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# Regulation of anthocyanin biosynthetic genes introduced into intact maize tissues by microprojectiles

(gene regulation/gene transfer/particle gun)

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ABSTRACT **We have employed microprojectiles to deliver genes involved in anthocyanin biosynthesis to cells within intact aleurone and embryo tissues of maize. Clones of the *A1* or *Bz1* genes were introduced into aleurone tissue that lacked anthocyanins due to mutations of the endogenous *A1* or *Bz1* gene. Following bombardment, cells within the aleurone developed purple pigmentation, indicating that the mutation in the *a1* or *bz1* genotypes was corrected by the introduced gene. To analyze the expression of these genes in different genetic backgrounds, chimeric genes containing the 5′ and 3′ regions of the *A1* or *Bz1* genes fused to a luciferase coding region were constructed. These constructs were introduced into aleurones of genotypes carrying either dominant or recessive alleles of the *C1* and *R* genes, which are known to regulate anthocyanin production. Levels of luciferase activity in permissive backgrounds (*C1, R*) were 30- to 200-fold greater than those detected in tissue carrying one or both of the recessive alleles (*c1, r*) of these genes. These results show that genes delivered to intact tissues by microprojectiles are regulated in a manner similar to the endogenous genes. The transfer of genes directly to intact tissues provides a rapid means for analyzing the genetic and tissue-specific regulation of gene expression.**

The biosynthetic pathway for anthocyanin production in maize has long been considered an ideal system for investigating the regulation of gene expression in plants (1, 2). Interest in this pathway stems from the fact that it is comprised of a group of structural genes that are coordinately regulated and expressed in response to genetic, developmental, and environmental cues (1, 3–5). The pathway is well characterized genetically and biochemically (6–12), with the order of structural genes deduced (11) to be *C2* (11), *A1* (13), *A2* (7), *Bz1* (10, 12), and *Bz2* (1, 6, 9).

The expression of the structural genes of the pathway is controlled by a number of regulatory genes that condition anthocyanin production in particular tissues (1). Pigment production in the maize aleurone requires the presence of the *C1* and *R* genes. These genes have been shown to regulate the appearance of the *Bz1* and *C2* gene products in the aleurone (11, 14). Additionally, there is evidence that *C1* is necessary for transcription of the *A1* and *Bz1* genes in this tissue (15). Other dominant alleles of *C1* or *R* can influence anthocyanin production (1). For example, the dominant *R* gene conditions anthocyanin production in the aleurone but not in the embryo. However, an allele of *R*, *Rscm2*, permits anthocyanin production in the scutellum of the embryo. An allele of *C1*, *C-I*, acts as a dominant inhibitor preventing anthocyanin production in the aleurone.

Much progress has been made toward understanding the pathway at the molecular level. Genomic clones have been isolated for *C2* (16), *A1* (17), *Bz1* (18), and *Bz2* (19, 20). In addition, the regulatory loci *C1* and *R* have also been cloned (15, 21, 22). Homology between the proteins encoded by *C1* and *myb*, a protooncogene from mammalian cells whose product is known to possess DNA-binding capacity, provides further evidence for the regulatory nature of the *C1* gene (23). Together with the *opaque2* locus of maize (24), the *C1* and *R* loci are among the few regulatory genes that have been isolated from plants.

A thorough understanding of the complex regulation of the anthocyanin biosynthetic pathway at the molecular level will expand knowledge of the factors that control gene expression in plants. However, it will be necessary to introduce modified structural or regulatory genes into maize to fully study the system. Unfortunately, a routine transformation system that gives rise to fertile plants has not yet been established for maize. Therefore, studies of the expression of foreign genes in maize, and in cereals in general, have relied on transient assays of genes introduced into protoplasts (25–31). However, foreign genes are often not properly regulated in protoplasts (32, 33). Therefore, it would be desirable to transfer exogenous DNA directly into cells within intact tissues so that proper gene regulation would be maintained.

It has previously been shown that microprojectiles can deliver DNA into cells within various intact tissues (34–37). In this paper we investigate whether microprojectiles can be used to introduce clones of the *A1* and *Bz1* genes into maize aleurone or embryo tissue and whether the expression of these clones can be detected phenotypically. Using the 5′ and 3′ regulatory regions of the *A1* or *Bz1* genes fused to a luciferase coding region (38), we examined whether the expression of the introduced DNA is properly controlled by the *C1* and *R* regulatory loci and their *C-I* and *Rscm2* alleles.

## MATERIALS AND METHODS

**Plasmid DNA.** The *A1* clone (pALC1, designated pA1 in this study) is the 4.3-kilobase (kb) *Eco*RI–*Hin*dIII fragment of the 8-kb *Eco*RI genomic clone (13, 17) in pUC9. The *Bz1* clone, pMBzR1, has been previously described (18, 39, 40). Fig. 1 shows the chimeric *A1*– and *Bz1*–luciferase constructs used in this study. pA1L is comprised of the following: (*i*) a 1.4-kb fragment from the 5′ region of the *A1* genomic clone. This *Hin*dIII–*Sst* I fragment was modified by adding a *Bgl* II site adjacent to the *Sst* I site at nucleotide 49 (ref. 13); (*ii*) a 0.165-kb fragment that includes the first intron of *Bz1* (nucleotides 584–749, ref. 40); (*iii*) the 1.8-kb coding region (*Bam*HI–*Bgl* II fragment) of the firefly luciferase gene (38), and (*iv*) the 3′ region contained in a 1.0-kb *Nhe* I (nucleotide 1693, modified to a *Bgl* II site) to *Eco*RI fragment of the *A1* genomic clone (17). pBz1L also includes the *Bz1* intron and the luciferase coding region but is under the control of the 5′ noncoding region of the *Bz1* genomic clone contained in a 2.3-kb *Eco*RI–*Nhe* I (nucle-

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Abbreviations: CAT, chloramphenicol acetyltransferase; DAP, days after pollination.

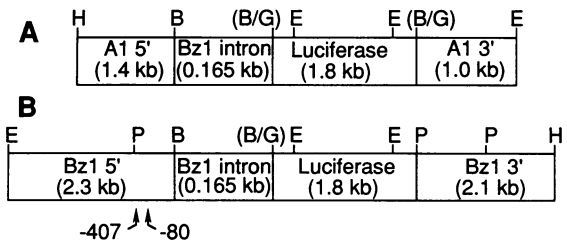


FIG. 1. Chimeric genes used in this study. (*A*) pA1L. (*B*) pBz1L. The arrows under *B* indicate the boundaries of the *Bz1* promoter deletion mutants. E, *Eco*RI; H, *Hin*dIII; B, *Bam*HI; G, *Bgl* II; P, *Pst* I.

otide 42, modified to a *Bam*HI site) fragment. The 3′ region of pBz1L consists of a 2.1-kb *Pst* I (nucleotide 1510; ref. 40) to *Hin*dIII fragment from the *Bz1* genomic clone. Both pA1L and pBz1L were cloned in pUC18 (41).

pBzΔ407L and pBzΔ80L are BAL-31 deletions (42) that extend from the *Eco*RI site in the *Bz1* promoter to position −407 and −80, respectively. pLBz3′ is a promoterless derivative of pBz1L that lacks the *Eco*RI–*Bam*HI *Bz1* promoter fragment. $pAI_1LN$ and $pAI_1CN$ have been previously described (31). For this study, $pAI_1LN$ and $pAI_1CN$ have been renamed pAdhL and pAdhC, respectively.

**Plant Material.** The genotypes and their sources are given in Table 1. Plants were grown in a greenhouse and either self-pollinated or crossed with sibling plants. Ears were harvested either 15 or 35–38 days after pollination (DAP) and surface sterilized by soaking them in distilled water containing bleach (2.5%, vol/vol) and sodium dodecyl sulfate (0.05%, wt/vol) for 30 min. Dissections for aleurone tissue were carried out by cutting the pericarp around the circumference of the caryopsis about half-way up from its base. The pericarp was then peeled back and removed to expose the aleurone. The aleurone and underlying endosperm tissue was excised by cutting horizontally through the tissue. Embryos were excised from caryopses 15–18 DAP. Twelve aleurones or embryos (embryo axis facing down) were placed in the center of a Petri dish containing agarose-solidified N6 medium (43) lacking 2,4-dichlorophenoxyacetic acid.

**Bombardment and Enzymatic Assays.** One day after dissection, the aleurones or embryos were bombarded with tungsten particles (average diameter of 1.2 μm) coated with plasmid DNA. The bombardment conditions and method for coating the microprojectiles with DNA have been described (35). In the experiments involving the chimeric genes, 5 μg of pBz1L, pA1L, or pAdhL and 2 μg of pAdhC were added to the microprojectiles prior to addition of the $CaCl_2$ and spermidine solutions. Following bombardment, the tissue was incubated under fluorescent light at 26°C for 1 day. The tissue from each bombardment (12 aleurones or embryos) was then ground in a mortar and pestle with 2 ml of buffer (100 mM potassium phosphate buffer, pH 7.0/1 mM dithiothreitol) at 4°C. The extract was centrifuged at 10,000 × *g* for 10 min. Luciferase activity was assayed as described (31) and is expressed as the number of light units recorded in 10 sec from 100 μl of extract by using a luminometer (Analytical Luminescence Laboratories, model 2001). Chloramphenicol acetyltransferase (CAT) activity was assayed as previously described (44) and is expressed as cpm observed from 25 μl of extract.

Table 1. Genotypes used in this study

| Genotype | Stock number | Permissive for expression of structural genes | |
|---|---|---|---|
| | | Aleurone | Embryo |
| *c1, r* | 291* | No | No |
| *c1* | 911A† | No | No |
| *r* | X13F† | No | No |
| *C-I* | 907E† | No | No |
| *bz, Rscm2* | G893-6‡ | Yes | Yes |
| *bz1* | P2414-2§ | Yes | No |
| *a1* | M476-27x471-12§ | Yes | No |

Those loci that are necessary for anthocyanin production and are not listed are present in a homozygous dominant condition.
*From Susan Wessler, University of Georgia.
†From Maize Stock Center, University of Illinois.
‡From Bill Sheridan, University of North Dakota.
§From Ed Coe, University of Missouri.

## RESULTS

**Expression of *A1* and *Bz1* Clones.** Aleurones from plants homozygous for a recessive allele of *a1* and dominant alleles of *C1* and *R* were excised 15 DAP, placed on agarose-solidified medium, and bombarded with microprojectiles coated with pA1 DNA. This plasmid contains the intact *A1* gene and flanking regions (17). After 1 day of incubation, some of the cells in the aleurone layer developed purple pigmentation. Isolated purple cells could be visualized, but, more generally, 3–5 adjacent cells developed pigmentation, with a central cell being the most pigmented. After 3–5 days, the pigmentation appeared to diffuse such that each cluster was generally composed of 5–10 cells (Fig. 2). The cells forming each cluster appeared to be pigmented to the same degree and were restricted to the aleurone layer. In general, half of the 12 aleurones exhibited 10–100 individual pigmented cells or clusters of cells following bombardment with pA1. Pigment production also was observed in pA1-bombarded aleurone tissue isolated from caryopses 35 DAP. Pigmented cells did not appear in *a1, C, R* aleurone tissue bombarded with pUC18 (Fig. 2). Additionally, purple cells did not develop in pA1-bombarded aleurones from plants homozygous for either or both of the recessive regulatory alleles (*C1, r; c1, R;* or *c1, r*).

Similar results were observed following introduction of the clone of the *Bz1* gene, pMBzR1 (18), into *bz1* aleurones dissected 15 DAP (Fig. 2). Purple spots were detected in permissive genotypes (*bz1, C1, R*) but not in aleurones from nonpermissive genotypes (*C1, r; c1, R;* or *c1, r*). Pigment was not produced in *bz1, C1, R* aleurones bombarded with pUC18 (Fig. 2).

To confirm that the purple pigment was anthocyanin, the bombarded tissue was soaked in 0.1 M HCl. Treatment caused the purple cells to rapidly change color to bright pink. This acid-induced change of color is characteristic of anthocyanin pigments (9). The production of the anthocyanin pigment in bombarded aleurones of the mutant *a1* and *bz1* genotypes indicates that the introduced genes corrected the respective genetic lesion and allowed the phenotypic expression of the complete pathway.

**Analysis of *A1* and *Bz1* Gene Expression in Tissue from Permissive and Nonpermissive Genotypes.** Phenotypic expression of the introduced *A1* or *Bz1* genes is dependent on the synthesis of the other enzymes of the anthocyanin pathway. Therefore, the lack of pigment production in the bombarded aleurones carrying a recessive regulatory allele (*C1, r; c1, R;* or *c1, r*) does not prove that the promoters of the introduced genes are inactive in these genotypes. To assess the activity of the *A1* and *Bz1* promoters, the firefly luciferase coding region was substituted for the *A1* or *Bz1* coding regions to produce pA1L and pBz1L, respectively (Fig. 1). These chimeric genes were delivered to aleurones (harvested 35–38 DAP) that carried either dominant or recessive regulatory loci. Aleurones bombarded with microprojectiles coated with pAdhL served as a positive control for luciferase expression. The luciferase gene in this plasmid is under the control of the promoter from the alcohol dehydrogenase 1 gene (*Adh1*) of maize (31). The expression of this gene is not influenced by

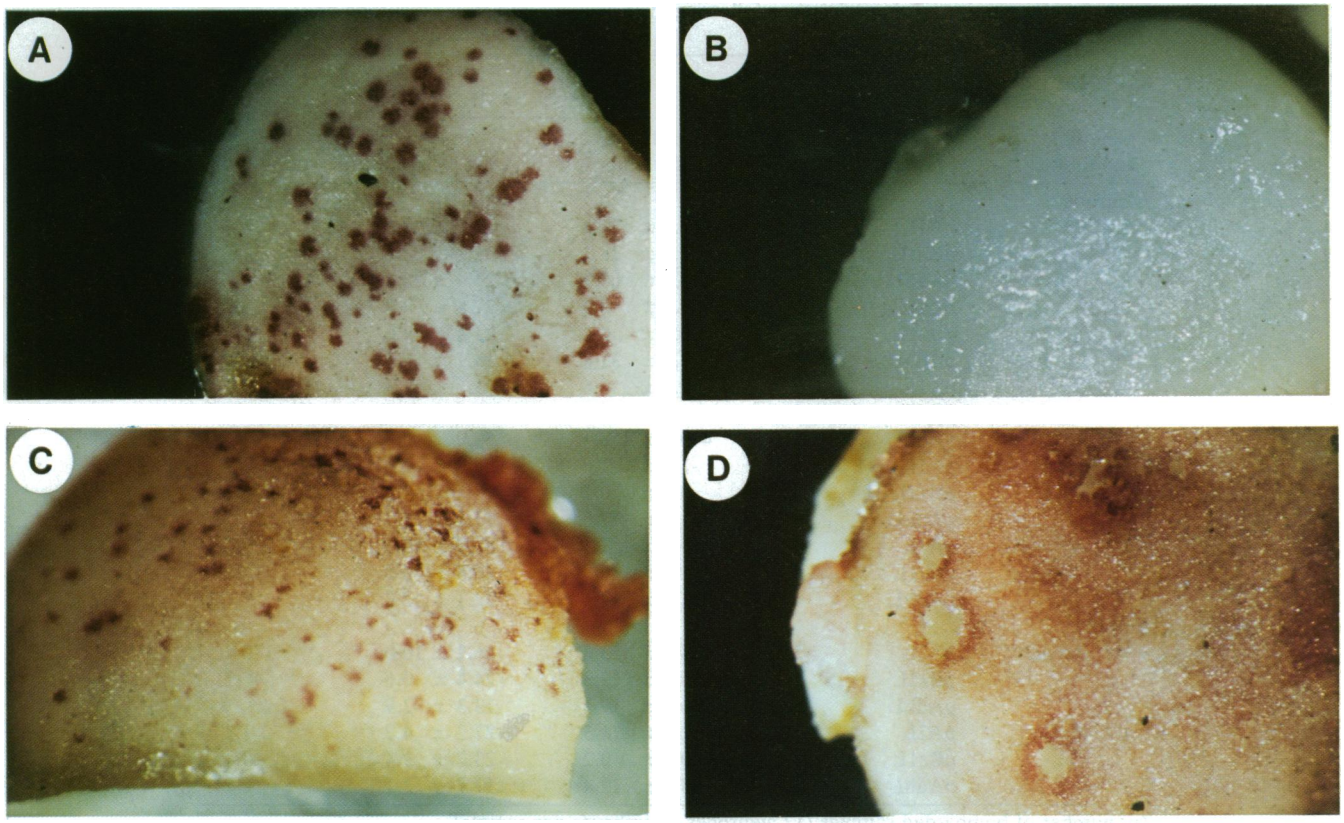


FIG. 2. Anthocyanin production in aleurone tissue lacking functional *A1* or *Bz1* genes following delivery of the appropriate genomic clone by particle bombardment. (*A* and *C*) Aleurone tissue (15 DAP) from an *a1* (*A*) or *bz1* (*C*) plant that was bombarded with microprojectiles coated with pA1 or pMBzR1, respectively. Following bombardment, the tissue was incubated in the light for 2 days. The purple spots represent clusters of about 5–10 cells. (*B* and *D*) Aleurone tissue from *a1* (*B*) or *bz1* (*D*) plants that was bombarded with pUC18 as a control. Debris from the bombardment apparently caused the damage to the bz1 aleurone (*D*), and some of this debris is apparent as small black spots on the bombarded aleurones.

the loci that regulate anthocyanin production. As a control to verify that DNA was being delivered with each bombardment, pAdhC was coprecipitated to the surface of the microprojectiles with the chimeric luciferase constructs. The CAT coding region in pAdhC is also under the control of the *Adh1* promoter of maize (31).

Table 2 shows the levels of luciferase and CAT activities observed following bombardment of aleurone tissue from each of the genotypes. Luciferase levels in pAdhL-bombarded material were relatively high in all the genotypes examined. Following bombardment with either pA1L or pBz1L, luciferase activity in aleurone tissue from plants containing *C1* and *R* but lacking a functional *a1* or *bz1* gene was also relatively high. The level of luciferase expression in *C1, R* aleurones bombarded with pA1L or pBz1L was 27% and 16%, respectively, of that observed in aleurones treated with pAdhL. In contrast, bombardment of aleurones homozygous for either or both of the recessive regulatory alleles (*C1, r; c1, R;* or *c1, r*) with pA1L or pBz1L resulted in relatively low levels of luciferase activity. Luciferase expression in aleurones with these genotypes was only about 0.1–0.9% of that observed in pAdhL-bombarded tissue (Table 2). Low levels of luciferase activity were also observed following delivery of pA1L or pBz1L to aleurones carrying C-I, an allele of *C1* that acts as a dominant inhibitor of the anthocyanin pathway (1). Levels of CAT activity from the codelivered control plasmid, pAdhC, were comparable among the various genotypes tested. This result confirms that DNA was delivered into cells of all the genotypes studied.

The genetic control of *A1* or *Bz1* gene expression was also investigated in embryos from *bz1, C1, R* or *bz1, C1, Rscm2* plants. *Rscm2* is necessary for anthocyanin production in the scutellum of the embryo. Purple pigmented cells and cell clusters developed on the scutellum of *bz1, C1, Rscm2* embryos following delivery of pMBzR1 (data not shown). However, such pigmented cells did not develop in *bz1, C1, R* embryos following bombardment with pMBzR1. This result was expected since embryos of *bz1, C1, R* plants do not produce the precursors necessary for phenotypic expression of the *Bz1* gene. Therefore, the regulation of *A1* and *Bz1* gene expression in embryo tissue was further investigated with the chimeric luciferase constructs. High levels of luciferase activity were observed in *bz1, C1, Rscm2* embryos bombarded with pBz1L, pA1L, or pAdhL (Table 2). In contrast, when *bz1, C1, R* plants were used as the source of embryos, levels of luciferase activity were far lower following bombardment with pBz1L or pA1L than with pAdhL (Table 2). CAT activity from the cotransferred plasmid, pAdhC, was consistently high indicating that the microprojectiles delivered DNA to both *bz1, C1, R* and *bz1, C1, Rscm2* embryos.

**Analysis of Expression of 5′ Deletions of the *Bz1* Promoter.** An experiment was conducted to determine if microprojectile-mediated delivery could be used in the analysis of promoter deletions. Two constructs containing deletions of the *Bz1* promoter of pBz1L (Fig. 1) were introduced into aleurones carrying *bz1, C1, R*. A deletion that leaves 407 nucleotides upstream of the mRNA start site (pBzΔ407L) produced luciferase levels comparable to those observed with pBz1L (Table 3). Another deletion of pBz1L, which leaves only 80 nucleotides upstream of the mRNA start site (pBzΔ80L), was also tested. This deletion retained the sequence AATAAAA found at position −32 (the putative TATA box of this gene) and the sequence CTAACT (a potential CCAAT box) at position −72 (39). Relatively low levels of luciferase activity were detected when this deletion was delivered into *bz1, C1, R* aleurones. Background levels

Table 2. Expression of chimeric genes following their delivery into maize aleurone or embryo tissue

| Genotype and tissue | Plasmid | n* | Enzyme activity × $10^{-3}$ (mean ± SEM) Luciferase, light units | CAT†, cpm | Relative activity‡, % |
|---|---|---|---|---|---|
| Aleurone | | | | | |
| *C1, R*§ | pA1L | 20 | 69.6 ± 22.4 | 21.1 ± 2.2 | 27.4 |
| | pBz1L | 7 | 72.4 ± 28.1 | 36.1 ± 8.1 | 16.7 |
| | pAdhL | 11 | 294.0 ± 60.9 | 24.4 ± 6.4 | |
| *c1, r* | pA1L | 5 | 0.8 ± 0.4 | 21.8 ± 8.0 | 0.9 |
| | pBz1L | 5 | 0.2 ± 0.02 | 24.4 ± 9.6 | 0.2 |
| | pAdhL | 5 | 77.5 ± 31.8 | 18.7 ± 6.4 | |
| *c1, R* | pA1L | 5 | 2.0 ± 0.8 | 31.7 ± 1.3 | 0.4 |
| | pBZ1L | 5 | 1.0 ± 0.2 | 42.1 ± 2.5 | 0.1 |
| | pAdhL | 5 | 449.5 ± 158.4 | 39.0 ± 0.8 | |
| *C1, r* | pA1L | 5 | 1.8 ± 1.0 | 29.8 ± 7.0 | 0.4 |
| | pBz1L | 5 | 0.3 ± 0.2 | 27.5 ± 12.2 | 0.1 |
| | pAdhL | 5 | 231.8 ± 67.6 | 13.8 ± 4.2 | |
| *C-I, R* | pA1L | 4 | 5.6 ± 2.4 | 41.9 ± 13.3 | 0.1 |
| | pBz1L | 4 | 1.4 ± 0.8 | 16.8 ± 0.8 | 0.1 |
| | pAdhL | 4 | 1381.8 ± 317.4 | 22.1 ± 2.2 | |
| Embryo¶ | | | | | |
| *C1, Rscm2* | pA1L | 5 | 134.5 ± 53.6 | 16.2 ± 10.0 | 170.4 |
| | pBz1L | 5 | 55.0 ± 12.3 | 24.4 ± 9.6 | 46.1 |
| | pAdhL | 5 | 64.8 ± 19.1 | 13.3 ± 2.9 | |
| *C1, R* | pA1L | 5 | 2.9 ± 1.3 | 27.2 ± 4.2 | 3.2 |
| | pBz1L | 5 | 0.3 ± 0.2 | 23.7 ± 3.4 | 0.4 |
| | pAdhL | 5 | 58.8 ± 12.4 | 17.7 ± 2.1 | |

*n, number of bombarded samples (12 aleurones or embryos per sample).
†CAT activity from pAdhC that was cointroduced with the chimeric genes in each bombardment.
‡Relative activity was calculated by dividing the luciferase-to-CAT ratio obtained from bombardment with either pA1L or pBz1L by the luciferase-to-CAT ratio obtained from bombardment with the pAdhL control.
§Aleurones were from plants carrying either *a1, C1, R* or *bz1, C1, R*. Levels of luciferase activity were not significantly different between these two genotypes and the results were pooled for this table. The other aleurone genotypes used carried both the dominant *A1* and *Bz1* genes.
¶Embryos were from plants carrying *bz1, Rscm2* or *bz1, R*.

of luciferase activity were observed following bombardment of aleurones with pLBz3', a promoterless derivative of pBz1L. These results indicate that most, if not all, of the cis-acting regulatory regions of the *Bz1* are within 407 nucleotides of the mRNA start site and that a critical region is located between position −407 and −80.

## DISCUSSION

Molecular studies of the regulation of genes involved in the anthocyanin biosynthetic pathway of maize have been restricted by the inability to transfer genes back into plants after their modification *in vitro*. This deficiency is particularly acute since a functional *in vitro* transcription system has not yet been developed for plants. Microprojectiles provide a means for overcoming these barriers by delivering genes directly into cells within tissues. The utility of using microprojectiles to study anthocyanin gene expression was initially demonstrated by delivering clones of the *A1* and *Bz1* genes into aleurones lacking functional alleles of these structural genes. The production of anthocyanin pigment in the bombarded cells provided evidence that these genomic clones possess sufficient 5' and 3' regulatory regions for efficient gene expression. Although the structures of these genes have been studied (39, 40, 45, 46), their phenotypic expression following introduction into aleurone cells provides the first clear evidence that the isolated clones are functional.

Table 3. Expression of *Bz1* chimeric genes containing deletions of the 5' region

| Plasmid | n* | Enzyme activity × $10^{-3}$ (mean ± SEM) Luciferase, light units | CAT†, cpm | Relative activity‡, % |
|---|---|---|---|---|
| pLBz3' | 5 | <0.01 | 5.6 ± 2.2 | <1.0 |
| pBz80ΔL | 10 | 1.1 ± 0.4 | 17.0 ± 8.0 | 3.1 |
| pBz407ΔL | 5 | 21.6 ± 12.6 | 22.4 ± 3.9 | 47.2 |
| pBz1L | 5 | 19.4 ± 4.2 | 9.5 ± 1.0 | |

*n, number of bombarded samples (12 aleurones per sample).
†CAT activity from pAdhL that was cointroduced in each bombardment.
‡Relative activity was calculated by dividing the luciferase-to-CAT ratio obtained from bombardment with one of the genes carrying a deletion of the *Bz1* promoter by the luciferase-to-CAT ratio obtained from bombardment with the gene carrying the nondeleted 5' region, pBz1L.

Genotypes unable to produce anthocyanins because of mutations in regulatory loci provided a means to stringently test the regulation of genes introduced by microprojectiles. Relatively high levels of expression of the chimeric anthocyanin/luciferase genes were observed following their introduction into aleurones of a permissive (*C1, R*) genotype. Conversely, far lower levels of expression were observed when these chimeric genes were delivered to aleurones carrying one or both of the recessive regulatory alleles (*c1, r*). The difference in the level of luciferase expression found for the chimeric *Bz1* gene was thus similar to the difference reported for the accumulation of the endogenous *Bz1* gene product, UDPglucose:flavonol $O^3$-D-glucosyltransferase, when compared in permissive (*C1, R*) and nonpermissive

(*C1, r; c1, R;* or *c1, r*) genotypes (14). In addition, pA1L and pBz1L were highly expressed in *bz1, C1, Rscm2* embryos but not in *bz1, C1, R* embryos. These results indicate that the introduced genes are properly regulated by the genotype of the target tissue. The introduced chimeric genes also showed proper tissue specificity as indicated by the differences in expression following their introduction into *bz1, C1, R* aleurones and embryos. Therefore, genes transferred to intact tissues by microprojectiles are regulated in a manner similar to the native genes.

The experiments involving 5′ deletions of the *Bz1* gene demonstrate that this system will be useful for a detailed analysis of promoter structure. We have defined a critical regulatory region of the *Bz1* promoter to be between nucleotides −407 and −80. Previous information concerning the functional organization of the 5′ region of the *Bz1* gene involved the influence of insertions on the transcriptional activity of the endogenous gene. For example, a 406-base-pair (bp) insertion 62 bp upstream of the transcription start site dramatically reduced the level of mRNA of the *Bz-wm* allele as compared to the dominant gene (45). However, a 437-bp insertion beginning 216 bp upstream of the transcription start site in an allele of the *Bz1* gene (*bz-R*) did not influence transcription of the gene (39). These results indicate that an important regulatory region is probably 3′ of position −216. Taken together, these results set the framework for finer analysis of the *Bz1* promoter and those of the other structural genes involved in anthocyanin production. In addition, it should be possible to use microprojectile-mediated delivery to study the interaction of mutant regulatory genes with the structural genes they influence.

Gene expression studies with microprojectiles should be applicable to many other types of tissues of various organisms and should facilitate investigations of gene function. The application of microprojectiles will aid studies with transgenic plants since the expression of constructs can first be tested in the desired target tissue on a transient expression basis. Transient expression assays of genes delivered by microprojectiles will be particularly useful for species for which stably transformed whole plants cannot yet be produced.

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