

been determined. The results of this test indicate that the two kinds of roots do not differ significantly in their pigment content.

The data so far obtained can be summarized as follows:

1. A significant decrease of R action in the plant tissues is obtained only after exposure of paramutable R to the repressive activity of a paramutagenic allele for two successive generations. These results suggest that paramutation, in the plant tissues, is weak and progressive in nature.
2. No corresponding decrease of pigmenting potential is observed in the roots even after two generations of R<sup>r</sup> R<sup>st</sup> heterozygosity.
3. The level of R gene action in the aleurone of paramutable r<sup>st</sup>R' individuals is not correlated to its level of action in the roots.

These data suggest that the R locus does not react as a whole to the action of an inducing allele. Rather, it seems that different R subunits react in different ways to the repressive activity of R<sup>st</sup>. However, the interpretation of these results requires a deeper knowledge of the structural organization of the R region and of the biosynthesis of anthocyanins.

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#### 5. Chromatographic analysis of pigments of the various plant tissues.

A chromatographic analysis of the various pigments has been undertaken with the aim of investigating the following points:

1. Distribution of different pigments in the sporophytic and aleurone tissues of a W22 A<sub>1</sub> A<sub>2</sub> C<sub>1</sub> C<sub>2</sub> Pr R<sup>r</sup> b pl stock, hereafter referred to as A C R<sup>r</sup>.
2. Chromatographic analysis of the anthocyanins extracted from different tissues of A C R<sup>r</sup> plants in order to establish whether they are the same or undergo changes in their chemical composition.
3. Variation in pigment distribution of A C R<sup>r</sup> plants carrying various allelic combinations at the R locus.

Table 1 shows the variation of spots in different parts of the tissues of plants genotypically A C R<sup>r</sup>. The chromatogram was run first downwards with Butanol--Acetic acid--water (4:1:5) and then from left to right with Acetone--Hydrochloric acid (1:3). Spots 1, 2, 3, 4 are anthocyanins. Spots 5 and 7, faint yellow at the visible light, turn blue after spraying with  $\text{FeCl}_3$  solution. Spots 8 and 9 react positively with p-toluene sulfonic acid (dark yellow). Spot 10 turns light blue after spraying it with  $\text{Na}_2\text{CO}_3$  solutions. Spots 15 and 16 react positively with both  $\text{AlCl}_3$  and  $\text{Na}_2\text{CO}_3$ .

Table 1  
The variation of spots in different parts of plants genotypically A C R<sup>r</sup>

Part	Spots present												n(*)	Total No. of spots	Spots missing	
	1	2	3	4	5	6	7	8	9	10	15	16				
Aleurone	+	+	+	+				+	+					48	6	5, 6, 7, 10, 15, 16
Roots	+	+	+		+	+	+	+	+	+				16	9	4, 15, 16
Internode	+	+	+			+	+	+	+					15	7	4, 5, 10, 15, 16
Anthers	+	+	+			+	+	+	+			+	+	8	7	4, 5, 10

(\*) n = No. of chromatograms analyzed.

Table 2  
Rf values of the anthocyanins present in various parts of plants genotypically A C R<sup>r</sup>

Spot No.	Rf(1)	Rf(2)	Rf(3)	Rf(4)
1	0.25	0.28	0.27	0.24
2	0.30	0.34	0.34	0.29
3	0.39	0.42	0.42	0.39

- (1) Internode (n = 8)  
 (2) Aleurone (n = 50)  
 (3) Roots (n = 20)  
 (4) Anthers (n = 16)

Table 3  
Rf values of the anthocyanins found in plants genotypically A C R<sup>r</sup> Pr and A C R<sup>r</sup> pr

Genotype	Spot No.	Rf values in			
		BAW (n = 50)	BuHCl (n = 18)	1% HCl (n = 18)	HAc-HCl (n = 16)
<u>A C R<sup>r</sup> Pr</u> (seeds)	1	0.28	0.17	0.03	0.27
	2	0.34	0.27	0.04	0.33
	3	0.42	0.40	0.04	0.36
	4		0.68	0.13	0.54
-----		(n = 30)	(n = 10)	(n = 10)	(n = 10)
<u>A C R<sup>r</sup> pr</u> (roots)	1	0.31	0.18	0.06	0.31
	2	0.41	0.28	0.08	0.38
	3	0.51	0.35	0.08	0.40

Table 4  
The variation of different spots in the roots and aleurones of plants homozygous and heterozygous for different R alleles

Part	Genotype	Spots present																n	Total No. of spots
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
Roots	$\underline{r}^{\underline{g}} \underline{r}^{\underline{g}}$					+	+	+		+	+							4	5
Roots	$\underline{r}^{\underline{g}} \underline{R}^{\underline{r}}$	+	+	+		+	+	+	+	+		?	?					32	11
Roots	$\underline{r}^{\underline{g}} \underline{R}^{\underline{r}'}$	+	+	+		+	+	+	+	+								32	9
Roots	$\underline{R}^{\underline{r}} \underline{R}^{\underline{r}}$	+	+	+		+	+	+	+	+								16	9
Roots	$\underline{G} \underline{r}^{\underline{g}} / \underline{G} \underline{R}^{\underline{1.sk}}$					+	+	+	+	+					+	+		14	7
Roots	$\underline{G} \underline{r}^{\underline{g}} / \underline{G} \underline{R}^{\underline{sk}}$					+	+	+	+	+								8	5
Roots	$\underline{G} \underline{r}^{\underline{g}} / \underline{G} \underline{R}^{\underline{st}}$					+	+	+	+	+								8	5
Roots	$\underline{G} \underline{r}^{\underline{g}} / \underline{G} \underline{R}^{\underline{sc}}$					+	+	+	+	+								8	4
Aleurone	$\underline{G} \underline{r}^{\underline{g}} / \underline{G} \underline{R}^{\underline{1.sk}}$	+	+	+							+							8	4
Aleurone	$\underline{G} \underline{r}^{\underline{g}} / \underline{G} \underline{R}^{\underline{sk}}$	+	+	+							+							8	4
Aleurone	$\underline{G} \underline{r}^{\underline{g}} / \underline{G} \underline{R}^{\underline{st}}$	+	+	+							+							8	4
Aleurone	$\underline{G} \underline{r}^{\underline{g}} / \underline{G} \underline{R}^{\underline{sc}}$	+	+	+							+							8	4

The  $R_f$  values of the first 3 anthocyanins determined with the BAW solvent are reported in Table 2. They appear to be rather constant in each of the tissues so far analyzed, thus suggesting that no qualitative change in the chemical composition takes place in the various tissues. On the other hand, qualitative changes are observed after substitution of  $Pr$  with  $pr$  (see Table 3) and  $C_1$  with  $c_1$ . In the latter case, anthocyanin biosynthesis is blocked in the aleurone while it occurs in the plant tissues leading to three different anthocyanins. Their  $R_f$  values in BAW are 0.34, 0.41 and 0.48 respectively. Table 4 shows the pigment distribution observed in plants carrying different  $R$  allelic combinations.

We are presently involved in the chemical identification of the various anthocyanins and in the genetic control of their biosynthesis in sporophytic tissues.

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1. Notes on tinged in chromosome 10.

The tinged character (Maize News Letter 40:106) was poorly expressed in all  $F_2$ , backcross, and increase progenies the past summer. In previous years it had been well expressed as seedlings, and still classifiable as adult plants. The character is not allelic to  $\underline{g}_1$ .

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2. Effects of colchicine treatment on multiple interchange heterozygotes.

Stocks homozygous for the T5-7-1-9-10 and T3-2-4-6-8 interchanges were crossed with normal stocks to produce  $F_1$ 's with  $\odot 10$ ; and with each other for 2  $\odot 10$ . The  $F_1$ 's were treated as seedlings with colchicine solutions of various strengths. Plants with sectors that extruded anthers and shed pollen were found among the treated  $F_1$  plants from the three crosses. Examination of pollen from these sectors with a pocket microscope indicated that more than half was normal in appearance, and considerably larger than the normal haploid pollen. These sectors are presumed to be  $4n$ . Some selfed seed was obtained. In untreated plants normal pollen was less than 10% in plants with a  $\odot 10$ , considerably less in plants with 2  $\odot 10$ .

The cross giving 2  $\odot 10$  may be used to test the effectiveness of agents and conditions for inducing polyploidy.

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