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1. Conditional colored and colorless alleles at the C_1 locus.

Besides the dominant colored allele, C_1, and inhibitor, C_1^I, recessive colorless, c_1, can be differentiated into two allelic forms: c^p (p for positive), the conditional colored which becomes colored in the light during germination (MGCNL 44:153, 1970), and c^n (n for negative), the colorless which fails to give color at any time. Their dominance relationship is $C^I > C > c^p > c^n$. It has been found that *c* testers derived from W22 and K55 contain conditional colored c^p alleles while most other *c* tester stocks and KYS contain colorless c^n alleles. Four point linkage data have been obtained to support the differentiation of *c* alleles into two forms (Table 1).

Some properties of this conditional colored c^p allele have been observed. Light is necessary for pigment formation in c^p tissue. Peeling off the husk to expose c^p tissue to the light does not induce pigment formation during normal seed development. However, c^p vp_1 kernels on the cob can synthesize pigments (although pigments are reduced) if the husk is removed. Thus, light and germination are two critical conditions for anthocyanin formation in c^p tissue. The c^p allele has many properties in common with *C*. It is inhibited by C^I and shows mottled expression with *R r r*. It also requires A_1, A_2, C_2, Bz_1, and Bz_2 to be present in the dominant form for pigment formation. Unlike *C*, c^p seeds from c^p/c^n F_2 segregating ears sometimes show a wide range of color variation, from very light to very intense. The variation is not heritable. It is possible that the variation is due to dosage effect at the *C* locus, to background factors, to varying physiology and vigor of germinating seeds, or to environmental factors.

The relative concentration of cyanidin to pelargonidin (obtained by acid hydrolysis of pigments) in c^p germinated seeds is much lower than that in normally pigmented *C* seeds (Kirby & Styles, Can. J. Genet. Cytol. 12:934, 1970). We observed similar results (lower cy/pg ratio) in c^p

Table 1

Four point linkage data for $\frac{+\ c^{p}\ +\ +}{yg\ c^{n}\ sh\ wx}$ x yg c^{n} sh wx

Test cross	Parental	R1	R2	R3	R1 & R2	R1 & R3	R2 & R3	R1, R2 & R3	Total
c^{p} (W22)	1731 1580 3311	584 510 1094 19.59%	63 105 168 3.01%	492 462 954 17.08%	14 5 19 0.34%	25 6 31 0.56%	2 0 2 0.04%	4 1 5 0.09%	5584
	Recombination percentages = yg c 20.58, c sh 3.48, sh wx 17.77								
c^{p} (K55)	963 892 1855	233 279 512 16.20%	68 79 147 4.65%	339 272 611 19.33%	1 5 6 0.19%	5 8 13 0.41%	4 5 9 0.28%	4 4 8 0.25%	3161
	Recombination percentages = yg c 17.05, c sh 5.37, sh wx 20.27								

tissue in K55 do not show a decrease in cy/pg ratio, compared with that of C tissue (Table 2). Within W22 background, F_1 (C C x $c^p c^p$) and F_2 C --

Table 2

Relative concentration of anthocyanidins from C and c^p tissues in different backgrounds (average O.D. of 4 replicates).

Background	W22			K55		
Anthocyanidin	cy	pg	cy/pg	cy	pg	cy/pg
Genotype						
A C R	2.64	0.17	15.53	1.60	0.39	4.10
A c^p R	1.69	0.92	1.84	0.64	0.14	4.57
F_1(C C x $c^p c^p$)	1.54	0.10	15.40			
F_2 C --	1.64	0.14	11.71			
F_2 $c^p c^p$	0.59	0.32	1.84*			

*two replicates
cy = cyanidin, pg = pelargonidin

seeds have a cy/pg ratio similar to that of the C C parent, while F_2 conditional colored $c^p c^p$ seeds have a ratio similar to that of the $c^p c^p$ parent. The data favor the hypothesis that physiological conditions of germination in W22 result in the increase in pelargonidin. Anthocyanidin constitutions of F_1 and F_2 between K55 c^p and W22 c^p have also been studied (Table 3).

Table 3

Relative concentration of anthocyanidins of F_1 c^p (W22) x c^p (K55), F_2, and backcrosses (average O.D. of 4 replicates)

Anthocyanidin family	Relative O.D.		
	cy	pg	cy/pg
F_1	1.64	0.70	2.35
F_2	0.71	0.38	1.87
F_1 x c^p (W22)	1.50	0.74	2.03
F_1 x c^p (K55)	1.12	0.44	2.54

The data indicate that the two $\underline{c}^p$ alleles present in W22 and K55 $\underline{c}$ testers have essentially the same effects. The materials used for K55 $\underline{c}^p$ were from very weak inbred ears, and this may be the reason for the higher cy/pg ratio observed in K55 $\underline{c}^p$ tissue. There seems to be a general trend such that the more vigorous the germinating seed, the lower the cy/pg ratio.

It is clear that there are two allelic forms present in $\underline{c}$ testers: $\underline{c}^p$, the conditional colored, and $\underline{c}^n$, the colorless. Light and germination are two required conditions for anthocyanin formation in the $\underline{c}^p$ tissue. The pigments formed in $\underline{c}^p$ tissue have a lower cyanidin/pelargonidin ratio than that in normally pigmented $\underline{C}$ tissue.

Shu-mei Chen

2. An unsuccessful search for mutations affecting anthocyanin distribution.

Large populations of one inbred line, Ky 27, grown in isolation, have been observed closely for mutations of factors controlling anthocyanin distribution. This inbred is $\underline{A}\ \underline{C}\ \underline{r}^r$ and has purple plumule; a search was conducted in the plants and in the seeds produced on them for pigment formation in new locations--for example, in culm, husk, glume bar, and aleurone tissue. Among more than 10,000 plants studied, no distinctive plants or sectors were found; among 3.62×10^6 kernels, several colored contaminations were identified, but no valid mutations were found, either as whole-kernel exceptions or as sectors down to the limit of naked-eye resolution. Considering that any single mutation-competent locus (for example, $\underline{r}^r$) is present in the aleurone in 3 doses (i.e., 10.86×10^6 chromosomes entering into the triple fusion), and that the twofold observation protocol used here should identify events through at least the first ten divisions (i.e., 10^3 sites for minimum detectable mitotic events; 10^6 mitoses per kernel), no mutation to anthocyanin synthetic capacity was found in around 10^{13} mitotic replications. This observing load was lightened by the help of Paul Bolen, Shu-mei Chen, John Cousins, Kenneth Hall, Henry Lee, Marion Murray, Donald Smith, Jean Spengel, and Charles Williamson.

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