Table 1. Quantitative determination of anthocyanin pigments from colored, pale and colorless derivatives of different a2 mutable alleles.

| Pedigree | Phenotype | Mean O.D. | % control | Origin |
|---|---|---|---|---|
| B 2561/sib | Colored | 0.588 | 100.00 | W22-Col (control) |
| 4 4409-4/2335 | Colored | 0.375 | 67.24 | a2-m78018 |
| 4 4409-1/2335 | Colored | 0.493 | 84.40 | a2-m78018 |
| 4 4409-1/2335 | Colorless | 0.005 | 0.85 | a2-m78018 |
| 4 4415-1/2340 | Colored | 0.373 | 63.79 | a2-m78018 |
| 4 4415-1/2340 | Colorless | 0.005 | 0.85 | a2-m78018 |
| 4 4474-4/2347 | Colored | 0.478 | 82.75 | a2-m78018 |
| 4 4417-4/2347 | Colorless | 0.005 | 0.85 | a2-m78018 |
| 308-78-7A x | Pale | 0.0715 | 12.00 | a2-m68144 |
| 4 3048 x | Pale | 0.0445 | 6.80 | a2-m68140 |
| 4 3048 x | Colored | 0.435 | 74.13 | a2-m68140 |
| 4 3036 x | Pale | 0.0265 | 5.17 | a2-m68140 |
| 4 3049 x | Colored | 0.403 | 68.96 | a2-m68140 |
| 8 3825 x | Pale | 0.075 | 11.20 | a2-m11511 |

arising from one source allele can be significantly different from the derivatives of other alleles, indicating that the processes involved in the origin of such derivatives may not be identical; 4) every colored derivative included in this study is significantly different from pale, colorless and colored control; thus, it can be concluded that all these colored derivatives represent a differential impairment of anthocyanin synthesis in the aleurone of kernels.

Similarly the pales, which are distinctly different from the colored and colorless types, also show significant differences in anthocyanin content. Also, significant differences exist between pales originating from the same source as well as pales originating from different sources. All the tested pales are significantly different from colorless and colored types. Again, these intermediate allelic types represent a higher degree of impairment of anthocyanin production, since these accumulate relatively low quantities of anthocyanins in the aleurone tissue (as little as 6 to 13% of control). Absolutely no anthocyanin pigments were present in the aleurone of colorless derivatives.

Qualitative analysis: Qualitative differences among the colored, pale and colorless types have been investigated by thin layer chromatography and spectroscopic techniques. The results suggest that there are no qualitative differences in terms of anthocyanin pigments between colored, pale and colored control. All of them accumulate the same anthocyanin pigments, namely cyanidin-3-glucoside and pelargonidin-3-glucoside in appropriate proportions depending on Pr and pr constitution.

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Pattern differentiation of mutable c alleles; a second factor conditioning pattern type

In the En controlling-element system, there are a large number of independently originated mutable c alleles with widely varied spotting patterns of aleurone, ranging from coarse to very finely spotted type. Some of these mutable alleles, for example, c-m55301, c-m55398, c-m55453 and c-m55351, express two distinct patterns: (a) coarse and (b) fine type in testcrosses (c-m Sh Wx/c sh wx x c sh wx/c sh wx).

Each of the pattern types was tested. When the plants grown from the coarse kernels of the above cross were testcrossed (c-m sh wx/c sh wx x c sh wx/c sh wx) only coarse types appeared in the progeny (Table 1). In tests of fine type kernels (c-m Sh Wx/c sh wx x c sh wx/c sh wx) both coarse and fine kernels were

Table 1. Segregation of second factor; pattern differentiation. Test crosses of coarse and fine plants. c-m Sh Wx/c sh wx x c sh wx/c sh wx.

| Cross | ♀♀ Genotype | ♀♀ Phenotype | Mutable allele | Sh Fine(%) | Sh Coarse | Sh Colorless | sh Fine | sh Coarse | sh Colorless | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| 8 3103-1/3314 | c-m Sh Wx/c sh wx | fine | c-m55301 | 73(4.48) | 74 | 16 | 3 | 2 | 118 | 402 |
| 8 3116-1/2903 | c-m Sh Wx/c sh wx | fine | c-m55301 | 85(38.29) | 104 | 33 | 3 | 8 | 226 | 459 |
| 8 3102-1/3322 | c-m Sh Wx/c sh wx | coarse | c-m55301 | 0 - | 72 | 88 | 0 | 0 | 147 | 307 |
| 8 3118-1/3330 | c-m Sh Wx/c sh wx | coarse | c-m55301 | 0 - | 49 | 37 | 0 | 0 | 75 | 151 |
| 8 3106-1/3314 | c-m Sh Wx/c sh wx | fine | c-m55351 | 136(46.74) | 125 | 30 | 0 | 0 | 214 | 505 |
| 8 3106-2/3314 | c-m Sh Wx/c sh wx | fine | c-m55351 | 70(28.46) | 108 | 68 | 1 | 1 | 142 | 390 |
| 8 3107-1/3324 | c-m Sh Wx/c sh wx | coarse | c-m55351 | 0 - | 203 | 27 | 0 | 6 | 150 | 386 |
| 8 3107-2/3324 | c-m Sh Wx/c sh wx | coarse | c-m55351 | 0 - | 167 | 30 | 0 | 2 | 140 | 339 |
| 8 3108-1/3317 | c-m Sh Wx/c sh wx | fine | c-m55398 | 90(35.29) | 114 | 51 | 6 | 8 | 242 | 511 |
| 8 3109-2/3320 | c-m Sh Wx/c sh wx | fine | c-m55398 | 74(30.0) | 69 | 97 | 0 | 1 | 250 | 491 |
| 8 3110-1/3322 | c-m Sh Wx/c sh wx | coarse | c-m55398 | 4 - | 52 | 123 | 1 | 2 | 140 | 322 |
| 8 3119-1/2904 | c-m Sh Wx/c sh wx | fine | c-m55453 | 68(40.96) | 74 | 24 | 1 | 1 | 130 | 298 |
| 8 3120-1/3302 | c-m Sh Wx/c sh wx | coarse | c-m55453 | 0 - | 189 | 59 | 0 | 10 | 231 | 509 |

Table 2. The determination of segregation of coarse and fine kernels in the sib cross progeny of c-m Sh Wx/c sh wx x c sh wx/c sh wx En (in each case, En was identified in the male parent).

| Cross | ♀♀ Phenotype | ♀♀ Genotype | Mutable allele | Total number tested | Number of ears segregating coarse and fine |
|---|---|---|---|---|---|
| 5 1719/1720 | coarse | c-m Sh Wx/c sh wx | c-m55301 | 9 | 7 |
| 5 1721/1722 | coarse | c-m Sh Wx/c sh wx | c-m55301 | 4 | 0 |
| 5 1723/1724 | coarse | c-m Sh Wx/c sh wx | c-m55301 | 1 | 1 |
| 5 1725/1726 | coarse | c-m Sh Wx/c sh wx | c-m55301 | 3 | 1 |
| 5 1727/1728 | coarse | c-m Sh Wx/c sh wx | c-m55301 | 7 | 2 |
| 5 1729/1730 | coarse | c-m Sh Wx/c sh wx | c-m55351 | 4 | 4 |
| 5 1731/1732 | coarse | c-m Sh Wx/c sh wx | c-m55351 | 4 | 1 |
| 5 1733/1734 | coarse | c-m Sh Wx/c sh wx | c-m55398 | 4 | 2 |
| 5 1735/1736 | coarse | c-m Sh Wx/c sh wx | c-m55398 | 6 | 3 |
| 5 1737/1738 | coarse | c-m Sh Wx/c sh wx | c-m55398 | 7 | 3 |
| 5 1739/1740 | coarse | c-m Sh Wx/c sh wx | c-m55398 | 4 | 2 |

segregating in the progeny in approximately a 1:1 ratio. In the light of these results, it is assumed that the c-m allele expresses a coarse pattern that is modified by a second factor resulting in a fine pattern. However, the nature of this second factor remains unknown.

Attempts have been made to determine if the second factor is En itself. Plants grown from the coarse kernels of the original testcross of fine plants (c-m Sh Wx/c sh wx x c sh wx/c sh wx) were sibcrossed by colorless shrunken sibs known to contain En (c sh wx/c sh wx En). The results are given in Table 2. Among the 53 tested, 26 showed segregation of coarse and fine patterns. In view of the absence of segregation for coarse and fine types in all of the progenies, even in the presence of En, there is a strong indication that the second factor modifying the coarse pattern is not En. Further tests to characterize the second factor are in progress.

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Initiation and maintenance of callus cultures of the En-Spm controlled allele, wx-m8

We are presently developing callus and cell cultures of homozygous recessive wx-m8 endosperm and scutellar tissue. Endosperm cultures were obtained by the following procedure. Immature ears were harvested (7 to 10 days post-pollination) and immediately processed for culture on solid media. The young ears were cut into small pieces and surface sterilized for 10 minutes in 0.5% Clorox solution. After rinsing three times in sterile distilled water, the tops of the kernels were cut off with a fine scalpel and the endosperm squeezed with a spatula onto the medium. The basal medium used consisted of the major and minor salts of revised Murashige and Skoog (1962): 0.5 mg of thiamine per litre of medium; 0.5 mg/L of pyridoxin HCl; 0.5 mg/L of niacin; 8 mg/L of glutamine; 2 gm/L of asparagin; 30 gm/L of sucrose; 8 mg/L of agar; 500 mg/L of yeast extract and 2 mg/L of 2,4-dichlorophenoxyacetic acid; pH 5.8 to 6; dark incubation 80 F. The callus was transferred to fresh medium every 21 days.

Callus induction was not successful either with (a) White's basal medium plus Nitsch's trace elements, 2% sucrose, 2 gm/L of asparagine; 9 gm/L of agar; and 2 gm/L of yeast extract or, (b) Linsmaier and Skoog medium: 0.4 mg/L of thiamine HCl; 100 mg/L of i-inositol; 2 mg/L of NAA; 2 mg/L of IAA; 30 gm/L of sucrose; 10 gm/L agar and 150 ml/L of coconut milk. In both of these cases, no callus growth was observed even after transferring to fresh medium.

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Further data on the location of the modifier gene of the cl locus

In the 1972 News Letter (M.G.C.N.L. 46:93-95, 1972) and the 1973 News Letter (M.G.C.N.L. 47:79-81, 1973) $F_2$ data were presented that indicated the modifier of cl (Clm) was linked to the breakpoint of T8-9(6673) in chromosome 8. The Clm locus has a series of alleles that partially or completely suppress the albino phenotype of cl seedlings. The pale yellow or white endosperm phenotype of this mutant is not suppressed. In the 1972 report, it was indicated that there were two modifiers of cl in the inbred M14. One was responsible for green seedlings (Clm-M14 gr) and the other for pale green or pastel seedlings (Clm-M14 pas). Allele tests had confirmed that Clm-M14 gr was allelic to the other modifier genes of cl. However, the Clm-M14 pas, which was involved in the 1972 linkage tests with T8-9(6673), had not actually been shown to be allelic to the other modifiers. In 1973 allele tests between Clm-3 and Clm-M14 pas were made by self-pollinating plants from the cross of cl cl Clm-3 Clm-3 X Cl cl-7716 Clm-M14 pas Clm-M14 pas.