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### **Linkage Test of Necrotic Root ( $Rn_1$ ) with Root Fluorescence ( $Fr_1$ )**

Five recessive allelic necrotic root mutants have been identified in cultivated soybean [*Glycine max* (L.) Merr.]. Four descended from germinal revertants in the *w4*-mutable line of soybean (Kosslak et al., 1996; Andersen and Palmer, 1979). The fifth necrotic root mutant was identified in family EMS-95, which was derived from EMS-treated cultivar AgriPro 1776 (Palmer and Wubben, 1998). Necrotic root is a programmed cell death mutant (Kosslak et al., 1997).

Linkage tests were done with the necrotic root mutant and phosphoglucomutase (E.C.2.7.5-1), malate dehydrogenase (E.C.1.1.1.37), aconitase (E.C.4.2.1.3), diaphorase (E.C.1.6.4.3), and isocitrate dehydrogenase (E.C.1.1.1.42) (Kosslak et al., 1996), and with seed coat peroxidase (Wubben and Palmer, 1998). The necrotic root mutant was not linked to any of the loci tested.

Our objective was to test determine if the root fluorescence  $Fr_1$  locus was linked to the necrotic root  $Rn_1$  locus.

### **Materials and Methods**

Cross pollinations were made between  $Rn_1 rn_1$  (Ames 1) T328H as female parent and Minsoy ( $fr_1 fr_1$ ) (PI 27890) as male parent. The  $Rn_1 rn_1$  heterozygous genotype plants were identified by evaluating self-pollinated progeny of wild-type plants in entries segregating normal and necrotic root plants. The  $rn_1 rn_1$  homozygous recessive plants usually die before flowering. The F1 seed were planted at the University of Puerto Rico-Iowa State University soybean nursery near Isabela.

The F2 seed were planted at the Bruner Farm near Ames, Iowa. The F2 plants were single-planted threshed and evaluated as F2:3 families. The seeds were placed on germination paper. The roots of homozygous necrotic root genotype turn brown 7 to 10 days after germination. The progenies were classified as nonsegregating or segregating normal and necrotic root.

Root fluorescence was classified while irradiating the seedling roots with ultraviolet light. The progenies were classified as nonsegregating fluorescent root, nonsegregating nonfluorescent root or segregating fluorescent and nonfluorescent root.

The F2:3 families were grouped into six phenotypic classes. The homozygous recessive necrotic root plants usually die before flowering or do not produce many seed. Thus the F2 necrotic root plants were not harvested.

## Results and Discussion

Progenies from 304 F2 plants from the cross  $Rn_1 rn_1, Fr_1 Fr_1 \times Rn_1 Rn_1 fr_1 fr_1$  were evaluated for normal/necrotic root and fluorescent/nonfluorescent root. Based upon F2:3 family segregation, the necrotic root locus,  $Rn1$  was not linked to the fluorescent root locus ( $Fr_1$ ) (Table 1). Within segregating F2:3 families, the phenotypic ratios were a good fit to the expected ratios (Table 2).

Collectively, the F2 and F2:3 data indicated that the  $Rn_1$  locus and the  $Fr_1$  locus were not linked.

## References

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Table 1. Segregation for normal and necrotic root, and fluorescent and nonfluorescent root from the cross of T328H ( $Rn_1 rn_1 Fr_1 Fr_1$ ) X Minsoy ( $Rn_1 Rn_1 fr_1 fr_1$ ). F2:3 family data.

No. F2:3 families	Phenotype	Genotype	$\chi^2(1:2:2:4:1:2)$	P
31	Normal root and fluorescent root	$Rn_1$ (Ames 1) $Fr_1$ $Rn_1$ (Ames 1) $Fr_1$	1.27	
46	Seg. necrotic root	$Rn_1$ (Ames 1) $Fr_1$ $rn_1$ (Ames 1) $Fr_1$	0.43	
49	Seg. root fluorescence	$Rn_1$ (Ames 1) $Fr_1$ $Rn_1$ (Ames 1) $fr_1$	0.06	
103	Seg. necrotic root and seg. root fluorescence	$Rn_1$ (Ames 1) $Fr_1$ $rn_1$ (Ames 1) $fr_1$	0.03	
26	Normal root and all nonfluorescent root	$Rn_1$ (Ames 1) $fr_1$ $Rn_1$ (Ames 1) $fr_1$	0.02	
49	Seg. necrotic root and all nonfluorescent root	$Rn_1$ (Ames 1) $fr_1$ $rn_1$ (Ames 1) $fr_1$	0.06	
Total	304		$\chi^2 = 1.86$	0.87

Table 2. Phenotypic ratios and number of F3 plants from the cross of T328H ( $Rn_1 rn_1 Fr_1 Fr_1$ ) X Minsoy ( $Rn_1 Rn_1 fr_1 fr_1$ ).

Phenotypic ratio within F2:3 families	F3 plant ratio	$\chi^2(3:1)$	$\chi^2(9:3:3:1)$	P
Normal root : necrotic root	512:170	0.001		0.96
Fluorescent root : nonfluorescent root	935:287	1.49		0.22
Normal root : necrotic root and fluorescent root : normal root and nonfluorescent root : necrotic root and nonfluorescent root	1122:343:378:115		2.74	0.43
Nonfluorescent root and normal root : necrotic root	773:234	1.67		0.20