standard stippled kernels. One of them has a very fine spotting pattern and has been called "smoky," symbolized $\underline{R}^{st}$ (sky).

Chromatographic comparison of pigment extracts of homozygous $\underline{R}^{st}/\underline{R}^{st}$ and $\underline{R}^{st}(sky)/\underline{R}^{st}(sky)$ seeds does not disclose any qualitative difference between their anthocyanin content. The smoky derivatives are strongly paramutagenic.

When $\underline{R}^{st}(sky)/\ \underline{r}^{g}$ is crossed with $\underline{r}^{g}/\underline{r}^{g}$, some of the resulting ears show, besides the expected colorless kernels (genotypically $\underline{r}^{g}/\underline{r}^{g}$) two kinds of smoky, darker and lighter, often in equal frequency. While the former breed true in successive generations, the lighter segregate again, when crossed with $\underline{r}^{g}/\underline{r}^{g}$, for darker and lighter smoky, in a ratio of 1:1.

Similar results seem to indicate that the lighter smoky phenotype results from the interaction of $\underline{R}^{st}(sky)$ with a Modifier of the smoky expression that assorts independently of $\underline{R}^{st}(sky)$.

Giuseppe Gavazzi

4. Chromatographic and spectrometric analysis of root and seed pigments.

Pigments are extracted from roots and seeds of a W22 $\underline{A}_1$, $\underline{A}_2$, $\underline{C}_1$, $\underline{C}_2$, $\underline{Pr}$, $\underline{R}$ stock carrying one of the following $\underline{R}$ combinations: $\underline{R}^{st}/\underline{R}^{r}$, $\underline{r}^{g}/\underline{R}^{r}$, $\underline{r}^{g}/\underline{R}^{r'}$.

The extracting solvent used is a 0.1% concentrated hydrochloric acid in 95% ethanol (v/v) solution. The pigment extracts are concentrated under vacuum and then chromatographed with the ascending method on Whatman paper #1. Two solvent systems have been used;
(1) n-butanol, acetic acid, water (4:1:5)
(2) ethyl acetate, t-butanol, acetic acid, water (3:4:1:3).
Both seed and root extracts are separated into three red bands that turn blue when exposed to ammonia vapours. They represent three different anthocyanins. An additional yellow component appears in chromatograms of root extracts.

The absorbance spectra of the four components chromatographically separated are then determined spectrometrically. In Table 1 the Rf values and the absorption peaks (λ max.) of the four components are reported and in Fig. 1 their absorption spectra, after chromatographic separation, are indicated. It appears, from the graphs, that the three anthocyanins chromatographically separated have slightly different peaks of absorbance and are present in quite a different proportion. Their concentration increases from compound 1 up to the third in band three.

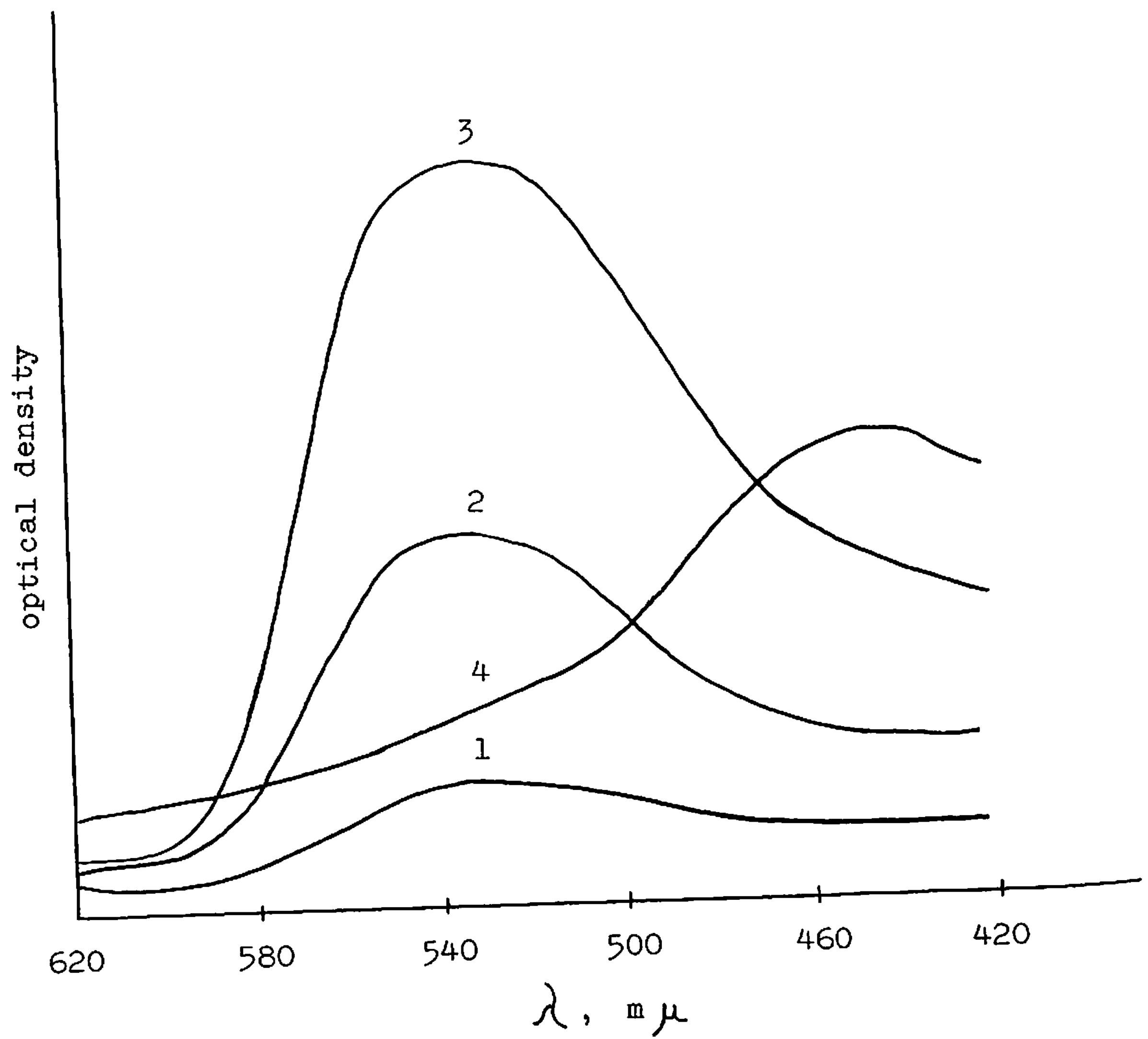

Fig. 1. Absorbance Spectra of the Root Pigments.

Table 1
Chromatographic and spectrometric identification of root pigments.

Compound	RF (1)	RF (2)	λ max (mμ)
1	0.28	0.18	535
2	0.36	0.25	533
3	0.45	0.37	530
4	0.71	0.58	448

The anthocyanins of seeds carrying other R alleles, have been chromatographed with the same procedure and they are all separated into three bands with the same Rf values reported in Table 1 for the first 3 compounds.

Compounds 2 and 3 have been tentatively classified, according to their Rf and λ max. as cyanidin-3-monoglucoside and pelargonidin-3-monoglucoside. Compound 1, present in much lower proportion, is still unknown.

The identity of pigment constitution of paramutable R and R' seeds seems to suggest that the phenotypic difference between the two rests only upon a difference in level of production of anthocyanins without a concomitant alteration in their single constituents.

Giusseppe Gavazzi

5. A test for the association of paramutation with roots.

Plant and seed pigments are controlled by the two subunits of the R gene, respectively symbolized P and S. If paramutation is not confined to the S component of the R locus but affects the R locus as a whole, it should also be possible to observe its expression in sporophytic tissues.

We compared the anthocyanin content of R^{st}/R^{r} with that of r^{g}/R^{r} roots with the intent of disclosing the existence of paramutation in sporophytic tissues. The former carry a paramutagenic allele (R^{st}) and a paramutable R, i.e. an R allele sensitive to the paramutagenic action of R^{st}; while the latter carry the same R^{r} allele in a heterozygote with r^{g}, i.e. an allele incapable of inducing paramutation. These roots derive from seeds obtained by parallel crosses of R^{st}/R^{st} and r^{g}/r^{g} plants with the same pollen parent R^{r}/R^{r}.

If paramutation takes place in roots, we expect to observe a decrease of pigment in R^{st}/R^{r} roots when compared to that of r^{g}/R^{r} roots. The determination of anthocyanin