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A Dominant Round Leaf Mutant in Cotton[1]

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ABSTRACT

In 1966, R. H. Dilday found a mutant cotton (*Gossypium hirsutum* L.) plant showing a combination of the characteristics of the marker mutants round leaf (*rl*), frego bract (*fg*), strap leaf (*s*), Smooth leaf loci (Sm_1,Sm_2, Sm_3), and open bud (*ob*). Inheritance and linkage tests showed that the mutation was conditioned by a dominant gene. In the homozygous state the new mutant is functionally lethal, and therefore it must be maintained as a heterozygote. Linkage associations were not found between the new mutant and 17 marker loci tested. However, the glabrous and abnormal bract expressions of the new mutant were epistatic to the expressions of the Pilose (H_2) and frego bract (*fg*) markers. It is proposed that this new mutant be named Round leaf-2 and assigned the gene symbols Rl_2Rl_2.

Additional index words: **Functional lethality, Epistasis, Linkage, *Gossypium hirsutum* L.**

IN 1966, R. H. Dilday found an aberrant cotton (*Gossypium hirsutum* L.) plant in B. A. Waddle's breeding nursery at Nodena, Arkansas. This plant was found in an F_3 progeny of a cross between a breeding line and a Yugoslavian introduction. It was determined that the abnormal phenotype was heritable, and self-fertilized seeds of this plant were sent to College Station, Texas, for genetic analysis. The mutant type was provisionally named "Yugo" at Arkansas, but in subsequent investigations at College Station it was assigned the working designation Arkansas-2 (Ark-2). Dilday et al. (1975) showed that Ark-2 is not an allele of any of the developmental leaf mutants crinkled leaf (*cr*), strap leaf (*s*), or veins-fused (*vf*) located on chromosome 15. This paper reports the results of inheritance and linkage tests on the Arkansas-2 mutant.

MATERIALS AND METHODS

Inheritance of Ark-2 was tested by classifying segregating backcross, testcross, and F_2 populations. The inbred tester line, Texas Marker-1 (TM-1) (Kohel, et al., 1970), was used as the normal stock both in inheritance studies and in phenotypic comparisons with Ark-2.

Linkage analyses were conducted with 16 published and one unpublished monogenic markers. The linkage tests included two multiple-marker lines, Texas 582 (T582) and Texas 586 (T586) (Kohel, 1972). Multiple recessive marker T582 carries cluster (cl_1), frego bract (*fg*), glandless boll (gl_1), cup leaf (*cu*), and virescent-1 (v_1). Multiple dominant marker T586 carries Petal spot (R_2), Brown lint-1 (Lc_1), Okra leaf (L^o), Red plant (R_1), Pilose (H_2), Naked seed (N_1), Pollen color (P_1), and Yellow petals (Y_1). The remaining three test mutants, which are recessives, were present in three separate lines: round leaf (*rl*) (Brown and Cotton, 1937), strap leaf (*s*) (Dilday and Waddle, 1974), and Ark-1, unpublished).

Seeds were germinated individually in peat pellets in a greenhouse and transplanted to field rows with 46-cm spacing between plants in 1-m wide rows. Seedlings were classified for mutant expression initially in the greenhouse and throughout the growing season in the field.

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Table 1. Segregation of Ark-2 mutant in F_1, backcross, and F_2 populations.

Populations	Segregation by class: Normal	Mutant	Semi-lethal	Total	χ^2 (ratio tested)
	No. of plants				
F_1					(1:1)
(Ark-2 × TM-1)	120	115	--	235	0.01
Backcross to nonmutant parent and testcross					(1:1)
(Ark-2 × TM-1) × TM-1	52	37	--	89	2.53
(Ark-2 × T586) × TM-1	46	48	--	94	0.04
(Ark-2 × T582) × T582	30	40	--	70	1.43
(Ark-2 × Ark-1) × Ark-1	57	63	--	120	0.30
(Ark-2 × ss) × TM-1	55	41	--	96	2.04
Total	240	229	--	469	0.26
Heterogeneity					6.08
F_2					(1:2:1)
(Ark-2 × r1r1)	21	47	29	97	1.41
(TM-1 × Ark-2)	23	60	34	117	2.14
(TM-1 × Ark-2)	33	55	31	119	0.75
Total	77	162	94	333	1.98
Heterogeneity					2.33

Description of the Mutant

Arkansas-2 plants have several modified morphological features, the most striking of which is the shape of the leaves. The leaf edges roll slightly downward; the leaf lobes are shorter, more rounded or blunted, and sometimes eliminated completely, which gives the leaf a rounded or obtuse appearance; the entire leaf has a leathery texture and prominent yellowish veins. The vein effect is most easily seen in young leaves. The plant (leaves, petioles, and stems) is almost devoid of hairs, and those few that are present are reduced in length. Plants are upright, are of medium stature, and have shorter than normal internodes. These characteristics are expressed individually in similar fashion by the recessive mutants round leaf (*rl*) (Brown and Cotton, 1937), strap leaf (*s*) (Dilday and Waddle, 1974), and Smooth leaf loci (*Sm*$_1$,*Sm*$_2$, *Sm*$_3$) (Lee, 1968). Mutant plants have slightly narrow bracts, rolled laterally towards the bud or boll. This effect is similar but not as pronounced as that of frego bract (*fg*) (Green, 1955). Floral buds have the stigmal apex and anthers exposed as in open bud (*ob*) (Kohel, 1973b). Ark-2 thus combines the specific expression of round-leaf (*rl*), strap-leaf (*s*), Smooth-leaf loci (*Sm*$_1$, *Sm*$_2$,*Sm*$_3$), frego-bract (fg), and open-bud (*ob*) mutants.

RESULTS AND DISCUSSION

Progeny of backcrosses involving Ark-2 with frego bract (*fg*) in T582, and F_2's of Ark-2 with round leaf (*rl*) each produced normal plants, indicating that Ark-2 is not an allele of these loci. Kohel (unpublished) has shown that it is not an allele of open bud (*ob*). Inheritance tests (Table 1) with selected Ark-2 mutant F_1's showed that the phenotypic expression of Ark-2 is controlled by one dominant gene. The progenies of the cross (Ark-2 × TM-1)F_1, of backcrosses with tester lines (to nonmutant parent), and of various testcrosses all segregated 1:1 (Ark-2:Normal). The F_2 populations segregated 1:2:1 (Semilethal:Ark-2:Normal).

The mutant is functionally lethal in the homozygous dominant state, and in this regard it can be compared to the Heritable Abnormalities 1, 2, and 3 of McNamara and Porter (1950). Heritable Abnormalities 1, 2, and 3 are alleles of the veins fused (*vf*), frego bract (*fg*), and strap leaf (*s*) loci, respectively (Kohel and Lewis, 1962; Kohel et al., 1965; Dilday and Waddle, 1974). In these abnormalities, as in Ark-2, a small rosetted plant with an irregular mass of buds and rudimentary leaves characterizes the homozygous mutant expression. Plants of homozygous Heritable Abnormalities 1 and 2 have little semblance of an epicotyl, but in homozygous Ark-2 plants the epicotyl elongates to about 12-15 cm, somewhat like Heritable Abnormality 3. With the exception of one plant observed in 1974, homozygous Ark-2 plants did not produce flowers under greenhouse or field conditions. The exception was a seedling that had a damaged terminal (topped) and an axillary branch growing below the break developed one open-pollinated boll. The foliage on this branch resembled that on plants intermediate in development between the homozygote and heterozygote Ark-2 plants. Brown and Cotton (1937) reported that a similar phenomenon occasionally occurred in populations of round leaf plants they had grown; however, the sport branches reported on the round-leaf (*rl*) plants were normal.

Table 2. Chi-square linkage deviation of Ark-2 with mutant loci in cotton.

Marker locus	No. plants	Ratio tested	Linkage χ^2
	[(T586 × Ark-2) × TM-1]	1:1/1:1	
R_1	94		0.68
H_2†	94		0.09
L^0	94		3.45
R_2	94		0.38
P_1	94		0.68
Y_1	94		1.06
Lc_1	94		2.09
N_1	94		0.58
Lg	94		0.38
	[(Ark-2 × T582) × T582]BC_1	1:1/1:1	
gl_1	70		0.91
fg†	69		3.26
v_1	70		2.80
cl_1	69		0.71
cu	70		2.80
	(Ark-2 × r1r1)F_2	2:1/3:1	
*r*1	68		2.67
	[(ss × Ark-2) × TM-1]	1:1/1:1	
s	96		3.38
	[(Ark-1 × Ark-2) × Ark-1]	1:1/1:1	
Ark-1	60		0.07

† H_2 and *fg* epistatic to Ark-1 (see Tables 3 and 4).

The linkage tests showed that Ark-2 is independent of the 17 markers with which it was tested (Table 2). In addition, it may be extrapolated that Smooth leaf-2 (*Sm*$_2$) is not an allele or linked with Ark-2, as Ark-2 was not found linked with Pilose (*H*$_2$). Pilose (*H*$_2$) has been found to be allelic with Smooth leaf-2 (*Sm*$_2$) (Endrizzi, personal communication). However, Ark-2 was found to be epistatic to the Pilose (*H*$_2$) and frego bract (*fg*) markers.

The glabrous condition of Ark-2 was epistatic to the expression of the dominant marker gene Pilose (H_2) (Table 3). In the testcross (Ark-2 × T586) × TM-1, Pilose did not segregate as expected. The Ark-2 /Pilose phenotypic class was missing, and the corresponding Ark-2/ (glabrous) class was about double the

Table 3. Testcross [(Ark-2 × T586) × TM-1] segregation, chi-square analysis, and recombination percentage of Ark-2 with Pilose (H_2) in cotton.

	Classification					χ^2 analysis		
	Ark-2/Pilose	Ark-2/Glabrous	Normal/Pilose	Normal/Glabrous	Total	Source	χ^2	P
	no. of plants							
Observed	0	48	22	24	94	Ark-2 vs. Normal	0.04	0.30-0.70
Expected	23.5	23.5	23.5	23.5	94	H_2 vs. h_2 linkage confounded (non Ark-2 phenotype, 46 plants)	0.09	0.30-0.50
Recombination = 52.2%		$s_p = \pm 5.2$						

Table 4. Backcross [(Ark-2 × T582) × T582] segregation, chi-square analysis, and recombination percentage of Ark-2 with frego bract (*fg*) in cotton.

	Classification					χ^2 analysis		
	Ark-2/Fg+fg	Ark-2/fg fg	Normal/Fg+fg	Normal/fg+fg	Total	Source	χ^2	P
	no. of plants							
Observed	0	40	11	18	69	Ark-2 vs. Normal	1.75	0.10-0.20
Observed (w/leaf interaction)	24	16	11	18	69	fg:Fg	0.01	0.90-0.95
Expected	17.25	17.25	17.25	17.25	69	Linkage	3.26	0.05-0.10
Recombination = 39.1%		$s_p = \pm 5.9$	17.25					

expected number. Both of the remaining non-Ark-2 classes segregated as expected. Thus, the test cross produced 3 classes of plants in the ratio 2:1:1 instead of the expected 4 classes in the ratio 1:1:1:1. The Ark-2/Pilose class was suppressed or "hidden" in what was classified as the Ark-2/ (glabrous) class. Thus, deviations due to linkage were computed from the two normal classes, which resulted in confounding of the Pilose segregation and linkage.

In the backcross (Ark-2 × T582) × T582, the homozygous recessive marker frego bract (*fgfg*) in T582 was indistinguishable from the aberrant bract caused by the Ark-2 mutant plus a single dose of frego Ark-2/ (Fg^++fg) (Table 4). The Ark-2/ (Fg^++fg) phenotype class was confounded with the Ark-2/ (*fgfg*) phenotypic class, because all Ark-2 plants also had a frego bract expression. However, the combination of Ark-2 and homozygous frego bract (*fgfg*) interacted to cause a leaf phenotype similar to the expression of the veins fused (*vf*) mutant. This extreme phenotype allowed the reclassification for frego bract based on the leaf interaction. Twenty-four of the 40 plants expressed the Ark-2 character in its recognizable form, and 16 expressed what was considered an extreme form of the mutant. These data suggest the possibility of loose linkage (RC = 39%). However, the small population and the interaction of Ark-2 and *fg* suggest the need to test potentional linkage between these two loci further.

In summary, the Ark-2 mutant is conditioned by a single dominant gene, and plants homozygous for this gene are severely stunted. Because throughout the growing season the round-leaf expression of Ark-2 is its most prolonged and prominent feature, we propose that this new mutant be named Round leaf-2 and assigned the gene symbol Rl_2 for the single locus (Kohel, 1973a).

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