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

Table 2

Comparison of mean anthocyanin content of:

I. $\underline{R}^{st}\ \underline{R}^{r}$ and $\underline{r}^{g}\ \underline{R}^{r}$ roots II. $\underline{r}^{g}\ \underline{R}^{r}$ and $\underline{r}^{g}\ \underline{R}^{r'}$ roots

Genotype selected	Pedigree	No. roots tested	Mean Score*	t value	P
	I. Comparison of pigment content of $\underline{R}^{st}\ \underline{R}^{r}$ roots with that of $\underline{r}^{g}\ \underline{R}^{r}$ roots				
	A - 7 days old roots				
$r^{g}R^{r}$	g 818 x g 830-3,-4,-6	100	0.28		
$R^{st}\ R^{r}$	g 780 x g 830-3,-4,-6	100	0.27	0.19	0.05
	B - 12 days old roots				
$r^{g}\ R^{r}$	g 818 x g 830-4	25	0.21		
$R^{st}\ R^{r}$	g 780 x g 830-4	25	0.24	1.36	0.05
	C - 9 days old roots (pieces)				
$r^{g}\ R^{r}$	g 818 x g 830-3,-5	60	0.39		
$R^{st}\ R^{r}$	g 780 x g 830-3,-5	60	0.41	0.35	0.05
	II. Comparison of pigment content of $\underline{r}^{g}\ \underline{R}^{r}$ roots with that of $\underline{r}^{g}\ \underline{R}^{r'}$ roots				
	D - 9 days old roots (pieces)				
$r^{g}\ R^{r}$	g 944 x g 940 - a	35	0.24	0.29	0.05
$r^{g}\ R^{r'}$	g 944 x g 940 - b	40	0.28		

*expressed as mean O.D. at 530 m

content is based on the spectrometric reading of the optical density of the pigment extracts of the roots.

The data of Table 2 (Part I) indicate that R^{st}/R^{r} and r^{g}/R^{r} roots do not differ significantly in their pigmenting potential level. The failure to observe a decrease in pigmenting level in $R^{st}R^{r}$ roots could be due to the insufficient time, in terms of cell generations, given to the roots before testing the paramutagenic effect of R^{st} upon R^{r}. It could be that at least one generation of R^{st}/R^{r} heterozygosity is required before paramutation becomes phenotypically manifest. Accordingly, the comparison of pigment concentration has been extended to $r^{g}R^{r'}$ and $r^{g}R^{r}$ control roots (Table 2, Part II). However, also in this case, when the pigment potential of $R^{r'}r^{g}$ roots is compared to that of $R^{r}r^{g}$ roots, no decrease in the level of anthocyanin is observed in the former.

The lack of reduction in pigment concentration of $R^{r'}$ roots suggests that the R component, controlling pigment formation in roots, is either insensitive or less sensitive than the S component to the paramutagenic action of R^{st}. The differential sensitivity of the two R sub-units to the R^{st} action is here considered as an indication that the R locus as a whole is not involved in paramutation.

Giuseppe Gavazzi
Nicole Zannini

ISTITUTO DI GENETICA VEGETALE
Università Cattolica
Piacenza, Italy

1. Location of Ga_8 in chromosome 9 linkage group.

Preliminary data for a close linkage relationship between a gametophyte factor and the waxy locus have been presented both by Schwartz and by Bianchi, in previous issues of MNL. However, the question whether the Ga factor was between the wx locus and the centromere or placed distally to the wx locus remained unanswered.

Some data from backcrossing plants heterozygous for Ga_8, as well as for wx and bz, on a multiple tester for chromosome 9 are as follows: