

# Inheritance of a Second Wrinkled Leaf Mutant in American Pima Cotton<sup>1</sup>

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## ABSTRACT

Leaf morphology mutants arise from time to time in cotton (*Gossypium* spp.). These mutants are useful as markers for the study of genetic systems and for developmental studies of leaf morphology. One such mutant with wrinkled leaves appeared as a single plant in a 1982 breeding row (82-748) of American Pima cotton (*G. barbadense* L.) at Phoenix, AZ. The objectives of the present study were to determine the inheritance of the wrinkled leaf trait and its allelic and linkage relationships with other *Gossypium* mutants. The wrinkled leaf phenotype is inherited as a monogenic recessive. It expresses on successive leaves beginning with the first sympodial branch produced from Nodes 6 through 8 on field grown plants. The wrinkled leaf trait was not allelic with three other leaf mutant genes nor was it linked with 22 *Gossypium* mutant genes. The name *wrinkled leaf-2* and the gene symbol *wr<sub>2</sub>* are proposed for the new leaf trait. The new trait should be useful in genetic and developmental studies as an easily scored recessive phenotype affecting leaf morphology.

**Additional index words:** *Gossypium barbadense* L., Genetic marker, Linkage, Leaf morphology, Allelism, Recessive trait, Mutations.

MORPHOLOGICALLY aberrant plants are occasionally found in breeding nurseries and growers' fields of Pima cotton (*Gossypium barbadense* L.). These can be the result of spontaneous mutations (2), cytologically conditioned phenotypes resulting from haploidy or aneuploidy (3), or nongenetic causes such as mechanical or insect damage. In addition to scientific interest and as a source of genetic markers, it is important to identify the aberrant phenotypes that are transmitted from parent to progeny so that these can be removed from breeding nurseries and commercial fields that are being grown for increases of planting seed.

A plant with atypical leaves was found in 1982 in Row 748 of the Pima cotton breeding nursery at Phoenix, AZ. The atypical leaves were evident beginning with the initial sympodial branch at Node 8 and successive leaves expressed the trait for the remainder of the growing season. The phenotype had not been observed previously in Pima cotton, the most similar described mutants being *rugate leaf* (*Ru*) (6), *wrinkled leaf* (*wr*) (7), and *crinkle dwarf* (*cr*) (4). Open pollinated seed were harvested from the aberrant plant, and these

were planted in a progeny row at Maricopa, AZ in 1983. All plants in this row expressed the aberrant phenotype, which indicated that the aberrant leaf appearance was under genetic control. The present paper describes the inheritance, linkage, and allelic relationships of the new leaf phenotype in Pima cotton.

## MATERIALS AND METHODS

Parental stocks with aberrant leaves were designated 82-748. The aberrant leaves had a wrinkled appearance due at least in part to the leaf veins developing more slowly than the leaf lamina, especially toward the periphery of the leaf (Fig. 1). The leaves also had more shallow sinuses than normal. The ratio of length from the pulvinus to the tip of the middle lobe to the width from the pulvinus to the sinus between the middle and the first lateral lobe on a sample of wrinkled leaves was 2.2, and 3.8 on a sample of normal leaves from adjacent plants. The leaf lamina near primary and secondary veins of several wrinkled leaves had small areas of tissue with a reddish color, which gave these leaves an even more distinctive appearance. The aberrant plants had an altered appearance due in part to reduced boll set; however, pollen shed appeared normal.

The original aberrant-leaf plant from 1982 Breeding Row 748 was transplanted to a greenhouse at Phoenix, self-pollinated, and also crossed reciprocally with normal leaf 'Pima S-5' for *F<sub>1</sub>* seed to begin inheritance studies. Successive progenies from 82-748 and *F<sub>1</sub>* progenies from the crosses were grown in the field in 1983 and 1984, and scored for leaf morphology. The *F<sub>1</sub>* plants were self-pollinated for *F<sub>2</sub>* seed.

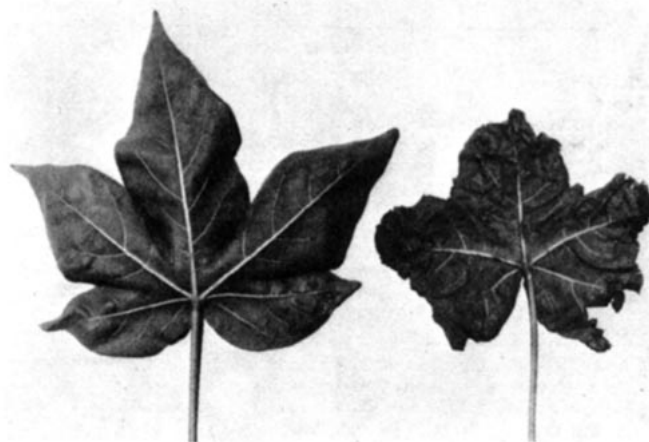


Fig. 1. Underside of normal leaf (left) and *wrinkled leaf-2* (right) of Pima cotton.

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The  $F_2$  populations from the cross of Pima S-5 with 82-748 were grown in the field in 1984, 1985, and 1986 and scored for leaf morphology. An  $F_2$  population from the reciprocal cross, 82-748 with Pima S-5, was grown in the field in 1986. The  $F_3$  lines from normal  $F_2$  plants from the cross of Pima S-5 with 82-748 were grown in 1985 and scored. In 1984,  $F_1$  plants from the cross of Pima S-5 with 82-748 were crossed, as female, with Pima S-5 and 82-748 for Backcross 1 ( $BC_1$ ) seed. These  $BC_1$  populations were grown in 1985 and scored for leaf morphology.

Crosses also were made between 82-748 and stocks with various *Gossypium* mutant genes for allelism and linkage studies. The crosses were made and progenies were grown and scored in the field in 1984, 1985, and 1986. Chi-square analyses were used for fit of observed/expected genetic ratios and for detection of linkage. Recombination values were calculated by the maximum likelihood method (1).

Field tests were conducted at the University of Arizona Maricopa Agricultural Center, Maricopa, AZ. Soil type was Mohall clay loam, a member of the fine-loamy, mixed, Hyperthermic Typic Haplargids. Conventional management practices including insect control were used in the field nurseries each year.

## RESULTS AND DISCUSSION

Plants grown from self-pollinated seed from the original aberrant leaf plant, 82-748, all expressed abnormal leaves, suggesting that penetrance was complete. The  $F_1$  plants from crossing Pima S-5 and 82-748 had normal leaves, which indicated that the aberrant leaf trait had a genetic basis and was recessive to normal leaf (Table 1).

The  $F_2$  and  $BC_1$  data showed that the trait was inherited as a monogenic recessive. The  $F_2$  populations from the cross of Pima S-5 and 82-748 were grown in 1984, 1985, and 1986. The  $F_2$  data from these years were pooled as a nonsignificant chi-square value for fit of observed/expected 3:1 ratio was obtained each year and a nonsignificant chi-square value for homogeneity also was obtained ( $\chi^2 = 0.27$ ;  $P = 0.90-0.80$ ). The  $F_2$  results were supported by a pooled  $F_2$  obtained by combining data from linkage tests that had various genetic markers as female parents and 82-748 as male parent. The  $F_2$  data from 16 linkage tests were combined based on a nonsignificant chi-square value for homogeneity of ratios ( $\chi^2 = 18.72$ ;  $df = 15$ ;  $P = 0.30-0.20$ ). A deficiency of plants with the recessive phenotype was obtained (Table 1), due primarily to segregations observed in 2 of the 16  $F_2$  populations. Deleting those populations from the pooled totals gave nonsignificant chi-square values for fit of observed/expected 3:1 ratio ( $\chi^2 = 1.06$ ;  $P = 0.50-0.30$ ) and for

**Table 1. The  $F_1$ ,  $F_2$ , and backcross data from crosses involving a Pima cotton aberrant leaf trait, 82-748, and Pima S-5.**

Parent		Generation	No. of plants		Ratio tested	$\chi^2$	$P$
Female	Male		Normal	Mutant			
Pima S-5	82-748	$F_1$	78	0			
82-748	Pima S-5	$F_1$	18	0			
Pima S-5	82-748	$F_2$	399	132	3:1	0.01	0.95-0.90
82-748	Pima S-5	$F_2$	83	38	3:1	2.65	0.20-0.10
Various genetic markers (Pima S-5 $\times$ 82-748)	82-748	$F_2$ pooled	1126	332	3:1	3.86	0.05-0.02
(Pima S-5 $\times$ 82-748)	Pima S-5	$BC_1$	106	0			
(Pima S-5 $\times$ 82-748)	82-748	$BC_1$	45	58	1:1	1.64	0.30-0.20

homogeneity of ratios ( $\chi^2 = 10.93$ ;  $df = 13$ ;  $P = 0.70-0.50$ ).

The  $F_3$  progenies from 11 normal  $F_2$  plants from the cross of Pima S-5 and 82-748 were grown and scored for leaf morphology. Nine  $F_3$  progenies segregated 3 normal/1 aberrant leaf plants ( $\chi^2$  for homogeneity of ratios = 13.43;  $P = 0.10-0.05$ ). Two  $F_3$  progenies had all normal leaf plants. One  $F_3$  progeny from an aberrant leaf  $F_2$  plant was grown and it contained 47 aberrant leaf and no normal leaf plants. These  $F_3$  data reinforce the conclusion that the aberrant leaf trait is inherited as a monogenic recessive. This trait probably arose by spontaneous mutation in a progenitor plant of Breeding Row 82-748.

The leaf phenotype of 82-748 resembles, but is not identical with, the *Gossypium* leaf morphology dominant mutant *rugate leaf*, and recessive mutants *crinkle dwarf* and *wrinkled leaf*. Allelism tests between 82-748 and the three described leaf traits were negative as evidenced by normal leaf plants occurring in each  $F_2$  population (Table 2).

Linkage tests of the aberrant leaf trait and 22 *Gossypium* mutant genes, 12 allotetraploid linkage groups and eight independent genes, were tested (Table 3). Chi-square values for detection of linkage were all nonsignificant. These results indicate that the recessive gene conditioning the aberrant leaf phenotype of 82-

**Table 2. The  $F_2$  data from crosses involving Pima cotton aberrant leaf trait 82-748 and the *Gossypium* mutant genes *rugate leaf* (*Ru*), *crinkle dwarf* (*cr*), and *wrinkled leaf* (*wr*).**

Parent		No. of plants		Ratio tested	$\chi^2$	$P$
Female	Male	Normal	Mutant			
82-748	<i>Ru Ru</i>	23	89	3:13	0.23	0.70-0.50
82-748	<i>cr cr</i>	110	76	9:7	0.63	0.50-0.30
<i>wr wr</i>	82-748	75	48	9:7	1.12	0.30-0.20

**Table 3. Linkage tests via testcrosses between a Pima cotton aberrant leaf trait, 82-748, and 22 *Gossypium* mutant genes.†**

Gene symbol	Linkage groups†	No. of plants				$\chi^2$ linkage	Recombination percentage
		AB	Ab	aB	ab		
<i>Gv</i>		55	18	22	9	0.27	46.75
<i>L<sub>1</sub><sup>L</sup></i>	VII	40	6	22	3	0.45	56.85
<i>L<sub>2</sub><sup>2</sup></i>	II	52	18	34	11	0.02	50.79
<i>Lc<sub>1</sub></i>	I	46	20	9	6	0.23	46.19
<i>M<sub>8</sub>, r<sub>2</sub>†</i>		66	79	82	64	3.30	55.33
<i>R<sub>2</sub><sup>V</sup></i>	I	45	7	13	4	0.71	41.03
<i>Ru</i>	XVII	74	11	23	4	0.06	47.43
<i>T<sub>1</sub></i>	IV	45	11	21	7	0.19	46.92
<i>fg</i>	VI	57	16	22	2	1.74	56.87
<i>gl<sub>1</sub></i>		42	9	17	4	0.00	50.00
<i>gl<sub>2</sub>, gl<sub>3</sub></i>	V, IX	62	15	7	3	1.39	57.50
<i>ml</i>	VIII	47	6	7	2	1.21	37.87
<i>p<sub>1</sub></i>	XI	64	22	15	5	0.82	49.44
<i>p<sub>2</sub></i>		58	15	21	6	0.02	51.04
<i>pm-2§</i>		67	25	17	5	0.19	46.37
<i>rl<sub>1</sub></i>	X	67	22	19	6	0.00	49.52
<i>rs</i>		73	25	17	4	0.27	45.14
<i>v<sub>1</sub></i>		30	13	8	7	1.72	59.51
<i>vf</i>	II	42	10	7	4	1.29	62.41
<i>wr</i>		75	20	24	4	0.40	44.17
<i>y<sub>1</sub></i>	XII	64	26	16	4	0.75	42.64

† Name, linkage group, and reference of cotton mutants given in Endrizzi et al. (2).

‡ Backcross data.

§ *pm-2* is an undescribed, monogenic recessive, virescent plant color trait in Pima cotton.

748 is inherited independently of the 22 mutant genes tested, or is beyond the limits of detection by the test used if on the same chromosome.

In view of the similarity of 82-748 and 'Pima S-4' *wrinkled leaf*(7), the name *wrinkled leaf-2* and the gene symbol  $wr_2$  (5) are proposed for the new monogenic recessive trait in Pima cotton.

*Wrinkled leaf-2* is an addition to the group of independent, simply inherited genes in *Gossypium* that are available for the study of genetic systems in cotton. One function may be to serve as an easily scored phenotype that expresses modified leaf morphology apparently resulting from differential growth of leaf vein and leaf lamina tissue. Noteworthy is the fact that expression of the phenotype begins with the onset of development of the sympodial branches and, once begun, persists throughout the life of the plant.

The recurrence of most spontaneous mutants in *Gossypium* is rare. Thus, plants with a *wrinkled leaf*

2 phenotype would be expected to appear infrequently as an off-type in breeding nurseries and commercial fields of Pima cotton.

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