

content. Seedlings were grown for three weeks at  $27\pm 3^{\circ}\text{C}$  under approximately 1000 ftc. of illumination on a 16 hour photoperiod. They were watered with tap water with supplements of Hoagland's #1 nutrient solution. Pigment analyses were carried out according to Arnon (Plant Phys. 24:1-15, 1949) and von Wettstein (Exptl. Cell Res. 12:427-506, 1957). Chlorophyll concentrations of pg<sub>11</sub>pg<sub>12</sub> and oy were 70% and 74% respectively of the normal line while the total carotenoids of the two mutant lines were 65% and 52%, respectively, of the normal line.

Free amino acids were extracted according to Block, Durrum and Zweig (1958) and purified on a Dowex 50-X8 ion exchange column. Total free amino acids were determined according to Barrolier (Naturwissenschaften, 48:554, 1961) and individual amino acids were separated on one- and two-dimensional thin-layer cellulose plates. The solvent systems used were chloroform/methanol/17% ammonium hydroxide (20/20/5,v/v) for the one-dimensional plates, and butanol/acetic acid/water (4/1/1,v/v) and the above solvent for the two-dimensional plates.

No significant differences were observed in the total free amino acid contents of either pg<sub>11</sub>pg<sub>12</sub> or oy when compared to the normal green plants. However, chromatographic analysis revealed that in both pg<sub>11</sub>pg<sub>12</sub> and oy, asparagine was very much reduced or completely missing while in the normal plants it was present in significant amounts. All other amino acids appeared to be unaltered.

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1. An oxidizable flavonoid difference in corn silks.

Last year (MGCNL, 1970) a polyphenol oxidase oxidizable flavonoid difference was reported in corn silks. When fresh silks were cut back prior to pollination, it was observed that the cut ends turned brown in a few minutes in certain lines while in others they did not brown but remained yellow-green (i.e. no change in color). Browning of cut ends

was due to the oxidation of polyphenols by polyphenol oxidase which resulted in a brown pigment formation. It was established that lines which did not brown after being cut back lacked the polyphenolic substrate. Both browning and non-browning types had the enzyme, polyphenol oxidase. Backcross ratios indicated a monohybrid segregation with the browning type being dominant to the non-browning. Further data involving larger numbers of progeny have confirmed the earlier genetic analysis (Table 1).

Table 1  
Backcross segregation results and  $\chi^2$  test for fit to a 1:1 ratio

Backcross	Brown type	Green type	Total	$\chi^2$	P
(NC236 x NC232) NC236 green brown green	316	331	647	.348	.5-.7
(Kys x T61) Kys green brown green	264	251	515	.328	.5-.7

Silks of the browning type have recently been studied to determine the type of polyphenolic compounds which are involved in the brown pigment formation. Fresh silks were ground and extracted in acidic 80% methanol. By means of PVP (polyvinyl-pyrrolidone) column chromatograph and paper chromatography in two solvent systems (BAW and 15% HOAc). We have found nine compounds present in the browning phenotype which were not present in the non-browning type. These nine compounds gave a positive reaction for ortho-dihydroxy flavones with Benedicts reagent on paper chromatograms. Although all nine compounds can be oxidized in the presence of polyphenol oxidase to form the brown pigment, three of the compounds occurred in much greater quantities than the remaining six. These three compounds were designated I, II, and III, in order of decreasing quantity.

Visible-ultraviolet spectroscopy studies have been carried out on compounds I, II, and III (Table 2). Spectra measurements were made in methanol alone and with the reagents, sodium acetate, sodium methoxide

Table 2  
Diagnostic chromatographic attributes and spectra absorption maxima

Compound	Rf		Color of spot			Absorption maxima in nm					
	BAW	HOAc	UV	UV + NH <sub>3</sub>	UV + Bened.	MeOH	MeOH- AlCl <sub>3</sub>	MeOH- AlCl <sub>3</sub> +HCl	MeOH- NaOMe	MeOH NaOAc	MeOH- NaOAc+ H <sub>3</sub> BO <sub>3</sub>
I	.52	.67	D	Y	D	349 270 255 242*	429 332 302* 277	387 363 297* 279 267*	409 336* 278* 270	392 327* 274	377 264
II	.55	.43	D	Y	D	351 271 258 246*	429 332 302* 278	388 366 296* 280 265*	408 278	407 269	376 264
III	.40	.67	D	Y	D	350 271 255	429 331 302* 277	386 365 296* 279 267*	410	393 325* 274	377 264

\*Shoulders

D = dark

Y = yellow

Bened = Benedicts solution

aluminum chloride, aluminum chloride-HCl solution and sodium acetate-boric acid solution. These results indicated the three compounds were derivatives of 5, 7, 3', 4' tetra-hydroxy flavone (luteolin). Acid hydrolysis experiments with these three compounds indicated that all had acid hydrolyzable O-glycosides. Of particular interest, acid hydrolysis of compound III yielded iso-orientin and orientin which are isomers. A milder hydrolysis of compound III gave primarily iso-orientin which indicated that compound III is an O-glycoside of iso-orientin. The isolation of iso-orientin established the presence of a C-glycosylflavone in corn.

The letters Fv have been selected to designate the dominant allele which results in browning of cut fresh silks because of the presence of at least nine oxidizable polyphenolics. Fv was chosen because the three compounds present in largest amounts have been identified as flavones. The recessive allele has been designated fv. In the homozygous recessive form, fv/fv, no browning of cut fresh silks occurs because the nine oxidizable polyphenols are absent. The dominant allele, Fv, appears to behave as a completely dominant allele since a quantitative distinction can not be demonstrated between the Fv/Fv and Fv/fv genotypes. The following commercial inbreds have been classified as Fv/Fv genotype: C121, Hy2, L317, NC7, NC232, T61, T204 and WF9, while the following inbreds are of the genotype fv/fv: Kys, NC34, NC45 and NC236. The open pollinated varieties Jarvis Golden Prolific and Indian Chief are segregating for Fv and fv alleles. Chromosomal aberration and marker stocks obtained primarily from the Coop carry both alleles, Fv and fv. It also appears that a third allele, which is intermediate in phenotype, has been isolated; however, genetic analysis is incomplete.

Several points can be made concerning the block to flavonoid synthesis imposed by the fv/fv genotype. First, several fv/fv genotypes have anthocyanin in their silk. Therefore, the fv/fv block doesn't prevent all types of flavonoid synthesis. Second, the fv/fv genotype prevents synthesis of at least nine different compounds, three of which are known to be flavones. The block may therefore occur early in flavone synthesis. Third, similar flavones appear to be produced in the leaves of plants which are of the fv/fv and Fv/Fv genotypes and some leaf

flavones appear identical to those produced in silk of the Fv/Fv genotype. These observations are based on  $R_f$  values obtained by paper chromatography in two solvent systems (BAW & 15% HOAc). Consequently, the block to flavone synthesis in the fv/fv genotype appears confined to the silks.

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1. A preliminary attempt to group Latin American races of maize.

Using a set of 30 characters including geographic characters as well as a set of ear, kernel, and cob characteristics known to be relatively slightly altered by environmental effects (see Goodman and Paterniani, 1969), principal components analysis and cluster analysis were employed to group 230 races of maize from Mexico, Venezuela, Colombia, Peru, Ecuador, Bolivia, Chile, Argentina, Uruguay, Paraguay, Brazil, the Guianas, and Cuba. The data were taken from the series of Races of Maize booklets describing these races, from Paterniani (1967), from the Ph.D. thesis of R. M. Bird (University of California, 1970), and from Goodman (1967, 1968, and unpublished). Distances between all possible pairs of races were calculated using the first 10 standardized principal components (those with eigenvalues of approximately one or greater). Unweighted cluster analysis (Sokal and Sneath, 1963) was then applied to those distances.

Thirty-six groups of races were obtained. (The following abbreviations are used: A = Argentina, Bo = Bolivia, Br = Brazil, Ch = Chile, Co = Colombia, Cu = Cuba, E = Ecuador, G = the Guianas, M = Mexico, Pg = Paraguay, Pu = Peru, U = Uruguay, and V = Venezuela). Brackets and parentheses indicate subgroupings. Races are listed within groups and subgroups in order of increasing average distance from those which precede them. Subgroups are also listed in order of increasing average distance.