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THE ALLELIC STATE AT *INTENSIFIER* INFLUENCES THE ACCUMULATION OF UDP GLUCOSE: FLAVONOID 3-*O*-GLUCOSYL-TRANSFERASE IN MAIZE

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Key Word Index—*Zea mays*; Gramineae; maize; biochemical genetics; *In/in* locus; flavonoids; anthocyanins; *Bz* locus.

Abstract—In a variety of genetic backgrounds in maize (*Zea mays*), homozygosity for the recessive allele *intensifier* (*in*) conditions strikingly deeper pigmentation of aleurone tissue. The precise function of *in* in anthocyanin biosynthesis is unknown. We have observed that homozygosity for *in* is correlated with an increase in the amount of *Bronze* (*Bz*) gene product. *Bz* encodes the enzyme UDP-glucose: flavonoid 3-*O*-glucosyltransferase which catalyses one of the terminal steps in anthocyanin biosynthesis. Possible mechanisms by which *in* influences the accumulation of UFGT are discussed.

INTRODUCTION

In maize at least 25 different genes are known to affect flavonoid and anthocyanin biosynthesis [1]. Some of these, *A1*, *A2*, *C2* and *Bz1*, are thought to encode enzymes of the biosynthetic pathway and are required for anthocyanin synthesis. Others, *C1*, *R*, *Vp*, *in*, appear to exert indirect or regulatory effects on pigment formation. There are increased accumulations of proanthocyanins and anthocyanins in the aleurone tissues of plants homozygous for the recessive allele *intensifier* (*in*) as compared to *In/In* or *In/in* stocks ([2] and refs. cited therein). The (protein) product of *in* is not known and the mechanism by which *in* modulates anthocyanin accumulation has not been established. Coe [3] concluded, based on genetic evidence, that *in* functions before *A1* in the anthocyanin synthetic pathway.

The *Bronze* 1 (*Bz*) locus specifies the enzyme UDP glucose:flavonoid 3-*O*-glucosyltransferase (UFGT) [4, 5]. We have determined that the allelic state at *in* also influences UFGT levels in mature aleurone: for *in* genotypes that were otherwise competent to synthesize UFGT, homozygosity for *in* leads to a 1.5- to 2-fold increase in the specific activity of UFGT. A preliminary account of this work was reported earlier [6].

RESULTS AND DISCUSSION

Dooner and Nelson [5] had previously demonstrated that the levels of UFGT in *a1* and *bz2* mutants are similar to that of wildtype W22 material. They had also shown that the levels of UFGT are reduced in *c1*, *r* and *vp* mutants (pale or colorless aleurone) [7]. Since homozygosity for *in* leads to deeper color and an increase in the accumulation of anthocyanins and proanthocyanins, we wondered if the allelic condition at the *intensifier* locus also influenced the synthesis of UFGT. We compared the amounts of UFGT in mature endosperms of three genotypes [(*A1*, *A2*, *Bz1*, *Bz2*, *C2*, *R*, *Vp*); (*a1*, *A2*, *Bz1*, *Bz2*, *C1*, *C2*, *R*, *Vp*); and (*A1*, *A2*, *Bz1*, *bz2*, *C1*, *C2*, *R*, *Vp*)] that are competent to synthesize UFGT and which were homozygous for either *in* or *In* (Table 1). The specific activity of UFGT was always 1.5- to 2-fold higher in *in* kernels compared to corresponding *In* kernels. This relationship was the same whether activity was calculated as enzyme units per mg protein or units per endosperm. In one experiment, samples from *In* and *in* endosperms were combined: the UFGT activity equalled the numerical average of the individual *In* and *in* samples. This suggests that the difference in enzyme activity between *In* and *in* is not due to the presence of an inhibitor in the *In* genotypes.

Table 1. Influence of the allelic state at the *In/in* locus on UFGT levels in mature endosperms.

Genotype	Phenotype	nkat/ mg protein	nkat/ endosperm
A1, *A2*, *Bz1*, *Bz2*, *C1*, *C2*, *R*, *pr*, *In*	Red	0.136 ± 0.032(3)	0.183 ± 0.044(3)
A1, *A2*, *Bz1*, *Bz2*, *C1*, *C2*, *R*, *pr*, *in*	Dark red	0.303 ± 0.029	0.319 ± 0.029
bz2, *In*	Bronze	0.172 ± 0.052	0.209 ± 0.064
bz2, *in*	Red	0.464	0.392
a1, *In*	Colorless	0.185 ± 0.028	0.294 ± 0.044
a1, *in*	Colorless	0.336 ± 0.061	0.389 ± 0.070

Table 2. UFGT levels of kernels segregating for *in/In* within the same ear*

Genotype	Phenotype	nkat/ mg protein	nkat/ endosperm
bz2, In/In/in	Bronze	0.164 ± 0.022(3)	0.107 ± 0.014(3)
bz2, in/in/in	Red	0.244 ± 0.022	0.220 ± 0.022
bz2, In/In/in	Bronze	0.207 ± 0.056	0.144 ± 0.039
bz2, in/in/in	Red	0.222 ± 0.033	0.240 ± 0.036

*$bz2/bz2$, $In/In \times bz2/bz2$, *in/in*.

In order to exclude the unlikely possibility that the *in* influence could be due to differences between plants, we also compared UFGT levels between *in/in/in* and *In/In/in* kernels from the same ear (Table 2). In a $bz2/bz2$ plant, kernels homozygous for *in* are red; heterozygous kernels, for *In/In/in*, are bronze. Females, *In/in*, $bz2/bz2$, were pollinated by *in/in*, $bz2/bz2$ males. The amount of UFGT in the triploid endosperm was higher for *in/in/in* kernels than for the heterozygotes. There was variation in the specific activities of UFGT between the two ears; this is probably due to differences in maturity between the plants. There was no clear linear proportionality between the number of copies of *In* and the amount of UFGT activity (compare lines 3 and 4 of Table 1 to line 1 of Table 2).

Dooner and Nelson [7] determined that the products of *C1*, *R* and *Vp* are required for function of the *Bz* locus. They speculated that UFGT may be precursor-inducible. This suggests that under the influence of *intensifier*, the increase in the synthesis of the *Bz* gene product may be due to an increased flow of intermediates through the major anthocyanin pathway (and not through a branch pathway requiring *In* gene product). Alternatively, these effects may be caused by some other regulatory mechanism controlled by *in* and acting directly on the genes for anthocyanin biosynthesis. It is not, however, due to the synthesis of an inhibitor of UFGT in *In* strains. These results further exemplify how the *Bz* locus responds to a range of signals which determine not only when the gene is expressed [8], but also the level of synthesis of *Bz* protein.

EXPERIMENTAL

Plant material. All genotypes used in these studies were in the common background of the inbred *W22*. A preliminary survey was carried out on mature kernels from plants grown in Madison prior to 1981; these experiments were repeated with material grown in the summer of 1981. For convenience, the genotype of the homozygote is designated by one symbol (e.g. *in*); it is understood that the plant is diploid (*in/in*) and the endosperm is triploid (*in/in/in*).

Enzyme preparation. The pericarps and embryos were removed and the endosperms were ground in a Wiley Mill fitted with a 40 mesh screen. Samples were extracted, with gentle stirring, for 1 hr at 4°, in 3 ml buffer/g dry wt. The extraction buffer contained 100 mM HEPES buffer (pH 7.5), 100 mM NaCl, 1 mM PMSF and 1 mM DTT with the ion exchange resin AGl-X2 (30 mg/l ml). Extracts were centrifuged for 20 min (10000 g), and the supernatant fraction was filtered through glass wool. These filtrates were used as the source of enzyme for various assays.

Enzyme assays. Isoquercitrin formation was monitored as described in ref. [5] with some modifications: each reaction contained 100 nmol HEPES buffer (pH 8.2), 310 nmol $CaCl_2$, 250 nmol β-mercaptoethanol and 3% polyethyleneglycol 6000, with 250 nmol UDP-[^{14}C]glucose (sp. act. 0.15 Ci/mol) and 250 nmol quercetin (dissolved in 5 μl ethyleneglycol monomethyl ether). 5 to 25 μl of enzyme extract was used in each assay. The total reaction vol. was 50 μl. Isoquercitrin was extracted into 300 μl of EtOAc and 200 μl of the organic phase was removed, spotted onto a glass fiber filter and dried in a scintillation vial. Chromatographic separation of the products indicated that isoquercitrin represented at least 95% of the labeled products formed under these conditions. Protein was determined by the method of Bradford [9].

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