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FLAVONOL GLUCOSYLTRANSFERASE ACTIVITY IN *BRONZE* EMBRYOS OF *ZEA MAYS*

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Key Word Index—*Zea mays*; Gramineae; maize; biochemical genetics; *Bz* locus; flavonoids; flavonol glucosyltransferase; isozymes.

Abstract—The major UDPG: flavonol glucosyltransferase (UFGT) in maize is an enzyme of strict positional specificity known to be coded by the *Bz* locus. In *bz* mature endosperms, no UFGT can be detected. However, *bz* embryos possess a residual flavonol glucosyltransferase activity which is independent of *Bz* locus control. The products of this activity have been identified as the 3′-, 7- and 3-glucosides.

INTRODUCTION

Flavonoid glucosylation in maize is predominantly controlled by the *Bz* locus in chromosome 9, which specifies the enzyme UDPG: flavonoid 3-*O*-glucosyltransferase (UFGT) [1, 2]. Homozygous *bz* plants accumulate a bronze, rather than purple, pigment in all plant parts, e.g. aleurone, coleoptile, leaf sheath and blade, silks, glumes, etc. [3]. Styles and Ceska [4] have detected in *bz* tissues increased amounts of luteoforol instead of cyanidin, which is the major pigment found in hydrolysed extracts of *Bz* plants that carry all the complementary anthocyanin genes.

The bulk of the flavonoid 3-*O*-glucosides found in maize is probably produced by action of the *Bz*-dependent UFGT. Such glucosides include isoquercitrin (quercetin-3-glucoside) which is known to occur in husks [5] and pollen [6, 7] and cyanidin-3-glucoside, which has been described in endosperm cultures [8] and aleurone tissue [9]. Recently, Styles [10] has found in *bz* pollen traces of flavonols glucosylated at either the 5- or 7-hydroxyl. This paper reports the presence in *bz* mature seed of a minor flavonol glucosylating activity which seems to be restricted to the embryo, being undetectable in the endosperm. The products of the reaction have been identified as the 3′-, 7- and 3-glucosides.

RESULTS

Assays of UFGT activity in endosperm preparations from *bz* mature seed reveal that, in contrast to *Bz* endosperm, most *bz* mutants lack the capacity to form isoquercitrin and hence can be considered as null mutants for this activity [11]. However, low levels of isoquercitrin (1.2–2.9% of *Bz*) were consistently detected in assays of embryo preparations from the same mutants (Table 1).

Since mature endosperm preparations of *bz* mutants lack UFGT activity and cross-reacting material (CRM) to anti-UFGT serum [11], the residual UFGT activity detected in embryo preparations of the same mutants is probably independent of *Bz*-locus control. To ascertain this, the effect of varying dosage of the *bz-R* allele on residual UFGT activity was examined. Homozygous (*bz-R*/*bz-R*) and hemizygous (*bz-R*/-) sib embryos can be generated by utilizing the *bz-x2* deficiency for the *bronze* locus [12], and taking advantage of the extremely tight linkage of the deficiency with the distal marker *sh* (shrunken endosperm). From the cross *Sh bz-x2*/*sh bz-R* × *sh bz-R*, two types of bronze kernels are produced. Shrunken kernels are almost exclusively *bz-R* homozygotes (*sh bz-R*/*sh bz-R*), whereas most plump kernels are *bz-R* hemizygotes (*Sh bz–x2*/*shbz-R*). The results of comparing one and two doses of *bz-R* are given in Table 2. As is evident, no effect of *bz-R* dosage on residual UFGT activity can be detected. Taken together, the data presented in Tables 1 and 2 indicate that the residual glucosyltransferase activity detected in *bz* embryos is independent of *Bz* locus control. Thus, different UFGT isozymes may be involved in isoquercitrin production in maize embryos.

Table 1. UFGT activity (isoquercitrin formation) in endosperms and embryos of *Bz* and *bz* mature seeds

Genotype	Endosperm m units* / mg prot.	Endosperm m units / endo.	Endosperm % *Bz*	Embryo m units / mg prot.	Embryo m units / embryo	Embryo % *Bz*
Bz	945.0	150.0	100.0	71.0	20.00	100.0
bz-R	0.0	0.0	0.0	1.3	0.33	1.7
bz-m2 (*DI1*)	0.0	0.0	0.0	1.2	0.23	1.2
bz-m4	5.3	1.2	0.8	3.1	0.58	2.9
Bz-wm	0.0	0.0	0.0	2.7	0.51	2.6

*nmol Isoquercitrin/hr.

Table 2. Effect of varying *bz-R* dosage in mature embryos on the residual UFGT activity (kernels obtained from the cross *Sh bz-x 2/sh bz-R* × *sh bz-R*)

Kernel phenotype	Embryo genotype	m units* / embryo
Shrunken	bz-R/bz-R	0.254
Plump	bz-R/-	0.217

*nmol Isoquercitrin/hr.

Isoquercitrin formation, as reported in Tables 1 and 2, was measured following our routine PC separation [2] which utilizes 15% HOAc as the developing solvent. In this solvent, UDPG-[^{14}C] moves close to the front, isoquercitrin has R_f 0.4, and quercetin barely leaves the origin. In assays of normal embryo preparations, essentially no counts can be detected at the origin. However, in assays of *bz* embryo preparations, a radioactive, yellow fluorescent spot, which did not separate well from quercetin, was consistently observed in addition to isoquercitrin. Since quercetin glucosides other than isoquercitrin have very slow mobilities in 15% HOAc, 2D PC was subsequently utilized to separate and characterize the novel radioactive products – presumably quercetin glucosides – which are formed uniquely by *bz* embryo preparations. Whether these products are not formed at all by *Bz* embryo preparations cannot be ascertained at this time. These preparations have the major, *Bz*-dependent UFGT activity, and after long incubation times (2 hr), about 40% of the label in UDPG has been incorporated into isoquercitrin, with very little, if any, radioactivity localized at the origin.

The two-dimensional analysis of the products formed by the *bz-R* embryo preparations revealed that, in addition to isoquercitrin, two radioactive, yellow fluorescent compounds, fully separable from quercetin, were present. The R_f values of these compounds and the relative amounts formed (cpm incorporated) are given in Table 3. These compounds were eluted from the paper and subjected to spectral analysis, the results of which are summarized in Table 4. From the R_f values and spectral properties, it is concluded that compounds B and C are, respectively, quercetin 3'-glucoside and quercetin 7-glucoside. The predominant product formed in the reaction has been found consistently to be the 3'-glucoside. The 3- and 7-glucosides, which are formed in roughly equal amounts, account together for less than 50% of the counts incorporated.

Attempts to establish whether the 3', 7- and 3-glucosides are formed by separate enzymes or by one enzyme of broad specificity for position of glucosylation have been hampered by the extremely low levels of activity seen in *bz* embryos. The activities coprecipitate during ammonium sulfate fractionation. However, efforts to recover activity following ion exchange column chromatography have so far been unsuccessful.

DISCUSSION

The genetic and biochemical evidence given in this paper argues for the presence in maize kernels of more than one flavonol glucosylating activity. The only activity seen in normal endosperms and embryos is that of the enzyme UDPG: flavonoid 3-*O*-glucosyltransferase, an enzyme of strict positional specificity known to be coded by the *Bz* locus. In *bz* embryos, but not endosperms, a residual glucosylating activity can be detected which is independent of *Bz* locus control. The glucosylation of quercetin by *bz* embryo preparation occurs predominantly at the 3' position. However, the 7- and 3-glucosides are also formed in the reaction. Assays with lengthy incubation periods (2 hr) of normal embryo preparations resulted in high yields of isoquercitrin, the product of the *Bz* enzyme, but gave no indication of formation of either the 3'- or 7-glucosides. Possibly, the enzyme(s) responsible for 3'- and 7-glucosylation is absent in normal embryos and is formed only in *bz* embryos. Styles and Ceska [13] have, in fact, suggested that the effect of the *bz* block is to favor the formation of any compound that stabilizes aglycones. Alternatively, the enzyme may be present in *Bz* embryos but cannot be detected due to the presence of the major UFGT. One question that remains unanswered is whether the

Table 3. Characterization of products formed by *bz-R* embryo preparations*

Compound	R_f(× 100) in BAW	R_f(× 100) in 15% HOAc	cpm†	Color UV
A (quercetin)	66	06	0	yellow
B	46	09	500	bright yellow
C	36	12	211	bright yellow
D (isoquercitrin)	56	40	263	brown

*Separation by 2D PC.
†Total cpm added: 11 500.

Table 4. Spectral properties of the yellow fluorescent products formed by *bz*-R embryo preparations

Compound	λ_{max} in 95% EtOH (nm) Band II	Band I	$\Delta\lambda$ NaOEt Band I	$\Delta\lambda$ $AlCl_3$ Band I	$\Delta\lambda$ $NaMoO_4$ Band I	$\Delta\lambda$ NaOAc Band II
B	253, 266*	368	−48, dec.	+58	+15	+18
C	256	375	+55, dec.	+50	+40	0

*Shoulder.

residual flavonol glucosylating activity seen in *bz* embryos is due to a single enzyme of broad positional specificity or to several enzymes. The glucosyltransferases of parsley [14], like the *Bz* enzyme in maize, have strict positional specificity. Unfortunately, the low activity present in *bz* embryos has militated against a more extensive biochemical analysis.

EXPERIMENTAL

Materials. All the genotypes studied were in the common genetic background of the inbred W22. Most *bronze* mutations have been described elsewhere [11]. The *bz-x2* deficiency was kindly provided by John Mottinger [12]. Other than *bz* mutations, the stock carried all the complementary factors required for anthocyanin formation.

Enzyme preparation. Mature kernels were dissected into endosperms and embryos which were then processed basically as per extraction of the major, *Bz*-controlled UFGT [2]. Embryo protein precipitating between 30 and 55% ammonium sulfate saturation was used in enzyme assays.

Enzyme and protein assay. Isoquercitrin formation and total protein were assayed as described previously [11]. Incubation times varied depending on the source of the preparation being assayed for enzyme activity. Endosperm and embryo preparations from *Bz* kernels were assayed for 15 min, whereas *bz* embryo preparations were incubated for 1–2 hr.

Product characterization. The different flavonol glucosides formed in the reaction by *bz* embryo preparations were separated by 2D PC on Whatman 1 or 3 MM paper. Solvents used were 15% HOAc for the first direction and *n*-BuOH–HOAc–H_2O (4:1:5, upper) for the second. Spots were eluted with 95% EtOH and spectral analysis was performed as suggested by Mabry *et al.* [15] and Swain [16].

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