytochemistry, 1975, Vol. 14, pp. 413–415. Pergamon Press. Printed in England.

GENETIC CONTROL OF 3-HYDROXY- AND 3-DEOXY-FLAVONOIDS IN *ZEA MAYS*

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(*Revised Received* 24 *July* 1974)

Key Word Index—*Zea mays*; Gramineae; maize; flavonoid genetics; apiforol; apigeninidin 5-glucoside; luteoforol.

Abstract—Genetic strains of maize have been derived that have strong concentrations of 3-deoxyanthocyanins, flavan-4-ols and *C*-glycosyl-flavones in silks and cob. The gene *Pr* controls the B-ring hydroxylation pattern of both 3-deoxy- and 3-hydroxy-flavonoids in these plants. Cob phlobaphenes are red in *Pr* plants and orange in *pr* plants, corresponding to the presence of the probable precursors luteoforol and apiforol.

INTRODUCTION

Two classes of flavonoid pigments commonly found in *Zea mays* are anthocyanins, which can be produced in almost any tissue, and "phlobaphenes", which are found predominantly in the cob and the pericarp. The dominant gene A_1 is required for both classes of pigments, but the genetic control differs in that phlobaphene production requires only an active *P* gene in addition to A_1, whereas anthocyanin synthesis requires A_1, A_2, and either *B*, R^r or r^r in the plant, and A_1, A_2, C_1, C_2 and *R* in the aleurone [1]. The gene *Pr* exerts a qualitative change in aleurone anthocyanins such that *Pr* stocks produce predominantly cyanidin derivatives and *pr* stocks produce predominantly pelargonidin derivatives [2]. We have now derived strains of maize that have strong concentrations of *P* locus dependent 3-deoxyanthocyanins, flavan-4-ols and *C*-glycosyl-flavones in silks and cob. In these same strains the *Pr* factor controls the B-ring hydroxylation pattern of both 3-deoxy and 3-hydroxy-flavonoids in all plant tissues. Cob phlobaphenes are red in *Pr* plants and orange in *pr* plants.

RESULTS AND DISCUSSION

Plants of the W22 inbred line of maize genetically P^{wr} R^r *Pr* (colorless pericarp, red cob, purple aleurone and red anthers) were crossed with plants of an early line of maize originating from the Gaspé region of Quebec, phenotypically colorless pericarp, white cob, colorless aleurone and brown anthers. Families derived from these crosses have strong concentrations of 3-deoxyflavonoids in both silks and cobs. Table 1 shows that these 3-deoxyflavonoids are independent of *R*, a gene

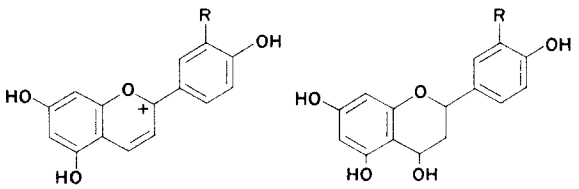
Table 1. Predominant flavonoids in genetic strains of maize that show strong concentrations of *P* locus dependent 3-deoxyflavonoids in cobs and silks. Note that the *Pr* gene controls the B-ring hydroxylation pattern in all predominant flavonoids

		Plant genotype							
		P^{wr}				P^{ww}			
		Pr		*pr*		*Pr*		*pr*	
Flavonoid class	Tissue	R^r	r^r	R^r	r^r	R^r	r^r	R^r	r^r
Anthocyanidins* (3-hydroxy)	Cob	—	—	—	—	—	—	—	—
	Silks	Cyanidin	—	Pelargonidin	—	Cyanidin	—	Pelargonidin	—
Anthocyanidins* (3-deoxy)	Cob and Silks	Luteolinidin	Luteolinidin	Apigeninidin	Apigeninidin	—	—	—	—
Flavan-4-ols	Cob and Silks	Luteoforol	Luteoforol	Apiforol	Apiforol	—	—	—	—
C-glycosyl flavones	Cob and Silks	Orientin type	Orientin type	Vitexin type	Vitexin type	—	—	—	—
Phlobaphenes	Cob	Red	Red	Orange	Orange	—	—	—	—
	Silks	—	—	—	—	—	—	—	—

* Mainly glycosidically bound.

which seems only to control the presence or absence of 3-hydroxyflavonoids in a tissue. They are, however, dependent on *P* action, as shown by the absence of 3-deoxyflavonoids from cobs of P^{ww} plants. All the predominant flavonoids appear subject to the control of the *Pr* locus. The genetic factors immediately involved in the production of the 3-deoxyflavonoids have not been characterized, but the gene *sm* (salmon silk) is known to be *P* locus dependent, and Levings [3] isolated two anthocyanins based on luteolinidin (**1**) from the silks and pericarp of *sm* P^{rr} plants, and identified one of these anthocyanins as luteolinidin 5-glucoside. He did not report obtaining any apigeninidin (**2**) glycosides.

The general expression of the *pr* factor in plant tissues is not typical for most strains of maize, but in these strains the composition and color of the phlobaphenes is clearly under *Pr* control. Bate-Smith [4] has obtained evidence that phlobaphenes in sorghum pericarp are formed by the conversion of luteoforol (**3**), and we have found parallel evidence in maize that, (a) there is a strong concentration of luteoforol in P^{rr} *Pr* cobs and pericarp



(**1**) R = OH
(**2**) R = H

(**3**) R = OH
(**4**) R = H

prior to the formation of the cob and pericarp color, (b) the luteoforol extract converts to a red pigment that has similar properties to red pigment extractable from the colored cob and pericarp, and (c) the concentration of luteoforol decreases as the red pigment develops in the maturing tissues of the cob and pericarp. The orange cob pigments of the P^{wr} *pr* families shown in Table 1 are probably phlobaphenes resulting from the complexing of apiforol (**4**), which appears in place of luteoforol in *pr* plants.

Evidence from this and other studies in maize favor a biosynthetic pathway that establishes the B-ring hydroxylation pattern prior to the formation of chalcone. There appears to be a requirement for two pathways beyond chalcone, one concerned predominantly with the synthesis of 3-deoxy compounds and the other with 3-hydroxy compounds. The two pathways are required in order to explain how the *P* locus can control the presence or absence of the 3-deoxy compounds without affecting the synthesis of the 3-hydroxy compounds. The A_1 locus must necessarily be involved in both pathways as the recessive a_1 allele effectively blocks the synthesis of both 3-deoxy- and 3-hydroxy-anthocyanins and -leuco-anthocyanidins. The a_1 allele does not prevent the production of *C*-glycosylflavones [5] and flavonols [6], however, and in fact the production of these two classes of compounds may be enhanced in specific tissues of a_1 plants.

Apiforol is not a common flavonoid and there is little published data on it. Bate-Smith [4] obtained a λ_{max} of 537 nm by treating apiforol prepared by reduction of naringenin with 43% methanolic H_2SO_4 at $-15°$. This compared with a λ_{max} of 550 nm obtained by similar treatment of luteoforol prepared by reduction of eriodictyol. An extract from sorghum pericarp gave a λ_{max} of 550 nm with this same test. We have also obtained a λ_{max} of 550 nm when this test was applied to a sorghum pericarp extract. However, the extract from our particular sample of sorghum yielded approximately equal amounts of luteolinidin and apigeninidin on hydrolysis. We have obtained the following values by applying this color test to extracts of maize cobs: (A) Table I strains: P^{wr} *Pr*: λ_{max} 563 nm (yielded only luteolinidin on hydrolysis); P^{wr} *pr*: λ_{max} 547 nm (yielded apigeninidin plus traces of luteolinidin on hydrolysis) (B) W22 inbred strain P^{wr} *Pr*: λ_{max} 562 nm (yielded only luteolinidin on hydrolysis). Mixtures of extracts from P^{wr} *Pr* maize cob and sorghum pericarp gave single shoulderless peaks varying in λ_{max} from 563 to 550 nm according to the relative concentration of each extract.

It should be noted that *Zea mays* is in the tribe Maydeae of the Gramineae. Previous reports of flavan-4-ols in the Gramineae have been confined to species of the tribe Andropogoneae [7], of which sorghum is a member.

The apigeninidin glycoside was shown to be apigeninidin 5-glucoside (gesnerin) by co-chromatography and spectral comparison with authentic material. This is the first report of gesnerin in maize.

EXPERIMENTAL

Tissue was extracted in 1% HCl in MeOH. The extract was concentrated and separation was by 2-D TLC with Avicel S.F. Microcrystalline cellulose as medium. Aglycones were obtained by hydrolyzing the concentrate with an equal volume of 4 N HCl in darkness for 30 min. *C*-Glycosylflavones were obtained by hydrolyzing the concentrate with an equal volume of 2 N HCl at 70° for 60 min. The main solvents used for glycosides and *C*-glycosylflavones were EtOAc–HCOOH–H_2O–Conc. HCl (85:6:8:1) [8] for the first direction and 15% HOAc for the second. Solvents used for aglycones were Forestal for the first direction and *n*-AmOH–HOAc–H_2O (2:1:1) for the second.

Acknowledgements—This study was supported by grants from the National Research Council of Canada and the University of Victoria.

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