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Selection for Resistance in *Phaseolus vulgaris* L. to White Mold Disease Caused by *Sclerotinia sclerotiorum* (Lib.) de Bary

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Abstract. Three populations of beans were screened for resistance to white mold using a mycelium/juvenile stem inoculation (JSI) method on 3-week-old plants and an ascospore/blossom test on plants in bloom was used to test resistance of survivors of the JSI test. There were few survivors in the JSI test: 0.8% in the 4-way cross of susceptible x susceptible, 2% in the intermediate x intermediate crosses and 3.8% in 10 P. coccineus lines with intermediate resistance. JSI tests of the progeny produced more survivors, 17% from the first 2 populations. There was good agreement between the JSI test on juvenile plants and the ascospore/blossom tests on blossoming plants respectively. The JSI test appears to be an efficient method with which to identify individual plants with moderate resistance.

White mold disease of beans has been neglected by bean breeders until recently. In 1973 Adams et al. (2) identified some *Phaseolus coccineus* lines with resistance. Abawi et al. (1) reported resistance in *P. vulgaris*, derived crosses with *P. coccineus*. Most *P. vulgaris* accessions were relatively susceptible. In field trials *P. vulgaris* cultivars Anderson et al. (3), Coyne et

Abawi et al. (1) used blooming plants that were sprayed with a suspension of ascospores. Hunter et al. (8) modified Abawi's ascospore-blossom inoculation (ABI) method (1). They also developed a second procedure, called limited term inoculation (8) which is referred to as juvenile stem inoculation (JSI) in this paper.

The high reliability of JSI and ABI (8) screening methods made possible the screening of many accessions of *Phaseolus* spp. (7), which led to the identification of a number of plant introductions with varying levels of resistance.

al. (5) and Schwartz et al. (10) reported some resistance which could be attributed to avoidance due to plant growth habit in some cases and resistance in others.

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When we initiated this study we had no sources of resistance that were very promising. Therefore, we decided to use Krupinsky and Sharp's (9) approach using large populations and selection over several generations to determine if minor-gene resistance could be accumulated additively from horticulturally acceptable beans. The use of commercially acceptable cultivars as parents would reduce the undesirable traits usually associated with plant introductions, which now appear to be the best alternate source of resistance. We intercrossed susceptible x susceptible lines and susceptible x slightly resistant lines to test this approach. In addition we intercrossed P. coccineus x P. coccineus with the highest levels of tolerance we had been able to identify at the initiation of this project.

We report herein the results for the F_3 generation and performance of F_4 generation plants from F_3 survivors of white mold tests. The basis of the study presumed very low intitial survival and could not be designed for a genetic analysis. However, Krupinsky et al. (9), using the same approach, were able to accumulate minor genes for resistance, eventually obtaining a high level of resistance.

Methods and Procedures

Vigorous 3-week-old seedlings grown at 20°C under metal halide lights $(2,800~\mu\text{Em}^{-2}\text{s}^{-1})$, were used for the JSI method. This avoided the increased susceptibility of plants grown under low intensity winter light. Colonized canned bean pods were wrapped around the stem above the cotyledonary node (8) when the first trifoliolate leaf was almost fully expanded. The plants were then placed in a mist chamber for 44 hr at $20^{\circ} \pm 3^{\circ}$ and the moisture was adjusted to maintain dampness but avoiding runoff. With the modified ABI method, plants were grown in the greenhouse until full bloom when a suspension of 2000 spores/ml was sprayed on 3 blossoms. Each blossom was then placed in the leaf axil of the three youngest nodes with fully expanded trifoliolates. The inoculated plants were placed in a mist chamber for 6 or 7 days at $20^{\circ} \pm 3^{\circ}$. The mist was adjusted to give a heavy fog that filled the chamber for a few seconds every 6 minutes.

Three segregating populations were screened for white mold resistance: Population 1 involved 19 F₂ populations of four-way crosses of 10 lines identified as having little or no resistance. The lines were 'State Half Runner' (SHR), 'Wisconsin RR83' (W83), OSU 1604, 'G8953' (obtained from P. Pryke, Melbourne, Australia as 102) 'Bush Blue Lake 274' (BBL 274), 'Early Gallatin' (EG), G 767, 76B1, 'Black Valentine' (BV) and 'Early Wax' (EW).

Population 2 was produced from eight combinations of lines (intermediate x intermediate resistance, and intermediate x susceptible). The parental lines with intermediate resistance, based on prior tests, were: 2821-3, 2823-1 (from crosses of *P. coccineus* B3749 x *P. vulgaris*), 76B1 and 6985 (from Norvell X35-3-110-7-30-4); the susceptible lines were: G6701, 'Bush Blue Lake 72-112' (72-112) and 'Bush Blue Lake 274' (BBL 274).

Population 3, involved 21 combinations of 10 *P. coccineus* lines considered to be moderately resistant to white mold. The parents were: PI 368710, 304749, 311950, 361520, 361328, 'Hammonds Dwarf Scarlet' (HDS), 'Kelvedon Marvel' (KM), 'Streamliner' (Str), 'White Monarch' (WM), B3749 (selection of PI175829) and 'Early Princess' (EP). All were *P. coccineus* lines previously identified as having moderate resistance to white mold (6).

The F₂ seeds for all 3 populations were produced in the greenhouse, planted in the field, and harvested at maturity on a

Table 1. Average disease reaction of 3 check cultivars of beans to white mold during five months of testing.

	Distribu disease	Survival		
Cultivar	Susceptible	Intermediate	Resistant	(%) ^y
Bush Blue Lake 47	87	13	0	0
Black Valentine	53	16	31	3
Kelvedon Marvel	17	30	53	10

²Susceptible, intermediate, and resistant classes represented 0-1, 2-3, and 4-5 plants surviving, respectively, out of 5 plants per pot after inoculation with the fungus and incubation for 44 hr in a mist chamber. YPercent of individual plants still alive 7 days after test was started.

single plant basis. In the resistance screening test, each pot contained five F_3 plants derived from a single F_2 plant.

Over a 5-month period from November to March 2 tests of approximately 150 pots (five plants/pot/line) were conducted weekly. In each test 3 pots each of KM, BV and 'Bush Blue Lake 47' with moderate, slight and no resistance (8) respectively, were used as checks.

P. coccineus plants surviving the JSI stem test were reinoculated after bloom, using the ABI method. For all surviving *P. vulgaris* plants, remnant F₃ seeds were planted (three plants/pot) and inoculated by the ABI method.

Results and Discussion

The juvenile stem tests were made over a period of 5 months; 150 pots of plants were evaluated in each of 23 tests. The consistency of the check lines (Table 1), especially BBL 47, indicated the uniformity of the results.

Resistance of F₃ lines derived from 4-way crosses among susceptible lines (Population 1) was very low (Table 2); survival within crosses ranged from 0 to 2.4%. Susceptible, intermediate

Table 2. Resistance to white mold of *Phaseolus* spp. F₃ lines derived from 4-way crosses among 10 susceptible parents (Population 1), when inoculated by the juvenile stem method.

	No. F ₃ lin	es in each dise	ase class ^Z	Overall survival
Pedigree	Susceptible	Intermediate	Resistant	
(SHRxW83)(OSU1604xG8953	3) 47	8	11	.9
(SHRxBBL274)(OSU1604xG8	3953)54	9	8	.8
(SHRxBBL274)(EGxG767)	31	5	1	0
(SHRxW83)(EGxBV)	43	21	9	1.1
(SHRxBL247)(W83x76B1)	25	2	2	.7
(G767x76B1)(G8953xBV)	34	6	3	.5
(G767x76B1)(SHRxW83)	63	7	5	1.3
(W83xEW)(OSU1604x8953)	58	16	9	.5
(W83xEW)(EGxG767)	59	8	7	.3
(W83xEW)(EGxBV)	52	28	15	1.5
(W83x76B1)(EGxG767)	34	1	2	2.2
(W83x76B1)(W83xEW)	44	6	1	1.2
(W83x76B1)(G8953xOSU160	4) 49	13	4	2.4
(EGxG767)(OSU1604xG8953) 100	3	2	0
(EGxBV)(OSU1604xG8953)	43	7	1	0
(OSU1604xEG)(OSU1604xG8	3953)42	4	0	0
(OSU1604xEG)(76B1xG767)	33	4	0	0
(76B1xBBL274)(EGxBV)	17	14	11	.8
(SHRxBBL274)(EWxW83)	38	6	4	.4
Distribution of total (%)	77	15	8	.8

^ZSusceptible, intermediate, and resistant classes represented 0-1, 2-3, and 4-5 plants surviving, respectively, out of 5 plants per pot after inoculation with the fungus and incubation for 44 hr in a mist chamber. Each line was represented by one pot of 5 plants.

Table 3. Resistance to white mold of *Phaseolus* spp. F₃ lines from crosses where one or both parents had intermediate resistance Population 2) when innoculated using the juvenile stem method.

	No. F ₃ 1	Overall survival			
Pedigree	Susceptible	Intermediate	Resistant	(%) ^y	
2821-1x76B1	7	2	0	8.8	
2825-1xBBL274	9	1	0	0	
BBL274x2821-1	4	2	1	0	
76B1x2823-1	54	10	1	2.2	
2823-1x2825-1	29	4	0	0	
6985x76B1	63	12	3	3.5	
G6701x6985	69	14	2	1.0	
BBL72-112x6985	5 57	20	4	1.7	
Distribution					
of total (%)	79	18	3	2.0	

^ZSusceptible, intermediate, and resistant classes represented 0-1, 2-3, and 4-5 plants surviving, respectively, out of 5 plants per pot after inoculation with the fungus and incubation for 44 hr in a mist chamber. Each line was represented by one pot of 5 plants.

and resistant lines were respectively defined as those with $0\!-\!1$, $2\!-\!3$, and $4\!-\!5$ plants of 5 plants per F_3 population that survived in the mist chamber after 44 hr. The bean pod inoculum was then removed from the stems of surviving plants, they were placed on a greenhouse bench, and survival was again recorded after 5 days. Many surviving plants collapsed before this time. Of 5645 plants only 46 survived seven days. Remnant seeds of 36 of these 46 F_3 lines were subsequently planted and inoculated by the ABI method, 22 lines were classified as resistant and 14 as susceptible. Conversely, for plants from remnant seeds of lines originally classified as susceptible in the JSI tests, that were inoculated by the ABI method, 13 were classified as susceptible and three resistant. This correlation between results of the two inoculation methods, suggests that survivors are not escapes but do have some resistance.

In the crosses for which one or both parents was considered to have intermediate reistance (Population 2) the overall number of plants that survived the juvenile stem test (Table 3) was low (36/1840 plants). The number was higher in some crosses (up to 8.8%) than in any of the four-way crosses among susceptible lines (only up to 2.4%). Lines 2821-1 (which has *P. coccineus* in its parentage), and 76B1 and 6985 were superior parents (Table 3) and these results were verified via the ABI method. Remnant seed of four lines classified as resistant in JSI tests were resistant in the ABI test. Sixteen lines classified as intermediate in the JSI test produced 5 susceptible and 11 resistant in the ABI test. Of eighteen lines classified as susceptible in the JSI test, 15 were susceptible and three resistant in the ABI test.

The survival ratio (185/4885 plants) was highest among the F_3 of the *P coccineus* crosses (Population 3) where it ranged from 0 to 22.5% within individual crosses (Table 4). *P. coccineus* has a tough fibrous stem, which may provide physical resistance resulting in delayed stem collapse. Many plants remained upright after 44 hr, but a large percentage of these plants died later. F_3 populations from Str x PI 304749 and Str x WM had a much higher rate of survival than other crosses, suggesting superiority of these parents. Eighty-five plants surviving the JSI test were then subjected to the ABI blossom test; 39 appeared resistant, whereas 46 were susceptible.

Only a small percentage of plants in populations 1 and 2 survived, but progeny tests of these survivors resulted in a far higher

survival rate than the complete F_3 populations. In the JSI test of 227 progeny of actual survivors, 75 plants survived for 44 hr and 39 or 17% survived after 7 days. In comparison, only 1 of 15 KM and no BBL 47 or BV plants survived 7 days in the same test. It is not possible to calculate heritablility based on regression of F_4 on F_3 as all susceptible F_3 died. Therefore, the relative percent survival in a JSI test of the F_4 population grown from F_3 survivors is the best indication of increase in level of resistance. The 17% F_4 survival far exceeded any P. vulgaris F_3 families.

These results would suggest that repeated selection should result in accumulation of genes for resistance and a greater genetic resistance. In Krupinsky and Sharp's study (9) with rust resistance in wheat it was not until the F_6 generation that survival exceeded 50%, thus the slight progress we have made by the F_3 generation appears to be promising.

The relative resistance of the parents used to develop the 3 populations reported in Tables 2, 3, 4 is shown in Table 5. All of the 10 parents used to develop populations 1 were highly susceptible, although 8953, 767, EW and BL 1604 had a few plants that survived the 7 day test. Based on prior tests, 2823-1, 2821-1, 2825-1, and 6985 which were used in population 2, had been considered to possess intermediate resistance. In these tests most were susceptible, although a relatively high percentage of 6985 plants survived the 7 day test.

These extensive screening trials indicate that the JSI test on juvenile plants is an efficient method for screening large populations of plants for resistance to white mold. Far fewer plants can be handled if the ABI method is used because it is necessary to grow the plants to bloom. For the ABI test 95% relative humidity or higher is necessary for 6 or 7 days, whereas only 44 hr in a

Table 4. Resistance to white mold of F_3 lines derived from crosses of $10 \ P$ coccineus parents when inoculated using the juvenile stem test (Population 3).

	No. F ₃ lines in each disease class ²			Overall survival
Pedigree	Susceptible	Intermediate	Resistant	(%) ^y
EPx368710	10	3	2	0
EPxB3749	23	9	3	4.0
KMxHDS	30	37	28	7.8
KMx368710	28	23	11	.6
WMxB3749	18	16	33	2.7
WMx368710	31	25	17	1.6
WMx304749	4	9	16	4.1
Strx304749	3	6	28	20.0
StrxB3749	8	10	31	6.1
StrxWM	0	1	15	22.5
B3749x311950	4	8	16	7.1
B3749x361520	16	14	6	1.1
B3749x361328	11	9	7	0
304749xKM	11	13	7	1.9
HDSx368710	15	13	21	1.2
HDSxWM	15	28	6	.8
HDSx304749	7	11	3	.9
368710xB3749	7	7	4	0
304749x361358	16	13	17	2.1
361520xKM	30	35	25	1.1
304749x361520	8	12	26	7.8
Distribution				
of total (%)	32	33	35	3.8

²Susceptible, intermediate, and resistant classes represented 0-1, 2-3, and 4-5 plants surviving, respectively, out of 5 plants per pot after inoculation with the fungus and incubation for 44 hr in a mist chamber. Each line was represented by one pot of 5 plants.

yPercent of individual plants still alive 7 days after test was started.

yPercent of individual plants still alive 7 days after test was started.

Table 5. Resistance to white mold of *Phaseolus vulgaris* and *P. coccineus* lines used as parents to develop the 4 populations, when inoculated with the juvenile stem method.

	Disease classification ^Z			Survival		
Parents	Susceptible	Intermediate	Resistant	(%) ^y		
	First population P. vulgaris					
State Half Runner	10	0	0	0		
Black Valentine	7	0	3	0		
Early Gallatin	11	1	0	0		
Wisconsin 83	7	4	1	0		
Blue Lake 274	13	0	0	0		
G8953	9	3	2	4		
G767	9	6	1	4		
Early Wax	6	4	3	6		
76B1	10	3	0	2		
OSU 1604	8	6	0	6		
Seco	nd population	P. vulgaris and P.	coccineus			
2823-1	14	3	3	1		
2821-1	6	1	2	2		
2825-1	8	0	1	2 2 9		
6985	10	3	3	9		
BBL 72-112	12	0	0	0		
Third population P. coccineus						
Early Princess	0	1	2	0		
White Monarch	0	1	2 3	13		
Hammonds Dwarf Scarlet	. 0	2	2	7		
PI201389	1	1	1	0		
PI304749	0	2	1	7		
PI368710	0	3	0	13		
PI361520	1	0	0	0		
PI1361328	2	1	0	0		
B3749	0	2	1	7		
Streamline	0	2	1	0		
Kelvedon Marvel	0	1	5	10		

^ZSusceptible, intermediate, and resistant classes represented 0-1, 2-3, and 4-5 plants surviving, respectively, out of 5 plants per pot after inoculation with the fungus and incubation for 44 hr in a mist chamber. Each line was represented by one pot of 5 plants.

^yPercent of individual plants still alive 7 days after test was started.

humid chamber is needed for the JSI test. This causes considerably less stress on the plants being tested and allows for a fast turnover of plants in the mist chamber.

Both *P. coccineus* and *P. vulgaris* parents were identified that combined to produce F₃ progeny, which appeared to be superior to their parents. If this trend continues, subsequent testing of further generations as well as recombinations of survivors should result in higher levels of resistance and gains similar to those experienced by Krupinsky and Sharp (9) with wheat striped rust resistance.

It is apparent from the small percentage of survivors among parents or F_3 lines that the level of resistance is low in any one parent, but by combining genes additively from several parents it seems to be possible to develop considerably improved levels of resistance. Heritability appears acceptably high based on the much higher survival (17%) of F_4 *P. vulgaris* progenies of F_3 plants surviving the JSI test, compared to the best F_3 population (3.5%).

In addition to following a modified recurrent selection program to select the highest level of resistance possible, we plan to combine resistance with a plant morphology designed to aid escape from the disease. Plants with a more upright, open canopy have been shown to escape diseases as a result of microclimate shifts in and around the plant canopy (4, 5, 11). Combining both resistance and improved plant morphology should produce beans with economic resistance to white mold and also produce a plant better adapted to mechanical harvesting than most current cultivars.

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