Critical field-collected clonal introductions of Tripsacum from these study areas are being maintained in the Maize Relatives - Genetics Garden of Tulane University.

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1. Biochemical study of anthocyanidins produced by different R alleles.

Analyses of the anthocyanins in maize are being carried out in this laboratory for the purposes of:

(1) Using anthocyanin formation as an indicator to study gene action at various developmental stages.

(2) Characterizing different allelic forms of the R locus with respect to anthocyanin production.

Five different alleles of the R locus were employed for this study:

(1) standard R^rR^r -red seedlings, red anthers and colored aleurone.

(2) R^gR^g Canada (P.I. 214199) -red seedlings, green anthers and colored aleurone.

(3) R^rR^r Ecuador (1172) -red seedlings, red anthers and colored aleurone.

(4) r^rr^r -red seedlings, red anthers and colorless aleurone.

(5) r^gr^g -green seedlings, green anthers and colorless aleurone.

All stocks used were strains of W22 carrying A_1 A_2 C_1 C_2 Pr pl. The W22 B allele is probably B^b (pigmented glume base and culm but otherwise weak plant-color).

Plants for this study were grown in the greenhouse. Pigmented tissues were collected and extracted with 1% HCl in MeOH, concentrated in vacuum and hydrolyzed with 4N HCl for 30 minutes. By adding a few drops of isoamyl alcohol, the hydrolysate was separated into an organic and an aqueous layer. The aglycones in the alcohol layer were spotted on a thin layer plate coated with Avicel S.F. Cellulose. The chromatograms were developed in two directions. First, formic acid: 4N HCl (2:1 v/v),

secondly with either acidified methanol water* (20:1 v/v, 0.5 ml of conc. HCl added per 100 ml of solvent), t-BuOH: 2N HCl: HAC: H_2O (6:1:1:2 v/v), or n-BuOH: HAC: H_2O (2:1:1 v/v). Essentially the same results were obtained with all these second solvents.

The number of different anthocyanidins obtained were as follows:

Genotype	Leaf Sheaths	Aleurone	Anthers
$\underline{R}^r\underline{R}^r$ Standard	6	4	5
$\underline{R}^r\underline{R}^r$ Ecuador	6	4	5
$\underline{R}^g\underline{R}^g$ Canada	6	4	-
$\underline{r}^r\underline{r}^r$	6	-	5
$\underline{r}^g\underline{r}^g$	6	-	-

Anthocyanidin spots in different tissues

Tissue	Spot number						
	1	2	3	4	5	6	7
Anthers	+	-	+	+	+	-	+
Leaf Sheaths	+	+	+	+	+	+	-
Aleurone	+	-	+	+	+	-	-

Spots 1 and 3 have been identified as cyanidin and pelargonidin, respectively.

Spot 4 has been tentatively classified as peonidin.

Spots 2, 5, and 6 have the characteristics of anthocyanidins. Some were purple and some magenta. When exposed to NH_3 vapor or sprayed with Na_2CO_3 they turn bluish.

Spot 7 is orange and turns bluish purple when exposed or sprayed with NH_3 or Na_2CO_3.

*Solvents developed by Dr. D. B. Mullick
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