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FLAVONOID PIGMENTS IN GENETIC STRAINS OF MAIZE

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Abstract—Cyanidin, pelargonidin, peonidin, luteolinidin and five unidentified purple pigments have been isolated from hydrolyzed extracts of maize plants. Luteolinidin may be present in extracts from plants recessive for genes controlling visible anthocyanin production, but plants of a_1 a_1 genotype yield no anthocyanidins at all. The luteolinidin is probably originally present as the leuco substance luteoforol. Luteoforol appears to be the precursor of the bronze pigment found in bz_1 bz_1 plants.

INTRODUCTION

THE ANTHOCYANINS commonly found in maize are derivatives of cyanidin and pelargonidin. Plants of *Pr Pr* constitution produce predominantly cyanidin derivatives in the aleurone and anthers, whereas *pr pr* plants produce predominantly pelargonidin derivatives. Seah and Styles[1] reported that seven pigments were isolated from *Pr Pr* strains of the W22 inbred line following hydrolysis of the methanol–HCl extract and separation by TLC. Two of these pigments were cyanidin and pelargonidin, a third was later characterized as peonidin.[2]

A number of genes are known to be involved in flavonoid production in maize, and Reddy (see Ref. 3), on the basis of cross-feeding studies with fresh aleurone tissue, placed some of these genes in sequence for the metabolic pathway to anthocyanin as follows: $C^I \rightarrow C_1 \rightarrow C_2 \rightarrow R \rightarrow In \rightarrow A_1 \rightarrow A_2 \rightarrow Bz_1 \rightarrow Bz_2 \rightarrow$ anthocyanin. The *C* genes are necessary for anthocyanin formation in the seed, but not for the plant tissues. A_1, A_2 and *R* are complementary genes. Certain *B* alleles replace *R* in the sequence; Bz_1 and Bz_2 result in a bronze pigmentation when either are recessive; *In* results in intensification of pigment when recessive, and *Pr* controls the hydroxylation pattern of the anthocyanin molecule as described above. This paper reports the characteristics of pigments isolated from W22 inbred strains differing with respect to *B*, *R*, A_1, A_2, *Pr* and Bz_1 loci.

RESULTS AND DISCUSSION

Pigments were isolated from hydrolysates of *Pr Bz In* strains carrying all genetic factors necessary for visible anthocyanin production; the plants were grown in the greenhouse during the fall when conditions maximize anthocyanin formation in the plant. Several strains differing with respect to *B* and *R* constitution were sampled (*B* and *R* genes control

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[2] K. T. SEAH, M.Sc. Thesis, University of Victoria Library, Victoria, B.C. (1969).
[3] G. M. REDDY and E. H. COE, *Science* **138**, 149 (1962).

both the amount and distribution of pigment in the plant and seed and some strains had pigment in tissues where others had none).

Nine pigments were obtained. Cyanidin was the predominant pigment as expected, and, in general, the other pigments were in relatively low concentration. In order of decreasing concentration, the other pigments were pelargonidin, peonidin, luteolinidin and five unknown purple pigments with R_f values ranging from 36 to 84 in Forestal and 9 to 54 in FHW; λ_{max} from 538 to 546 nm and 265 to 275 nm; E_{440}/E_{max} ratios from 32–55. All gave positive aluminum shifts. The unknown purple pigments were usually at highest concentrations in the mature plant.

One strain, carrying a null factor r^g at the *R* locus and a factor at the *B* locus conditioning strong pigment in the seed ('R_2:Peru') is green throughout most of the plant life and has only a slight amount of pigment in the tassel and ear husks of the very mature plant. Despite the absence of visible pigmentation in most tissues, this strain yielded as much luteolinidin as any of the other pigmented strains. No other anthocyanidins were isolated in the plant tissues of this strain, except for the very mature tissues referred to above. This suggests that in maize the presence of luteolinidin is independent of the presence of the other anthocyanidins, and perhaps either the pathways involved are separate, or the *R* and *B* genes control steps subsequent to the formation of some common precursor, such as has been suggested for the *R* gene in Sorghum.[4]

That the pathways concerned may be different, but not necessarily independent, is supported by information obtained from other plants of different genotypes grown under field conditions and sampled at anthesis. Relevant points are the following: (1) Anthers from *pr* strains yield pelargonidin as the predominant anthocyanidin, and small amounts of cyanidin and peonidin, but they yield the same amounts of luteolinidin as do the *Pr* strains. There is no detectable amount of apigeninidin as might be expected if the *Pr* locus controlled 4′-hydroxylation in both pathways. (2) Strains carrying the recessive a_2 gene (no anthocyanin, but some accumulation of leucoanthocyanidins[5]) have the same concentration of luteolinidin as A_2 strains with normal amounts of anthocyanin. Thus it appears that the 3-deoxy pathway is independent of the A_2 gene also. (3) Anthers from a_1 strains (no anthocyanin, but flavonol formation unaffected[6] or enhanced[7]) have no detectable luteolinidin, nor any other 3-deoxyanthocyanidin. Reddy's proposed gene action sequence for the pathway to anthocyanin in maize (see above) places the A_1 gene after *R* but before A_2. This presumably means that the *Pr*, *R* and A_2 actions are required only for the pathway to the common anthocyanins, whereas the A_1 action is apparently required for the pathway to the 3-deoxyanthocyanins as well. (4) Luteolinidin is present in moderate amounts in the hydrolyzed extracts of silks from all plants except those of a_1 genotype, and it is particularly concentrated in the extracts from the silks of bz_1 plants, which lack other visible pigmentation. Chromatography of unhydrolyzed bz_1 silk extract did not yield an orange spot as expected of a luteolinidin glycoside, but a test for the presence of luteoforol in the fresh silk extract by the method of Bate-Smith[8] was positive. Thus it appears that the silks, and probably the other tissues also, contain luteoforol which converts to luteolinidin upon hydrolysis of the methanol extract. (5) There were increased amounts of luteoforol in all bz_1 tissues. This is particularly interesting from the standpoint of the possible origin of the bronze pigment in

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the mature bz_1 plants. If bz_1 silks are extracted in 1% HCl in MeOH prior to their emergence from the leaf sheath, the extract is light green. After storage at 5° for a period of time (2 or 3 weeks), the extract becomes orange–brown. When this extract is then chromatographed, the brown pigment in the extract behaves in the same manner as the grown pigment which can be extracted from the bronze coloured mature plant tissues. It seems probable that luteoforol forms a precursor to the bronze pigment in maize, much as Bate-Smith[8] found occurs in some varieties of Sorghum.

We have not yet been able to obtain evidence as to whether the unknown purple pigments exist in the plant as glycosides or whether they are products of the hydrolysis procedure. The anthocyanins in maize are very complex, and are almost certainly acylated.[9] Trace amounts of the unknown pigments, if they existed as glycosides, would be masked by the major pigments. However, their presence or absence is predictable in the sense that at certain stages of the plant's development they are present and at other times they are absent, and this is independent of whether the plant's genotype conditions strong or weak pigmentation. Apart from the fact that all five of the pigments undergo bathochromic shifts, indicating that they have at least one catechol group in their structure, there is little else that can be deduced at this stage as to their relationship with the common anthocyanidins.

EXPERIMENTAL

Tissue was extracted in 1% HCl in MeOH, concentrated, and hydrolyzed with an equal volume of 4 N HCl at 97–100° in darkness for 30 min. Separation was by two-dimensional TLC, using Avicel S.F. microcrystalline cellulose as a medium, Forestal as solvent for the first direction and *n*-AmOH–HOAc–H_2O (2:1:1) for the second.

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