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The *C1* locus in maize (*Zea mays* L.): effect on gene expression

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Abstract The focus of this genetic study is on the expression of two wild-type alleles [Wisconsin (W22 C) and Cornell (Cornell)] at the *C1* locus, functioning in their color-producing roles, that can be distinguished by assaying the gene expression in their competitive capacity against a third allele at that locus. The third allele is a transposon-induced color-suppressing allele with a deficiency that suppresses anthocyanin color. This color suppressing allele *(C1-lΔ*), however, is not as fully potent as the standard color suppressing allele *C1-I (std*) and, thus, is competed more readily against the *C1* allele in the regulatory control of anthocyanin coloration. Both the *C1-lΔ* and *C1-I*(*std*) alleles have somewhat similar deficiencies and produce truncated transcripts. In the measurement of anthocyanin production in heterozygote genotypes of the two wild-type alleles against the *C1-lΔ* color suppressor, the following could be shown genetically: that the inhibitor allele reduces color; the two wild-type alleles are different in their competitive capacity against *C1-lΔ*; and the genetic background of the two lines influences the degree of color expression. A hypothesis is presented that this study provides a genetic demonstration of the competition for transcription sites on the promoter between two different wild-type alleles that does lead to differences in gene expression. Further, this study illustrates how a genetic investigation could uncover subtle differences among seemingly similar wild-type alleles.

Keywords *C1* locus · Gene expression · Maize · *C1* inhibition · Modifiers

Communicated by F. Salamini

This paper is dedicated to Dr. Derek Styles of Victoria, British Columbia, an active early investigator of the anthocyanin pathway

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Introduction

The expression of a phenotype can generally be assigned to two broad categories of loci controlling gene products; namely, *qualitative* (QLs) and *quantitative trait loci* (QTLs). QL-type loci, generally identified by monogenic inheritance, lead to discrete phenotypes depending on genetic background. QTL-type loci, on the other hand, contribute in a major or minor way to particular phenotypes that lead to continuous graded variation such as color, yield or size measurement, also depending on genetic background.

Although QTLs were considered early in the history of Genetics (East 1910; Emerson and East 1912), QLs were the subject of most early genetic investigations because of their ease in facilitating inheritance and subsequent segregation studies (Emerson et al. 1935).

The gene products and specific interactions that control most traits under polygenic control are virtually unknown. Many of these genes in polygenic pathways are most likely very significant players in phenotypes that lead to traits of economic value. One significant difference between the phenotypes assigned to QLs vs QTLs is that polygenic traits (such as the genes of the anthocyanin cascade) have known functions, whereas most QTL-designated phenotypes are limited in their known enzyme functions. In this report, QTLs will be used, though the term could be polygenic.

Though genes controlled by QLs segregate as monogenic traits, they are part of multiple gene pathways. If wide crosses were made where a number of loci in the

pathway were heterozygous in the F1 generation, their segregation pattern would appear as polygenic or QTLs expressing a graded series contributing to a phenotype. But, because of the control of the genotypes involved in the cross, they segregate as monogenic inheritance. With respect to the anthocyanin color phenotype in maize, each of the participating genes adds a component to the final anthocyanin molecule, not as a QTL but as a qualitative contribution (hydroxylation or hydrogenation, a hydrogen molecule, etc.) to the final molecule. Yet, if a wide cross is made where a number of genes controlling the anthocyanin phenotype are heterozygous, the segregation in the F_2 would appear polygenic. Because the phenotypes are abrupt and distinct, they would be considered as polygenic rather than a typical QTL phenotype with a graded expression.

The anthocyanin pathway contributing to a QTL-type phenotype was illustrated in the study of genetic resistance to the corn earworm (*Helicoverpa zea*) by McMullen et al. (1998). Here, other additional genes contribute to the resistance phenotype. Another case is the study of length size in pepper, *Capsicum annum* (Petterson 1958), where a monogenic inheritance could be demonstrated for fruit-size inheritance though most crosses of varied graded pepper length sizes indicated a QTL-type of inheritance. A monogenic type could be demonstrated in the *Capsicum* study because the two parents in the cross were distinguished by only one major gene.

In addition to the multiple gene complexes that are responsible for a QTL trait, there is now as Koshland (1998) notes "*the era of pathway quantification*." The genes involved in a pathway provide individual steps that have increased metabolic rates contributing to increased yield of the final product. This is supported by RNA polymerase studies (Wang et al. 1998) and the box II effect on anthocyanin coloration (Hattori et al. 1992; Scheffler et al. 1994).

These increases in RNA polymerase in the Wang et al. (1998) study could come from components of the promoter that enhance mRNA production, providing abundant proteins for specific activities. Such an example is box II in the *C1* promoter that is present in some maize lines. Because *C1* is a regulatory gene, this gene is responsible, along with others, for activating transcription in the structural genes *a1, a2, c2,* and *bz1*. This active promoter (box II) is found in the super *C1* allele (^{s}*C1*) that is prevalent in many indigenous maize populations in South America. The consequence of this specific promoter represents an advantage (stronger expression) of the final product for the structural genes under this cascade of the anthocyanin pathway.

The anthocyanin pathway has been promoted as an ideal system to illustrate pathways and how a multiple set of genes is coordinated to produce a final product (McMullen et al. 1998). Especially noteworthy is that the anthocyanin pathway does yield a product(s) that leads to valuable economic traits.

Maize transposons inserted in genes undergo excision, leaving footprints, altered nucleotide sequences, and minor or major deletions. Many of the resultant products of these excisions are null alleles (Peterson 1970; Nowick and Peterson 1981; Seo and Petterson 1996) that arise from new mutant gene sequences and the new mutant proteins they encode (Schwarz-Sommer et al. 1984; Wessler 1988; Franken et al. 1994). This report describes a functional gene that mutated by a transposon insert, followed by some excision events, to became a dominant allele that suppresses anthocyanin coloration.

Gene clone availability and the augmentation of transient assays have uncovered the basic mechanisms that activate gene expression. Transcription factors have been described that contain domains that function to bind DNA at specific regions (Goff et al. 1990; Franken et al. 1994).

An example is the *C1* gene product. Wienand et al. (1991) proposed the *myb*-homologous domain (basic amino-terminus) as the DNA-binding unit of this protein, and Paz-Ares et al. (1990) considered the acidic carboxy terminus as the activating domain. Mutations in these units could lead to a nonfunctional *C1* gene product.

Dominant suppressor mutations similar to *C1-I* (Paz-Ares et al. 1990) have been described. In addition to the *C1-I* allele, others include the *Kn1* allele (Veit et al. 1990, Greene et al. 1994) and the *ae1–5180* allele (Stinnard et al. 1993).

The *C1-I(std*) allele (East and Hayes 1911), an allele of the *C1* locus – a regulatory gene controlling the anthocyanin pathway, suppresses all anthocyanin coloration in the kernel, and this allele is a potent suppressor in all crosses. This is especially evident when used as a female where there are two *C1-I*(*std*) alleles against one *C1* allele in the endosperm (Coe 1962). Paz-Ares et al. (1990) described the *C1-I*(*std*) product as a truncated transcript and hypothesized that the resulting truncated protein product with a basic DNA-binding domain was able to bind to promoters but, lacking an acidic carboxy terminus domain, the *C1-I* protein was unable to activate a transcript. It was hypothesized that competitive binding of the *C1-I(std)* protein occluded the functional *C1* protein from the available binding sites of the structural genes. This would prevent transcription of the structural genes, which explains the action of *C1 -I*(*std*) as a dominant suppressor of multiple gene functions.

This paper focuses on the *C1* gene of the anthocyanin pathway and the demonstration of the specific-allele genotype and the effect of the background genotype in controlling gene expression at the promoter of this regulatory gene. The example portrayed illustrates how background genotype and the specific-allele genotype can control gene expression at the transcription level.

This report describes the effect of two *C1* alleles [Cornell and Wisconsin (W22 C)] and the effect of genetic background (Cornell and W22 C) on the ability of a newly induced *C1-I* allele deficiency (*C1-IΔ*) (Singer et al. 1998) to suppress color expression in maize kernels. This study was triggered by the observation of the differing expression of anthocyanin coloration when this *C1-IΔ* line was crossed to various lines. The effect of the *C1*

allele and effect of the genetic-line background could be measured. Knowing the role of the *C1* protein in activating transcription, we posed two questions: (1) what is the effect of the two different alleles (*C1*^{W22C} and *C1*^{Cornell}) on challenging the color suppression of *C1-IΔ*; and (2) what is the effect of the *C1*^{W22C} vs the *C1*^{Cornell} genetic background on color expression?

Materials, Methods, Designations, and Statistical Procedures

Genetic materials

The original allele (*c1-m763103*) arose in a 1975 *En*-containing isolation plot that was developed to tag new *c1-m* alleles (Peterson 1963, 1978). This *c1* mutable allele was confirmed as a *c1*-mutable allele in 1976. Franken et al. (1994) placed the *En* insert of *c1-m611702* in the third exon (+921) of the *C1* locus, and the *c1-m763103* allele with an *En* insert is in the same general area about 20 bp 3′ to the *c1-m611702* insert (Singer et al. 1998). Three *C1-IΔ* alleles were sequenced (Singer et al. 1998). (Same as *C1-I*).

Three transposon-induced deficiencies of the *C1* gene (*C1-I*) (Singer et al. 1998) were backcrossed by two inbred lines (Wisconsin designated W22 C. and a Cornell flint line, designated Cornell, Fig. 1). Only one *C1-I* was used in further crosses for this study. The *C1-I* allele suppresses anthocyanin color in the aleurone in a graded series when used as a female but as opposed to the *C1-I*(*std)* allele is not expressed when inherited from the male parent. The recurrent testers included a Wisconsin line [W22 color line (identified as W22 C and originated from Inbred W22 and color converted by R.A. Brink, University of Wisconsin)] and a second color line (identified as Cornell) with a northern flint background from the 1950 s Cornell Maize collection. The transposon-induced deficiency was repeatedly backcrossed to the two genetic lines in the following series of crosses:

C1-I/C1 (Line)×*C1/C1* Line) – *C1-I/C1* and *C1/C1* Cross 1

The partially suppressed *C1-I/C1* kernels in four generations of backcrossing were selected and recurrently backcrossed to the recurrent lines (W22 C or Cornell, Fig. 1). This was to homogenize the background in the study of genotype effect on the suppressive expression of the alleles. Final percentage of Cornell line background in each genotype is given in Table 1. Progenies from two progeny ears from lines carrying the eight genotypes in Table 1 were analyed for color expression.

Spectrophotometric measurements

The focus of this study is the assay by spectrophotometric measurement of anthocyanin color expression as influenced by specific alleles and/or the background genotype. Materials in these measurements include mature dry kernels from each progeny ear of cross 1

Cross 1: *C1-IΔ* by Cornell by Cornell as tester

C1-IΔ/–×*C1-C*/*C1-C*
↓
1/2–/*C1-C*
1/2 *C1-IΔ* /*C1-C* × *C1-C*/*C1-C*
↓
1/2 *C1-IΔ* /*C1-C*
1/2 *C1-C*/*C1-C*

Cross 2: *C1-IΔ* by Cornell by W22C as tester

C1-IΔ/–×*C1-C*/*C1-C*
↓
1/2–/*C1-C*
1/2 *C1-IΔ* /*C1-C* × *C1-W*/*C1-W*
↓
1/2 *C1-IΔ* /*C1-W*
1/2 *C1-C*/*C1-W*

Cross 3: *C1-IΔ* by W22C by Cornell as tester

C1-IΔ/–×*C1-W*/*C1-W*
↓
1/2–/*C1-W*
1/2 *C1-IΔ* /*C1-W* × *C1-C*/*C1-C*
↓
1/2 *C1-IΔ* /*C1-C*
1/2 *C1-W*/*C1-C*

Cross 3: *C1-IΔ* by W22C by W22C as tester

C1-IΔ/–×*C1-W*/*C1-W*
↓
1/2–/*C1-W*
1/2 *C1-IΔ* /*C1-W* × *C1-W*/*C1-W*
↓
1/2 *C1-IΔ* /*C1-W*
1/2 *C1-W*/*C1-W*

Fig. 1 Crosses and genotypes for experiment 1

Table 1 Genotypes assayed in experiment 1: expected proportion of genetic background derived from the Cornell Line and coefficients for effects in the linear model used in the data analysis

Genotypes		% Cornell-line background	Model-effect coefficients							
			μ [a]	b_1 [b]	b_2 [c]	b_3 [d]	b_4 [e]	b_5 [f]	b_6 [g]	b_7 [h]
$C^I/C^{Cornell}$	g_1	75%	1	0.375	1	0	0	0	1	0
$C^{Cornell}/C^{Cornell}$	g_2	75%	1	0.375	0	0	1	1	0	0
$C^I/C^{Cornell}$	g_3	50%	1	0.125	1	0	0	0	–1	0
$C^{Cornell}/C^{W22C}$	g_4	50%	1	0.125	0	0	0	–1	0	1
C^I/C^{W22C}	g_5	25%	1	–0.125	0	1	0	0	–1	0
$C^{Cornell}/C^{W22C}$	g_6	25%	1	–0.125	0	0	0	–1	0	–1
C^I/C^{W22C}	g_7	0%	1	–0.375	0	1	0	0	1	0
C^{W22C}/C^{W22C}	g_8	0%	1	–0.375	0	0	–1	1	0	0

[a] μ=model intercept
[b] b_1=effect of Cornell-line background
[c] b_2=effect of Inhibitor allele when heterozygous with *C1*^{Cornell} allele
[d] b_3=effect of Inhibitor allele when heterozygous with *C1*^{W22C} allele
[e] b_4=1/2 difference between *C1*^{Cornell} and *C1*^{W22C} homozygotes (i.e.), 'additive' effect of *C1*^{Cornell} allele)
[f] b_5=contrast to test dominance of *C1*^{Cornell} allele over *C1*^{W22C}
[g] b_6=inhibitor allele by background interaction
[h] b_7=*C1*^{Cornell}/*C1*^{W22C} heterozgyote by background interaction

Table 2 Expected proportion of genome originating from the Cornell line. Genetic background effect, b_1 in the model, is a regression on the expected proportion of the genome originating from the Cornell line, adjusted to be orthogonal to the intercept. Table includes percentages of Cornell background in each genotype (these are the coefficients in parameter b_1 in the model)

Treatment	Genotype [a]	Experiment 1	Experiment 2			
			Family 1	Family 2	Family 3	Family 4
1	I/A	0.75	0.15625	0.15625	0.03125	0.140625
2	A/A	0.75	0.15625	0.15625	0.03125	0.140625
3	I/C	0.50	0.65625	0.65625	0.53125	0.640625
4	A/C	0.50	0.65625	0.65625	0.53125	0.640625
5	I/A	0.25	0.328125	0.40625	0.28125	0.390625
6	A/C	0.25	0.328125	0.40625	0.28125	0.390625
7	I/C	0.75	0.828125	0.90625	0.78125	0.890625
8	C/C	0.75	0.828125	0.90625	0.78125	0.890625

[a] Allelic desingations are: I=*C1-IΔ*; A=$C1^{Cornell}$; C=$C1^{W22C}$

and successive backcrosses placed in the following: [six kernels in 25 ml 1% MeOH/HCL as solvent, (e.g., 97 ml MeOH and 3 ml HCL (37%)]. The solvent-covered kernels were placed in a flask and shaken in an unlighted Brunswick Shaker at 100 rpm at 25° (centigrade/celsius) for 3 days. After extraction 1 ml of the colored MeOh/OH solution was used for spectrophotometric (Milton Roy Spectronic 601) examination at 509 nm using the solvent as a blank. The range for the *prpr* derivatives (pelargonidin) was 505– 512 nm.

Linear model

A linear model was chosen for the analysis to reflect the genetic effects of greatest biological interest. Eight genotypes were available, so a model was developed to include an intercept plus seven estimable linear functions (b_1 to b_7 in Table 1) of the eight means. An attempt was made to maintain orthogonality among effects in the model so that independent effects were estimated, but the treatment design did not allow meaningful effects to be partitioned while maintaining complete orthogonality (Table 1). The following is a description of all effects included in the model:

Genetic background (b_1)

The effect of the genetic background was quantified by the effect b_1 (Table 1). All genotypes in experiment 1 included a 25% unknown background, the remaining 75% was divided between W22 C and Cornell (Table 2). Each family in experiment 2 had a different proportion of the unknown background, with the remaining portion being derived from W22 C and Cornell (Table 2). The effect of genetic background in the model, b_1, quantified the relative proportion of the genome derived from the Cornell line. Coefficients for this effect were made orthogonal to the intercept (Table 1).

Effects of the inhibitor allele (b_2 and b_3)

Two heterozygous genotypes with the inhibitor allele were available in this experiment, *C1-IΔ*/$C1^{Cornell}$ and *C1-IΔ* /$C1^{W22C}$. The effect of the inhibitor allele was estimated separately for each of the two heterozygous genotypes. The reduction in color due to the inhibitor allele was quantified by b_2 when the inhibitor was in heterozygous combination with the Cornell-line allele (*C1-IΔ*/$C1^{Cornell}$) and by b_3 when the inhibitor was in heterozygous combination with the W22 C allele (*C1-IΔ*/$C1^{W22C}$).

Additive and dominance effects of the Cornell-line allele and the W22 C allele (b_4 and b_5)

The effects b_4 and b_5 quantified the effects of the Cornell-line allele and the W22 C allele. The effect b_4 was an estimator of one-half of the difference between the $C1^{Cornell}$/$C1^{Cornell}$ homozygote and the $C1^{W22C}$/$C1^{W22C}$ homozygote, i.e., the additive effects of the $C1^{Cornell}$ allele (Table 1). The effect b_5 was a test for dominance between these two alleles (Table 1).

Interaction with genetic background (b_6 and b_7)

The remaining two degrees of freedom were used to test interactions between specific genotypes and genetic background. The effect b_6 was a test of the inhibitor allele by genetic background interaction and b_7 was a test of the interaction of the $C1^{Cornell}$/$C1^{W22C}$ heterozygote with genetic background.

Data analysis

Color measurements were standardized on a 0 to 1 scale by dividing by the maximum value of 1598. Standardized measurements were then transformed using a fourth root transformation. This transformation was used to homogenize error variances. The importance of unequal error variances was heightened by the use of ordinary least squares to estimate nonorthogonal effects in the model.

In experiment 1, generalized least-squares means were obtained for each of the eight genotypes in Table 1 (g_1..g_8) by fitting the following model:

$$y_{ijk}=\mu+g_i+f_j+gf_{ij}+e_{ijk},$$

where:

g_i=fixed effect of the ith genotype [i=1..8 – see Table 1 (g_1..g_8) for the eight genotypes],
f_j=random effect of the jth family (j=1..14),
gf_{ij}=random interaction effect for the ith genotype, jth family, and
e_{ijk}=random residual for the kth observation on the ith genotype and jth family.

In experiment 2, each family differed in the composition of its genetic background. Therefore, each family was analyzed individually according to the model:

$$y_{ij}=\mu+g_i+e_{ij},$$

where:

g_i=fixed effect of the ith genotype (i=1..8 – see Table 1 for the eight genotypes)
e_{ij}=random residual for the jth observation on the ith genotype

In both experiments, the error variance was considered heterogeneous by tester and genotype combination, providing four separate error terms for the eight genotypes. The following pairs of genotypes had the same error: g_1 and g_3, g_2 and g_4, g_5 and g_7, g_6 and g_8.

Table 3 Estimates of effects μ and b_1..b_4 in the model described in Table 1

Effect [a]	Experiment 1	Experiment 2			
		Family 1	Family 2	Family 3	Family 4
μ	0.836±0.008	0.898±0.017	0.906±0.040	0.858±0.038	0.901±0.019
b_1	−0.263±0.039	−0.583±0.119	−0.413±0.252ns [b]	0.010±0.239ns [b]	−0.186±0.122ns [b]
b_2	−0.490±0.013	−0.489±0.042	−0.470±0.095	−0.341±0.083	−0.425±0.044
b_3	−0.262±0.015	−0.141±0.042	−0.096±0.094ns [b]	−0.162±0.085ns [b]	−0.179±0.044
b_4	−0.148±0.016	−0.252±0.047	−0.176±0.110ns [b]	−0.013±0.107ns [b]	−0.117±0.053ns [b]

[a] Effects are described in Table 1
[b] ns=nonsignificant

Ordinary least-squares solutions were obtained for the effects μ, and b_1..b_6 in Table 1 as functions of the eight genotype means g_1..g_8 according to the following equation:

$$\hat{\beta}_1=(W'W)^{-1}W'\gamma, \quad (1)$$

where:

W=the matrix of coefficients on effects μ and b1..b6 (Table 1),
γ=the vector of generalized least-squares genotype means (g1..g8 in Table 1), and
$\hat{\beta}_1$=the vector of solutions for parameters μ and b1..b6.

The variance-covariance matrix of $\hat{\beta}_1$ was computed from equation (1) and the variance-covariance matrix of the eight genotype means, g_1..g_8. Coefficients for the effect b_7 were orthogonalized to m and b_1..b_6 using the following equation:

$$L=[W_w(I-W(W'W)^{-1}W')'],$$

where:

L=the row vector of coefficients for b_7, orthogonalized to other effects,
W=the matrix of coefficients described in equation (1),
W_2=the vector of coefficients for b_7, and
I=the identity matrix.

The same model was used to estimate genetic effects in both experiments, except for the effect b_1, because coefficients for b_1 had to be adjusted for the exact contribution of the Cornell-line background in each respective family of experiment 2 (Table 2). A t-test was used at P=0.05 to test whether each of the effects b_1..b_7 were significantly different from zero, and to test for differences between effects b_2 and b_3.

Results

Genetic background, quantified as the expected proportion of the genome derived from the Cornell line, reduced color expression in experiment 1 and family 1 of experiment 2 (b_1 in Table 3). No effect of genetic background was found in families 2–4 in experiment 2 (b_1 in Table 3). Reduction in color expression, as a function the expected proportion of Cornell-line genetic background, suggests that modifiers in the genetic background affect the expression of color in addition to the effects of alleles at the *C1* locus. The least-squares estimates of reduction in color caused by the inhibitor allele (*C1-IΔ*) in heterozygous combination with the Cornell allele were significantly greater than zero for both experiments, including all families in experiment 2 (b_2 in Table 3). The estimated reduction in color caused by the inhibitor allele in combination with the W22 C allele was significant in experiment 1, and in families 1 and 4 in experiment 2 (b_3 in Table 3). The reductions in color caused by the inhibitor allele demonstrate that the inhibitor allele had a strong effect on reducing color when combined with either the Cornell allele or the W22 C allele. The inhibitor allele caused a greater reduction in color in combination with the Cornell-line allele (b_2 in Table 3) than in combination with the W22 C allele (b_3 in Table 3) in every case. The difference between b_2 and b_3 was significant at P<0.05 in every case except for family 3 in experiment 2 (difference not shown).

Differences in color between the Cornell-line allele and W22 C allele homozygotes at the *C1* locus were found (b_4 in Table 2) in experiment 1 and family 1 of experiment 2, demonstrating an inherent difference in color expression between the Cornell-line and W22 C alleles, in homozygous genotypes. The test of dominance, i.e., deviation of the heterozygote from the midpoint of the two homozygotes for the Cornell line and W22 C alleles, was significant in experiment 1 (b_5=0.014±0.006) but not for any families in experiment 2. Even in experiment 1, the significant value of 0.014 was relatively small compared to the deviation of 0.148 (b_5) of the Cornell-line allele and W22 C allele homozygotes from the midpoint value, demonstrating that dominance for the Cornell-line and W22 C alleles was relatively unimportant. The remaining interactions in the model, b_6 and b_7, were not significant except for b_7 in family 3 of experiment 2 (b_7=0.151±0.061, remaining values of b_6 and b_7 not shown).

Discussion

There are several aspects of this study that include transposon products, origin of a gene expressing dominance, the effect of different alleles in competing for transcription sites and an explanation of the origin of the long-known *C1-I* (*std*) dominant suppressor of color. Transposon excisions lead to varied types of products, which have been significant in the evolution of maize (Schwarz-Sommer et al. 1984).

The structure of the *C1* locus

With the use of transient assays, numerous investigators explored the protein domains of *C1* locus expression.

Wienand et al. (1991) and Goff et al. (1990) identified the myb homologous domain that binds to promoter DNA, and Paz-Ares et al. (1990) recognized that the C-terminus represents the activation function of the *c1* protein. By domain swapping, Goff et al. (1991) further defined the basic functions of different *C1* domains. With these revelations, the expression of different patterns for the different phenotypes of the various mutants could be explained (Franken et al. 1994).

C1-I suppressive effect

With the cloning of the *C1* gene (Paz-Ares et al. 1986) it became possible to examine the *C1-I*(*std)* allele and the basis of the suppressive effect (Paz-Ares et al. 1986). The *C1* gene is a regulatory locus and a transcription activator for members of the anthocyanin pathway (Paz-Ares et al. 1986; Cone et al.1986; Goff et al. 1990; Paz-Ares et al. 1990; Goff et al. 1991). Although the *C1-I*(*std)* gene product is able to start transcription and, because of a frameshift mutation that leads to a premature stop codon the allele does lack an acidic domain, it is thus unable to activate transcription. It was hypothesized that, by competitively occupying these structural gene promoter sites, the functional gene *C1* product is prevented from functioning as a transcription activator (Paz-Ares et al. 1990). The consequence is the lack of activation of members of the anthocyanin pathway and final absence of color expression.

What is clear in this analysis of *C1-IΔ* is that there are significant differences caused by the two recurrent tester lines in the suppressive capacity of the *C1-IΔ* deficiencies in anthocyanin coloration. Given with this, is the difference in color expression among the progenies of the two lines in affecting color suppression potency due to the *C1* alleles of the two testers or is it the genetic background of the testers?

Allele effect

It is clear from the data presented that the individual *C1* alleles of the two testers have an effect on the suppressive role of the *C1-I* allele. The role of the *C1* allele in regulation of the genetic cascade leading to anthocyanin coloration has been well documented by early demonstration of the regulatory role of the *C1* gene (Cone et al. 1986; Paz-Ares et al. 1986) and in later protein-binding studies on promoters by Sainz et al. (1997), Williams and Grotewold (1997), and Lesnick and Chandler (1998). According to these studies, the protein derived from the *C1* gene activates transcription of the *a1, a2, c2, bz1*, and *bz2* anthocyanin genes leading to coloration. Furthermore, promoters differ in effecting coloration intensity (Hattori et al. 1992; Lesnick and Chandler 1998).

The results of this study with the *C1-IΔ* alleles demonstrate that the process of *C1* control of transcription of the anthocyanin cascade affects the opposite allele in the heterozygous condition (*C1-IΔ*/$C1^{Line}$). Although it is recognized that the *C1* allele is not the sole regulator of flavonoid transcription, because it requires members of the *R* or *B* gene families (Grotewold 1995), the role of the opposite allele in the heterozygous *C1* condition has a place in this transcription machinery. This indicates that the two alleles are involved, most likely in their competition for the transcription sites, because there is no evidence that they occur as heterodimers. This latter feature of differences in *C1* allele composition may be the distinguishing feature of the effect of the two alleles rather than the heterodimer influence (Fincham 1966) on transcription. Of course, a determination of the sequences of the Wisconsin and Cornell alleles would be more definitive in determining the differences in the two alleles

Genetic background of the two lines (Wisconsin and Cornell)

Because the breeding scheme was initiated to homogenize the genetic background for our assay purposes, the color-suppression potency of the background effect could be measured. The genetic background, i.e., the expected proportion of the Cornell vs the W22C genetic background, directly affected kernel color. However, the degree of suppression of color by the *C1-IΔ* allele was not measurably affected by the genetic background.

Lack of color suppression potency from a male-derived *C1-IΔ* allele

There is no obvious phenotypical suppression of color when the contributed *C1–IΔ* allele in the heterozygote is derived from the male. This cross, *C1*/*C1*×*C1-I*/*C1*, yields two *C1* genotypes in the aleurone, *C1-IΔ*/*C1*/*C1* and *C1*/*C1*/*C1*, but the resulting phenotypes are completely colored. Here the *C1-IΔ* allele is challenged by two *C1* alleles in this triploid aleurone and is not able to suppress coloration as in the manner of a female-derived allele.

There are two observations that bear on this feature of allelic differences in the suppression of anthocyanin coloration when the *C1-lΔ* allele is male transmitted. First, the *C1-IΔ* allele reported in this study when derived from the female parent does not suppress color when in the heterozygous condition with the $^{s}C1$ allele (data not presented). Here, the $^{s}C1$ allele predominates in coloration. Secondly, differing from these *C1-I*(*Δ*) alleles, the *C1-I*(*std*) suppresses color when the allele is derived from the male. These two features are explained by the promoter composition of the differing alleles. Both $^{s}C1$ and *C1-I*(*std*) contain a Box 2, the promoter that makes those alleles overexpressors (Hattori et al. 1992; Scheffler et al. 1994). This identifies the promoter content as the contributor to this difference and is a significant feature in plant breeding concepts.

Transcription effects and yield of product

This study provides an example of how a well-known gene product which regulates a cascade of genes that leads to a final phenotype is affected. This expression, as assayed by color potency determination, is influenced both by the heteroallelic makeup and the background genotype. This study also provides an example of how the yield of a product leading to a phenotype is affected both by the dominance of the allele in the heteroallellic makeup and the background genotype of the lines combined in the cross.

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