

descent light and the greenhouse temperature was maintained at 15 to 24°C.

### Results and Discussion

The modified one-quarter strength Hoagland and Arnon solution appeared to be the most suitable concentration for culture of Kentucky bluegrass in this system. When higher concentrations were used, a ring of salt crystals formed around the base of the sheath causing injury to the tissue and sometimes death of the plants. Increasing iron to 40.3  $\mu\text{M}$  corrected a chlorosis problem associated with the use of one-quarter strength Hoagland solution.

During the 7 day period between replacements, evapotranspiration caused approximately a 1.0 cm lowering of the solution level. However, one of the assets of this system is that the floating flats maintain a constant plant-solution contact at all times. The evapotranspiration, in addition to removal of nutrients by the plants, caused some change in the nutrient concentrations. However, the pH of the solution remained constant at  $6.0 \pm 0.1$  throughout. The conductivity of the solution decreased somewhat over time, reflecting the removal of nutrients, but the change was minimal (approximately 10 percent). Therefore it was concluded that changes in the solution concentration during the week period was not great enough to adversely affect the plants.

The method of solution aeration apparently provided a sufficient dissolved oxygen level as vigorous root growth occurred. Within one month after transplanting, the majority of the root mass had grown through the paper and was suspended in the solution

below. Even though the paper had disintegrated by this time, the roots held the sand cartridge intact within the individual cells.

With the exception of powdery mildew, (*Erysiphe graminis* D.C.), no other disease incidence was observed. Under a 14-h photoperiod, more severe powdery mildew infestation occurred. However, switching to a 16-h photoperiod reduced the incidence of powdery mildew. Foliar application of cyclohexamide (3-[2-(3,5 dimethyl-2-oxocyclohexyl)-2-hydroxyethyl] glutarimide) or benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbonate] at label-recommended rates provided excellent disease control. When the nutrient solution was exposed to light, algal blooms occurred. However, light exclusion by close packing of flats and spacers eliminated algal contamination.

After removing plants from the flats, the roots were easily cleaned by dipping in a container of water which allowed the sand to wash off leaving clean and intact tissue. The hydroponic system described above has been used successfully to maintain healthy Kentucky bluegrass plants for periods of over 1 year. Therefore, the large-scale propagation and maintenance of plants for subsequent physiological experiments has been accomplished while deriving the benefits of hydroponic culture.

### References

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## LINKAGE ANALYSIS OF THE MALE-FERTILITY RESTORER GENE, *Rf*, IN COTTON<sup>1</sup>

R. J. KOHEL, J. E. QUISENBERRY,  
AND R. E. DILBECK<sup>2</sup>

### Abstract

The gene *Rf* partially restores male fertility to upland cottons, *Gossypium hirsutum* L., that have the cytoplasm of the wild diploid species *G. harknessii* Brandg. The *Rf* gene is linked with the deleterious Cracked root gene, *Rc*. The chromosomal location and additional linkage associations of *Rf* have not been identified. Knowledge of such associations could be useful in the transference and manipulation of the *Rf* gene. In this study we tested 13 genetic marker loci distributed on at least nine chromosomes, for possible linkage with *Rf*. No such associations were detected.

**Additional index words:** *Gossypium hirsutum*, *G. barbadense*, *G. harknessii*, Cytoplasmic male sterility, Genetic markers.

MEYER (1973,1975,1980) induced cytoplasmic male sterility (CMS) in cotton (*Gossypium hirsutum* L.) by transferring the cytoplasm of the wild diploid cotton (*G. harknessii* Brandg.) to the tetraploid species. The *G. harknessii* cytoplasm conferred complete male sterility to the cultivated cotton. Restoration of male fertility has been more of a problem.

Table 1. Name, gene symbol, chromosome association, and linkage group of cotton mutants in T582 and T586 testers (Endrizzi et al., 1984).

Name	Gene symbol	Chromosome	Linkage group
Brown lint	<i>Lc</i> <sub>1</sub>	7	I
Petal spot	<i>R</i> <sub>2</sub>	7	I
Green lint	<i>Lg</i>	15	II
Okra leaf	<i>L</i> <sub>2</sub>	15	II
cluster fruiting	<i>cl</i> <sub>1</sub>	16	III
Red plant	<i>R</i> <sub>1</sub>	16	III
Pilose	<i>H</i> <sub>2</sub>	6	IV
frego bract	<i>fg</i>	3	VI
Yellow pollen	<i>P</i> <sub>1</sub>	5	XI
Yellow petals	<i>Y</i> <sub>1</sub>	A	XII
Naked seed	<i>N</i> <sub>1</sub>	12	XIII(V)
virescent	<i>v</i> <sub>1</sub>	20	XVII
cup leaf	<i>cu</i>	--	--
glandless boll & stem	<i>gl</i> <sub>1</sub>	--	--

A restorer gene from *G. harknessii*, *Rf*, was described as partially dominant (Weaver and Weaver, 1977; Weaver and Weaver, 1979). Genetic modifiers were required to restore complete male fertility, and *G. barbadense* L. was identified as a source for such modifier alleles.

<sup>1</sup> Contribution from USDA-ARS, College Station, and Lubbock, TX, in cooperation with the Texas Agric. Exp. Stn. Received 13 Feb. 1984.

<sup>2</sup> Research geneticists and agronomist, USDA-ARS, College Station, TX 77841 and Lubbock, TX 79401.

**Table 2. Analyses of backcross populations grown to study linkage of *Rf* and the genetic markers of T582 and T586 in cotton.**

Genotype	Segregation no. of plants	Chi-square analysis†	
		Source	$\chi^2$
<i>Rfrf h<sub>2</sub>h<sub>2</sub></i>	65	<i>Rf</i> vs. <i>rf</i>	0.51
<i>Rfrf H<sub>2</sub>h<sub>2</sub></i>	71	<i>H<sub>2</sub></i> vs. <i>h<sub>2</sub></i>	0.06
<i>rfrf h<sub>2</sub>h<sub>2</sub></i>	79	Linkage	0.90
<i>rfrf H<sub>2</sub>h<sub>2</sub></i>	69		
	284	Recombination percent 52.82	
<i>Rfrf r<sub>1</sub>r<sub>1</sub></i>	69	<i>Rf</i> vs. <i>rf</i>	0.51
<i>Rfrf R<sub>1</sub>r<sub>1</sub></i>	67	<i>R<sub>1</sub></i> vs. <i>r<sub>1</sub></i>	0.22
<i>rfrf r<sub>1</sub>r<sub>1</sub></i>	77	Linkage	0.06
<i>rfrf R<sub>1</sub>r<sub>1</sub></i>	71		
	284	Recombination percent 50.70	
<i>Rfrf l<sub>1</sub>l<sub>1</sub></i>	66	<i>Rf</i> vs. <i>rf</i>	0.51
<i>Rfrf L<sub>1</sub>l<sub>1</sub></i>	70	<i>L<sub>1</sub></i> vs. <i>l<sub>1</sub></i>	0.13
<i>rfrf l<sub>1</sub>l<sub>1</sub></i>	73	Linkage	0.01
<i>rfrf L<sub>1</sub>l<sub>1</sub></i>	75		
	284	Recombination percent 50.35	
<i>Rfrf r<sub>2</sub>r<sub>2</sub></i>	67	<i>Rf</i> vs. <i>rf</i>	0.51
<i>Rfrf R<sub>2</sub>r<sub>2</sub></i>	69	<i>R<sub>2</sub></i> vs. <i>r<sub>2</sub></i>	0.06
<i>rfrf r<sub>2</sub>r<sub>2</sub></i>	77	Linkage	0.23
<i>rfrf R<sub>2</sub>r<sub>2</sub></i>	71		
	284	Recombination percent 51.41	
<i>Rfrf y<sub>1</sub>y<sub>1</sub></i>	67	<i>Rf</i> vs. <i>rf</i>	0.51
<i>Rfrf Y<sub>1</sub>y<sub>1</sub></i>	69	<i>Y<sub>1</sub></i> vs. <i>y<sub>1</sub></i>	0.06
<i>rfrf y<sub>1</sub>y<sub>1</sub></i>	73	Linkage	0.00
<i>rfrf Y<sub>1</sub>y<sub>1</sub></i>	75		
	284	Recombination percent 50.00	
<i>Rfrf lc<sub>1</sub>lc<sub>1</sub></i>	69	<i>Rf</i> vs. <i>rf</i>	0.51
<i>Rfrf Lc<sub>1</sub>lc<sub>1</sub></i>	67	<i>Lc<sub>1</sub></i> vs. <i>lc<sub>1</sub></i>	1.14
<i>rfrf lc<sub>1</sub>lc<sub>1</sub></i>	82	Linkage	0.69
<i>rfrf Lc<sub>1</sub>lc<sub>1</sub></i>	66		
	284	Recombination percent 52.46	
<i>Rfrf n<sub>1</sub>n<sub>1</sub></i>	68	<i>Rf</i> vs. <i>rf</i>	0.51
<i>Rfrf N<sub>1</sub>n<sub>1</sub></i>	68	<i>N<sub>1</sub></i> vs. <i>n<sub>1</sub></i>	1.70
<i>rfrf n<sub>1</sub>n<sub>1</sub></i>	85	Linkage	1.70
<i>rfrf N<sub>1</sub>n<sub>1</sub></i>	63		
	284	Recombination percent 53.87	
<i>Rfrf lglg</i>	48	<i>Rf</i> vs. <i>rf</i>	0.00
<i>Rfrf Lglg</i>	73	<i>Lg</i> vs. <i>lg</i>	7.67**
<i>rfrf lglg</i>	51	Linkage	0.20
<i>rfrf Lglg</i>	69		
	241	Recombination percent 51.45	
<i>Rfrf V<sub>1</sub>v<sub>1</sub></i>	70	<i>Rf</i> vs. <i>rf</i>	1.73
<i>Rfrf v<sub>1</sub>v<sub>1</sub></i>	71	<i>V<sub>1</sub></i> vs. <i>v<sub>1</sub></i>	0.16
<i>rfrf V<sub>1</sub>v<sub>1</sub></i>	79	Linkage	0.08
<i>rfrf v<sub>1</sub>v<sub>1</sub></i>	85		
	305	Recombination percent 49.18	
<i>Rfrf Cucu</i>	73	<i>Rf</i> vs. <i>rf</i>	1.73
<i>Rfrf cucu</i>	68	<i>Cu</i> vs. <i>cu</i>	0.08
<i>rfrf Cucu</i>	77	Linkage	0.74
<i>rfrf cucu</i>	87		
	305	Recombination percent 47.54	
<i>Rfrf Gl<sub>1</sub>gl<sub>1</sub></i>	80	<i>Rf</i> vs. <i>rf</i>	1.73
<i>Rfrf gl<sub>1</sub>gl<sub>1</sub></i>	61	<i>Gl<sub>1</sub></i> vs. <i>gl<sub>1</sub></i>	1.44
<i>rfrf Gl<sub>1</sub>gl<sub>1</sub></i>	83	Linkage	0.95
<i>rfrf gl<sub>1</sub>gl<sub>1</sub></i>	81		
	305	Recombination percent 47.21	
<i>Rfrf Cl<sub>1</sub>cl<sub>1</sub></i>	80	<i>Rf</i> vs. <i>rf</i>	1.73
<i>Rfrf cl<sub>1</sub>cl<sub>1</sub></i>	61	<i>Cl<sub>1</sub></i> vs. <i>cl<sub>1</sub></i>	3.15
<i>rfrf Cl<sub>1</sub>cl<sub>1</sub></i>	88	Linkage	0.16
<i>rfrf cl<sub>1</sub>cl<sub>1</sub></i>	76		
	305	Recombination percent 48.85	
<i>Rfrf Fgfg</i>	90	<i>Rf</i> vs. <i>rf</i>	1.73
<i>Rfrf fgfg</i>	51	<i>Fg</i> vs. <i>fg</i>	12.20**
<i>rfrf Fgfg</i>	93	Linkage	0.95
<i>rfrf fgfg</i>	71		
	305	Recombination percent 47.21	

† Chi-square values for  $P = 0.05$  and  $0.01$ ,  $ldf$ , are 3.84 and 6.64, respectively.

Weaver and Weaver (1979) documented the linkage of Cracked root, *Rc* with *Rf*. Knowledge of additional linkages with *Rf* could be useful for manipulating the *Rf* gene, inasmuch as the *Rc* gene is seriously debilitating and thus cannot be used in plant improvement programs. This paper reports the linkage analyses of *Rf* with 13 genetic marker loci.

### Materials and Methods

The restorer line, DES HAF 277, contributed the genes *Rf/Rf* and the male-sterile cytoplasm from *G. harknessii*. The multiple marker tester lines used were: i) the multiple recessive line T582 (*cu*, *fg*, *cl<sub>1</sub>*, *gl<sub>1</sub>*, and *v<sub>1</sub>*) and ii) the multiple dominant line T586 (*R<sub>2</sub>*, *Lc<sub>1</sub>*, *L<sub>2</sub>*, *R<sub>1</sub>*, *H<sub>2</sub>*, *Y<sub>1</sub>*, *N<sub>1</sub>*, *Lg*, and *P<sub>1</sub>* (Kohel, 1978). The markers used and chromosomal locations are listed in Table 1. Both tester lines were nonrestorers, and DES HAF 277 had Yellow pollen, *P<sub>1</sub>*. The male-sterile conditions precluded classification of pollen color segregation.

The initial crosses were made at Lubbock, TX with DES HAF 277 as female parent, and the  $F_1$  was used as the female parent for the backcrosses. One backcross population was produced and classified at Lubbock under a combination of field and greenhouse conditions. Subsequent crosses and scorings were performed in the Cotton Genetics Nursery at College Station, TX.

### Results and Discussion

At Lubbock, plants were scored for genetic markers in the field and transplanted to the greenhouse to verify male fertility classification. The populations at College Station were scored for all characters in the field. A minimum of five flowers per plant were classified and at no time was complete male fertility observed in the field. Anther development varied within the season, but there were two distinctly different types of anthers formed. Some plants produced flowers with small under-developed anthers, genotype presumed to be *rf rf*, and others produced large anthers that occasionally shed pollen, genotype presumed to be *Rf rf*. There was no indication that the genetic tester lines contained modifiers to increase the restoration of male fertility.

The results of the linkage analyses are presented in Table 2. There were no indications of linkage between the *Rf* locus and any of the genetic marker loci. Disturbed segregation ratios were observed for Green lint, *Lg*, cluster fruiting *cl<sub>1</sub>*, and frego bract, *fg*. We could think of no obvious reason for the deviant segregation of *Lg*. The classification of *cl<sub>1</sub>* and *fg* was somewhat obscured by the short internode and close fruiting growth habit characters of the DES HAF 277 parent. The expression of *cl<sub>1</sub>cl<sub>1</sub>* and *fg fg* phenotypes in the crosses were not typical of those noted on TM-1 background (Kohel et al., 1970).

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## DIFFERENCES IN MYCORRHIZAL COLONIZATION OF MAIZE SELECTIONS FOR HIGH AND LOW EAR LEAF PHOSPHORUS

RONALD TOTH, TERESA PAGE, AND RON CASTLEBERRY<sup>1</sup>

### Abstract

Mycorrhizal colonization may contribute to the uptake of P in crop plants, particularly under nutrient limited conditions. This study was done to evaluate the hypothesis that inbreds of maize (*Zea mays* L.) previously selected for differences in ear leaf P at silking, may differ in degree of mycorrhizal colonization. Roots of up to six inbreds (three high P and three low P) were assayed for mycorrhizae after 10 weeks growth under field conditions. A positive rank correlation was observed between ear leaf P content of the inbreds and their percent mycorrhizal colonization. The high P inbreds were more mycorrhizal (18% mean colonization) than the low P inbreds (10% mean colonization). The same six maize inbreds were also grown in the greenhouse under nonmycorrhizal conditions in 1983. Neither tops or roots showed growth or P content characteristics under nonmycorrhizal conditions which would explain the differences observed in the field in ear leaf P content or mycorrhizal colonization. These data suggest mycorrhizal colonization may be a significant component of the genetic differences expressed as ear leaf P content in these maize lines.

**Additional index words:** Genetics, *Glomus fasciculatum*, Lines.

UNDER P deficient conditions, vesicular-arbuscular (VA) mycorrhizae can cause a net increase in dry matter due to increased uptake of nutrients, primarily phosphorus (Gerdemann, 1968; Mosse, 1973). These mycorrhizae are widely distributed and occur on crop species in most agricultural soils (Gerdemann, 1968). The percent of mycorrhizal colonization increases with lower soil and root P concentrations (Menge, et al., 1978). However, the addition of P to the growth medium of a plant with mycorrhizae will not necessarily eliminate the mycorrhizal association (Abbot and Robson, 1978; Hall, et al., 1977; Porter, et al., 1978). A mechanism relating mycorrhizal colonization to membrane leakage associated with low P conditions has been proposed to account for these observations (Ratnayake, et al., 1978).

A number of factors indicate a genetic relationship between the host plant and mycorrhizae. Low P con-

ditions will not cause plants which normally do not form mycorrhizal associations to become mycorrhizal since many plants will grow under low P conditions in the presence of fungal inoculum without becoming mycorrhizal (Gerdemann, 1968). Basidiomycetes will not form mycorrhizal associations with grasses but will with diverse groups such as the Pinaceae and orchids (Gerdemann, 1968; Hayman, 1978). Different species of mycorrhizal fungi colonize a crop cultivar to varying degrees and individual species of mycorrhizal fungi colonize different crop cultivars to varying degrees (Mosse, 1973; Schenck, et al., 1975). These interactions between genotypes led to differences in yield and plant growth. Three lines of the wheat (*Triticum aestivum* L.) cultivar Centana, isogenic except for dwarfing genes, varied in their percent mycorrhizal infection, with the dwarf lines being the most mycorrhizal (Bertheau, et al., 1980).

Inbreds of maize (*Zea mays* L.) which are routinely developed for commercial use vary somewhat in their content of mineral nutrients (Gorsline, et al., 1964; Barber, et al., 1976). In addition, inbreds of maize have been developed, as part of a special study at DEKALB, which vary widely in their ear leaf (leaf subtending the uppermost ear shoot) content of N, K, and P at silking (Castleberry, et al., 1978). These lines were produced through selfing from breeding composites and breed true for the relative level of these characteristics. The ear leaf P content at silking of the low P inbreds ranged from 0.17 to 0.23% and of the high P inbreds ranged from 0.50 to 0.77% of leaf dry weight in the studies reported (Castleberry, et al., 1978). Ear leaf content of comparable unselected maize inbreds at this stage of growth was approximately 0.4%. Total P content of the above ground portion of these lines, as an indication of total P uptake, was related to ear leaf P concentration (Castleberry, et al., 1978).

The mechanism(s) controlling ear leaf P content in these inbreds is not known, although localization of P was eliminated as a possible mechanism (Castleberry, et al., 1978). Possible mechanisms include more extensive root systems or more efficient nutrient uptake and translocation (Barber, 1976). It is possible these inbreds differ in their ability to form mycorrhizal associations, the various P contents being related to differential colonization. To test this hypothesis, several high and low P inbreds were grown under field conditions and assayed for percent mycorrhizal colonization. These same inbreds were also grown under nonmycorrhizal conditions in the greenhouse and the P content and biomass of shoots and roots was determined.

## MATERIALS AND METHODS

Five replicates of two plants of each inbred were planted at DeKalb, IL in 1978 through 1981 in a randomized complete block design. The soil contained 510 mg/kg total P, 1160 mg/kg total Kjeldahl N, 80 mg/kg available K, 3000 mg/kg Ca (local soils have been heavily limed in the past), 6.5 mg/kg Zn, 35 mg/kg Fe, and had a pH of 8.0. A randomized grid system was constructed to determine planting placement of seeds of each inbred. Soil collected at the site was sieved and analyzed for spores of mycorrhizal

<sup>1</sup> Associate professor and research assistant, Dep. of Biological Sciences, Northern Illinois Univ., DeKalb, IL 60115 and corn physiologist, DeKalb-Pfizer Genetics, 3100 Sycamore Rd., DeKalb, IL 60115. Received 20 Oct. 1983.