

XII. APPENDIX II: CONTRIBUTED PAPERS

II - A. Rust Resistant Isolines Developed at the University of Illinois at Urbana-Champaign

Richard L. Bernard, Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL

Since the early 1980's the USDA, ARS soybean breeding program at the University of Illinois at Urbana-Champaign has been transferring genes for rust resistance from various germ plasm sources by means of backcrossing to the adapted soybean cultivar Williams 82. The objectives were: (i) to put resistance into adapted material where it could be used by midwest growers or more readily used in developing adapted cultivars (all known sources of rust resistance were unadapted for commercial production in the U.S.) and (ii) to provide useful lines for genetic and disease research.

The procedure was to make backcrosses to William 82 to the BC5 with selection for rust resistance at every one or more backcross cycles. Testing for rust resistance was done on a sample of F2 or F3 plants to identify lines carrying resistance and was done by S. Melching, Plant Pathologist at the USDA, ARS laboratory at Fort Detrick, MD. After BC5, a few true- breeding resistance lines were identified, compared for agronomic traits (appearance,

yield, maturity, etc.) and the most resembling cultivar William 82 was selected for germ plasm release.

The following have been developed to date with final testing and selection remaining to be done for three of the populations. All are BC5 Williams 82 (see table below).

LITERATURE CITED

1. Bernard, R. L., Nelson, R. L., and Cremeens, C. R. 1991. USDA Soybean Genetics Collection: Isoline Collection. Soybean Genetics Newsl. 18:27-57

Line to designation	Donor cv.	Donor PI no.	Origin of donor	Gene transferred
L85-2378*	Komata	200.492	Japan	R _{pp1}
L86-1752*	unkown	230.970	Japan	R _{pp2}
L87-0842	Bing Nan Dou	459.025	Fugian, China	R _{pp4}
L90-7874	unknown	230.971	Japan	---
inc.	wild	339.871	Jeju I.,	
S. Korea	---			
inc.	Qui Dou 1	459.024	Hunan, China	---
inc.	Ankur	462.312	Florida via India	R _{pp3}

II - B. Resistance to Soybean Rust

E. E. Hartwig, USDA, ARS, Jamie Whitten Delta States Research Center, Stoneville, MS

Soybean rust (*Phakopsora pachyrhizi*) is considered a serious disease of soybeans in southeast Asia. Although the disease had not been identified in the U.S., we considered it to be important that we have information as to whether or not we had genetic resistance to the causal organism within the soybean germ plasm collection. A planting of the southern germ plasm collection was made at Asian Vegetable Research and Development Center in Taiwan on 16 September 1975. A total of 1,675 germ plasm lines of maturity groups V through X were evaluated. The nursery was inoculated at two dates but only a moderate level of infection developed. Ten or 12 strains showed some evidence of resistance. These strains were the basis for screening work by Ken Bromfield at the USDA, ARS containment facilities at Frederick, MD. Because of the seriousness of the disease in southeast Asia and the fact that the disease had not been observed in the continental U.S. all work had to be conducted within the containment facilities at Frederick. The value of this work has increased with the identification of soybean rust in Hawaii (Plant Dis. 78:1216).

Bromfield used isolates India 73-1, Taiwan 72-1, Australia 72-1, and Philippines 77-1. PI 200492 gave an immune reaction to India 73-1 and PI 462312 (Ankur developed in India) gave a resistance reaction. Plants classified as resistant developed dark reddish brown lesions with few or no spores. Both were susceptible to all other rust isolates. PI 230970 was resistant to all of the rust isolates available at Frederick. However, it was later shown susceptible to Taiwan isolate 80-2. A more recent plant introduction from central China, PI 459025, was identified as resistant to Taiwan isolate 80-2.

As resistant or immune germ plasm was identified, it was used as parents in crosses with the cultivar Centennial. F1 plants were grown in the greenhouse at Stoneville and F2 populations were evaluated at Frederick. Because of limited space, minimum size populations were grown. In each case resistance was dominant. A portion of the resistant plants were grown to maturity for progeny testing. Four major genes for immunity or resistance have been identified.

The germ plasm line D86-8286 has been released. It is basically a cultivar Forrest type and represents a third cycle of breeding. In developing the germ plasm line D86-8286 we recognized that a major gene for seed shattering was closely linked with the gene for rust resistance.

We anticipate a germ plasm release of a second cycle line P1 459025 as a parent. We hope to have a further test at Frederick to verify resistance. It must be emphasized that the containment facilities at Frederick are limited and work involves diseases of several crop plants as well as potentially damaging weeds.

In Ted Hymowitz's work with the perennial *Glycine*, he found *Glycine tomentella* (P1 483218) was resistant to the soybean rust pathogen in Australia and Taiwan. Fertile soybean types from backcrosses to soybeans were susceptible when tested in Australia. At present 47 fertile ($2n=41$) lines are being evaluated for reaction to the soybean rust pathogen in Australia.

D86-8286 and the lines having the gene for resistance from P1 459025 will be inoculated with an isolate of the rust pathogen from Hawaii and what may be an isolate from Brazil.

LITERATURE CITED

1. Bromfield, K. R., and Hartwig, E. E. 1980. Resistance to soybean rust and mode of inheritance. *Crop Sci.* 20:254-255
2. Bromfield, K. R. Meiching, J. S., and Kingsolver, C. H. 1980. Virulence and aggressiveness of *Phakospora pachyrhizi* isolated causing soybean rust. *Phytopatholgy* 70:17-21
3. Hartwig, E. E., and Bromfield, K, R. 1983. Relationship among three genes conferring specific resistance to rust in soybeans. *Crop Sci.* 23:237-239
4. Hartwig, E. E. 1986. Identification of a forth major gene conferring resistance to soybean rust. *Crop Sci.* 26:1135-1136

II - C. Soybean Projects in Thailand (1985-1995)

Montha Nuntapunt, Chiangmai Field Crops Research Center, Maejo, Chiangmai, Thailand

Since 1985, we screened over 500 soybean lines under natural infection at Royal King Project Pangda Station. The height is about 600 meters above the sea level. Abundant and frequent precipitation with quite cool temperatures are favorable for rapid rust development, high rust severities, and downy mildew epidemics during the rainy season. Experiments start during the second week in August. The criteria used for the preliminary selection are:

- A. Resistance to downy mildew disease (no lesion).
- B. RB-type or RB- and Tan-type on the same leaf or the same plant.
- C. Less severe rust infection.
- D. Moderately severe rust infection, but still green leaves and full-pod formation at R6 growth stage.
- E. Good organic plant type, no lodging, uniform in maturity, and no shattering.

In some years, bacterial pustule occurs. We also try to select resistant lines to this bacterial disease. Later we selected lines which gave a high rating for disease resistance. They were evaluated for rate-reducing rust resistance and tolerance under fungicide or nonfungicide treatment regime. The main criteria used to determine the rust-tolerant lines or cultivars are as

above, including slow rust development and high yield.

At present we have the three rust-tolerant lines: 8517-3-4, 8523-11-2 and 8520-102-7-1. They were screened and selected since 1987. Now they are included in the soybean standard yield trials of some breeders. They gave higher yields than cultivar SJ-5 and CM-60. The cultivar CM-60 is the same as 7500-50-10 and was released in 1987.

A new group of rust-tolerant lines are SRE-C-56C, CM-60-IOKr-Y1, 8402-14, 8402-26 and the line ACIAR-BR-1-8-22 were released in 1993-94.

The management of chemicals use for rust control in the north was done during the 1989-90 season. The results showed that two applications of Triadimefon 25 WP (10 gm/20 l water) at 25 and 40 days after planting provided the best control.

Control of rust by an integrated control program was tested early and late rainy season during 1993-1995. Two cultivars, SJ-5 and CM 60, were planted. The results from the 2 years showed no difference in rust severities in either cultivar whether they were planted early or late. It is necessary to spray with Triadimefori 25 WP at 25 and 40 days after planting.

II - D. Testing for DNA Markers Associated with Rust Resistance in Soybean

Lila Vodkin, Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL

The objective of this research is to find DNA markers near genes for rust resistance. If such markers are found they would have several applications. These markers would provide a way to introgress chromosomal regions that carry rust resistance without having to screen for the disease during every phase of the backcrossing. This is especially useful since the rust pathogen cannot be tested in the continental U.S. without special containment facilities. In addition, markers near a rust resistance gene provide the first step toward mapping and isolation of the resistance genes by recombinant DNA methods. An understanding of the resistance mechanisms would aid in designing novel strategies to provide resistance to this fungal pathogen.

Background

Using short arbitrary primers, we have tested the cultivar Williams isolines in the USDA germ plasm collection that contain resistance to rust using polymerase chain reaction (PCR). Line L85-2378 ($R_{pp1} R_{ps1-k}$) was released by R. L. Bernard. It was constructed by crossing the plant introduction line, PI 200.492 as the source of the rust resistant R_{pp1} allele to cultivar Williams 82 (R_{ps1-k}) for six generations with cultivar Williams 82 as the recurrent parent and selecting for the rust resistant plants but otherwise selecting for characteristics similar to the cultivar Williams 82 recurrent parent. Likewise, isolate L86-1752 ($R_{pp2} R_{ps1-k}$) was created with cultivar Williams 82 (R_{ps1-k}) as the recurrent parent and PI 230-970 as the source of R_{pp2} resistance. We extracted DNA from cultivar Williams 82 and rust resistant L85-2378 and L86-1752 and subjected the DNA samples to the RAPD procedure (random amplification of polymorphic DNA) using 10 basepair primers. One primer of

over 150 that were tested showed a polymorphism between the L85-2378 line and cultivar Williams 82 recurrent parent. This DNA marker may be located near the R_{pp1} resistance gene.

Proposed research

The focus of the proposed research is to determine if the RAPD marker that we have found is located near the R_{pp1} gene. The isolates were made in the 1980's. We have recently reconstructed crosses between the two lines to derive F_2 and F_3 lines. We have shown that the RAPD marker is segregating in the F_2 individuals (Fasoula and Vodkin, unpublished). Because we cannot test for the rust pathogen in Illinois, we propose to have these lines tested for resistance in the USDA, ARS facility in Frederick, MD, if a collaborator is found.

A sample of the isolines will first be tested with various strains of the rust pathogen to define the resistant and susceptibility response. The F_3 lines will then be tested to determine whether they are homozygous for resistance, segregating, or homozygous for susceptibility to the pathogen. These results will then be correlated with the RAPD data that will be obtained on the F_3 lines at UIUC. In this manner, we will determine whether the RAPD marker is linked to the resistance gene.

In addition, we are cloning the RAPD marker and will determine whether this marker can be converted to a RFLP (resistance fragment length marker). This would provide an additional way to screen for the resistance gene. The RAPD marker indicated the presence or absence of a DNA band; and thus heterozygotes cannot be determined from the homozygous dominant condition. RFLP markers are more often codominant and if so they provide a way to distinguish the heterozygous condition.