INTROGRESSING WHITE MOLD RESISTANCE FROM THE SECONDARY GENE POOL OF COMMON BEAN

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ABSTRACT: Yield losses from white mold (caused by *Sclerotinia sclerotiorum* Lib de Bary) in common bean (*Phaseolus vulgaris* L.) vary from 30% to 90%. Only low levels of resistance exist in common bean, and chemical and cultural controls alone are often inadequate. However, high levels of resistance exist in the secondary gene pool. Our goal is to introgress and pyramid high levels of resistance from across *Phaseolus* species to provide a sustainable solution to white mold. The specific objectives are to (i) screen inbred genotypes derived from interspecific populations in the field and greenhouse, (ii) screen known white mold resistant *P. coccineus* and common bean genotypes in the greenhouse, (iii) develop and screen a new group of recombinant inbred and inbred-backcross breeding lines from interspecific populations between susceptible pinto bean and recently identified white mold resistant *P. coccineus* accessions PI 433246 and PI 439534, (iv) determine the inheritance of white mold resistance found in PI 433246 and PI 439534, and (v) pyramid white mold resistance from across *Phaseolus* species. Four hundred thirty-three F_2, inbred-recurrent, and inbred-congruity backcross derived breeding lines from 10 interspecific populations of 'ICA Pijao' with the three species in secondary gene pool (*P. coccineus*, *P. costaricensis*, and *P. polyanthus*) were evaluated in the field in Idaho and in greenhouse in Colorado in FY2002. Approximately 75 breeding lines resistant (disease scores ≥3) in both field and greenhouse screenings and an additional 325 interspecific breeding lines were planted in the field at Hazelton, Kimberly, and Rupert, Idaho for white mold evaluation in FY2003. Single-row plots 10 ft long with 5 to 9 replications were used for evaluation. Despite two ascospore and one mycelial inoculations during flowering, and use of solid-set sprinkler system to maintain humidity, no white mold infection of any consequence occurred at any site due to prolonged hot and dry weather. The greenhouse evaluation of these interspecific breeding lines using the straw-test is in progress at Fort Collins, Colorado. Twenty-one previously known white mold resistant *P. coccineus* and common bean accessions along with susceptible cultivar Bill Z were screened using the straw test in greenhouse at Fort Collins, Colorado in FY 2002. Our results were similar to those reported by earlier researchers. Of these two *P. coccineus* accessions, namely PI 433246 and PI 439534 were crossed and backcrossed twice with pinto Othello and UI 320, respectively. Two inter-gene pool single crosses and one double-cross were made to pyramid white mold resistance from across *Phaseolus* species. All interspecific breeding lines will be evaluated in fields in Idaho, Washington and/or Wisconsin in 2004. Highly resistant genotypes will be screened in greenhouse at Fort Collins, Colorado. New interspecific crosses and backcrosses will be grown in field in Idaho for selection for photoperiod insensitivity. Inheritance of white mold resistance in PI 433246 and PI 439534 will be determined in the greenhouse at Fort Collins, Colorado. At least one multiple-parent cross will be made for pyramiding white mold resistance. Resistant genotypes from all five experiments will be tested nationally and information shared with bean growers, researchers, and other clientele. Research results will be published in refereed journals.

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GOALS and OBJECTIVES:

Our goal is to introgress and pyramid high levels of white mold resistance from across *Phaseolus* species into common bean cultivars to provide a low input, sustainable, and ecosystem-friendly solution to this devastating disease. Our specific objectives are to:

- Screen interspecific breeding lines from crosses of common bean with *P. coccineus*, *P. costaricensis*, and *P. polyanthus* under heavy white mold pressure in the field and greenhouse.
- Screen known white mold resistant *P. coccineus* and common bean in the greenhouse.
- Introgress white mold resistance alleles and QTL from recently identified selected white mold resistant *P. coccineus* accessions PI 433246 and PI 439534.
- Determine the inheritance of white mold resistance found in *P. coccineus* accessions PI 433246 and PI 439534.
- Pyramid alleles and QTL imparting white mold resistance from across *Phaseolus* species.

MATERIALS AND METHODS:

Experiment # 1.

Source of Interspecific Breeding Lines. The F_2, recurrent backcross, and congruity backcross derived 433 breeding lines from 10 interspecific crosses of common bean cultivar ICA Pijao with *P. coccineus* (G 35171 and G 35172), *P. costaricensis* (S 33720), and *P. polyanthus* (G 35877) were developed and introduced by the PI from CIAT, Cali, Colombia in 2000. None of these genotypes were ever tested for their reaction to white mold before initiating this project.

Field Evaluation in FY 2002. ICA Pijao, white mold susceptible (e.g., Bill Z, Top Crop, UI 114, and UI 537) and resistant (e.g., Ex-Rico 23, G 122, I 9365-25, 92 BG-7, and MO 162) common bean, and 433 interspecific breeding lines from 10 populations were grown in a heavily white mold infested-field at Hazelton, Idaho. Each plot consisted of a single row 3 meters long spaced 56 cm apart without replication. Each plot was flanked on either side by a susceptible cultivar to enhance white mold and facilitate evaluation. A solid set sprinkler system was used three times per day (2 hr run each), in addition to the standard 10 to 12 hr run every 10 days, during the flowering and reproductive periods. Reaction to white mold was recorded on a plot-basis at the R 8 and R 9 growth stages, using a 1 to 9 scale, where 1 = no visible white mold symptoms on stem and pods, and 9 = severely damaged.

Field Evaluation in FY 2003. Approximately 400 genotypes including interspecific breeding lines selected in FY 2002, and resistant and susceptible checks were grown in field at Hazelton, Kimberly, and Rupert, Idaho. Single-row plots 3 m long with 5 to 9 replications were used for evaluation. Despite two ascospore and one mycelial inoculations during flowering, and use of solid-set sprinkler irrigation system to provide humidity, no white mold infection of any consequence occurred at any site due to prolonged hot and dry weather.

Greenhouse Evaluation in FY 2002. Each of 433 interspecific breeding lines and resistant and susceptible checks had two seeds per pot, with 5 pots per breeding line for a total of 10 replicates per test were used for the straw test in the greenhouse at Fort Collins, Colorado. Three to five day-old mycelial inoculum + agar plugs in 6-mm diameter plastic straw pieces 3 cm long and sealed on one end were placed on the cut end of the growing point of each of two 14 to 21 day old seedlings in a pot in the greenhouse. Inoculated plants were placed on a misting bench (30 second misting every 3 – 5 min) to maintain free moisture on the plant foliage at 24 – 27°C with 12 – 14 hr lighting for 7 to 10 days until disease evaluation. Individual plants were evaluated on a 1 to 9 scale, where 1 = no sign of disease beyond contact with the straw to 9 = total plant collapse.

Greenhouse Evaluation in FY 2003. The greenhouse evaluation of the interspecific breeding lines and resistant and susceptible checks, using the straw-test, is in progress at Fort Collins, Colorado. Partial results may be available at the time of the annual meetings in Minneapolis in January 2004.

Experiment # 2

Greenhouse Evaluation of Resistant *P. coccineus* and Common Bean Genotypes. Twenty-one known white mold resistant *P. coccineus* and common bean accessions along with susceptible cultivar Bill Z were screened, using the straw test as described above, in the greenhouse at Fort Collins, Colorado in 2002. Seed of these genotypes was obtained from USDA-ARS, Pullman, Washington.

Experiment # 3

New Interspecific Crosses: Resistant *P. coccineus* and Susceptible Pinto Bean Cultivars. Two single crosses between two white mold resistant *P. coccineus* accessions, namely PI 433246 and PI 439534, and susceptible pinto bean cultivars Othello and UI 320 were initiated in FY 2002. PI 433246 is highly photoperiod sensitive (taking 82 d to flower even in a 12-hr light) with indeterminate extreme climbing growth habit Type IV from Guatemala. PI 433246 has scarlet color flowers and a mixture of large seeds of brown, purple, and red colors. PI 439534 is also indeterminate climbing Type IV with scarlet flowers, but it is photoperiod insensitive (taking 38 d to flower in a 12-hr light). PI 439534 is from Netherlands and it has large pink spotted seeds. The first and second backcrosses of Othello and UI 320 with *P. coccineus* accessions PI 433246 and PI 439534, respectively, were completed in FY 2003.

Experiment # 4

Some seed from the four parents, F_1 , F_2 , and the two first backcrosses made for Experiment # 3 were saved for investigating the inheritance of resistance to white mold in interspecific progenies derived from crosses of *P. coccineus* accessions PI 433246 and PI 439534. With pinto cultivars Othello and UI 320.

Experiment # 5

Pyramiding White Mold Resistance From Across *Phaseolus* Species. Two inter-gene pool single crosses, namely MO 162/I 9365-25 and Ex-Rico 23/G 122, and the resulting double-cross to pyramid white mold resistance from across *Phaseolus* species were made in FY 2003. All four parents have extensively been tested in the USA, and are known to possess moderate levels of white mold resistance across environments. However, their genetic basis of resistance and interrelationship are not fully understood. Large-seeded Andean MO 162 and G 122 have a determinate growth habit Type I. I 9365-25 derives its resistance from *P. coccineus*. I 9365-25 has an indeterminate upright growth habit Type II with small pink seeds. Ex-Rico 23 (synonymous ICA Bunsi) has an indeterminate prostrate growth habit Type III, and small white seeds. Ex-Rico 23 and I 9365-25 possess characteristics of the Middle American gene-pool.

RESULTS and DISCUSSION:

Experiment # 1

Evaluation of Interspecific Breeding Lines for White Mold Resistance

Highly resistant and susceptible breeding lines were found in all interspecific populations (Table 1). Breeding lines derived from *P. costaricensis* (S 33720) seem to be slightly superior for white mold resistance. Breeding lines derived from two or more recurrent backcrosses did not seem to offer any advantage over breeding lines derived from single crosses and the first backcrosses. Similarly, breeding lines from congruity backcrosses were not superior to recurrent backcrosses. Only breeding lines exhibiting a score of ≤3 (invasion only to the first node) in greenhouse test at Fort Collins, Colorado, and ≤3 scores (≤5% plants showing mild white mold infection) in the field screening at Hazelton, Idaho were selected (approximately 20%) for the subsequent evaluation in 2003, and their growth habit and seed characteristics were recorded.

Table 1. Number of F_2 recurrent backcross and congruity backcross derived interspecific breeding lines from crosses between common bean cultivar ICA Pijao and *P. coccineus* (G 35171 and G 35172), *P. costaricensis* (S 33720), and *P. polyanthus* (G 35877) screened for white mold resistance in the field in Idaho and in the greenhouse in Colorado in 2002.

Interspecific population	No. of breeding lines	Range and mean for white mold ratings in 2002			
		Field, Idaho		Greenhouse, Colorado	
		Range	Mean	Range	Mean
ICA Pijao/G 35877	24	1-8	5.8	1-9	6.2
ICA Pijao// ICA Pijao/ G 35877	121	2-8	6.1	1-9	5.8
ICA Pijao/S 33720	35	1-7	5.0	1-9	4.9
ICA Pijao // ICA Pijao/ S 33720	138	1-8	5.2	1-9	5.0
ICA Pijao/G 35171// ICA Pijao	15	1-8	5.5	1-9	5.3
ICA Pijao/G 35171// ICA Pijao /3/ICA Pijao	6	1-9	5.7	1-9	5.1
ICA Pijao/G 35171// ICA Pijao /3/G 35171	36	1-9	5.9	1-9	5.4
ICA Pijao/G 35172// G 35172	19	1-8	5.3	1-9	5.0
ICA Pijao / G 35172// ICA Pijao	32	1-9	5.8	1-9	5.5
ICA Pijao / G 35172// ICA Pijao/3/ G 35172/4/ ICA Pijao	7	1-9	6.1	1-9	5.9
Mean (all interspecific genotypes)	433	1-9	5.6	1-9	5.4
ICA Pijao (Parent)		-	4.4		
UI 114 (Susceptible cultivar)				3-9	7.9

Experiment # 2

Evaluation of Resistant *P. coccineus* and Susceptible Common Bean Genotypes

Our results were similar to those reported by earlier researchers (Table 2). However, these genotypes may still need to be screened using the oxalate and other tests before maximizing their use in breeding for white mold resistance.

Table 2. Disease scores from the straw-test of known white mold resistant *Phaseolus coccineus* and *P. vulgaris* germplasm in the greenhouse at Colorado State University, Fort Collins in 2002.

Genotype	Species	No. of plants	Percentage of plants with white mold scores†					Mean score
			1	3	5	7	9	
Susceptible Bill Z	*P. vulgaris*	9	0	0	0	11.1	88.9	8.8
PI 189023	*P.coccineus*	16	0	50.0	37.5	6.3	6.3	4.4
PI 201304	*P.coccineus*	12	33.3	33.3	8.3	25.0	0	3.5
PI 201320	*P.coccineus*	15	13.3	66.7	20.0	0	0	3.1
PI 311985	*P.coccineus*	15	33.3	60.0	6.7	0	0	2.5
PI 317551	*P.coccineus*	14	42.9	35.7	14.3	7.1	0	2.7
PI 433236	*P.coccineus*	12	25.0	58.3	8.3	0	8.3	3.2
PI 433237	*P.coccineus*	13	23.1	61.5	15.4	0	0	2.9
PI 433242	*P.coccineus*	15	20.0	60.0	20.0	0	0	3.0
PI 433246‡	*P.coccineus*	7	57.1	42.9	0	0	0	1.9
PI 433247	*P.coccineus*	11	0	90.9	9.1	0	0	3.2
PI 433250	*P.coccineus*	14	57.1	28.6	7.1	0	7.1	2.4
PI 433251	*P.coccineus*	16	0	87.5	12.5	0	0	3.3
PI 439534‡	*P.coccineus*	10	0	80.0	20.0	0	0	3.4
PI 201354	*P. vulgaris*	15	6.7	73.3	20.0	0	0	3.3
PI 263596	*P. vulgaris*	15	6.7	86.7	6.7	0	0	3.0
PI 311974	*P. vulgaris*	15	0	60.0	33.3	6.7	0	3.9
PI 312018	*P. vulgaris*	15	20.0	40.0	40.0	0	0	3.6
PI 313348	*P. vulgaris*	14	7.1	64.3	28.6	0	0	3.4
PI 313425	*P. vulgaris*	15	13.3	66.7	20.0	0	0	3.1
PI 319683	*P. vulgaris*	14	7.1	78.6	14.3	0	0	3.1
PI 325653	*P. vulgaris*	13	7.7	69.2	15.4	7.7	0	3.5
Total/mean		286	16.3	60.0	17.3	2.4	1.0	3.2

† Pinto bean isolate S20 was used to inoculate seedlings on a mist bench covered by plastic at 20 – 23° C and diffuse lighting. Reactions scored on a 1 to 9 scale at 5 – 7 days post-inoculation.
‡Selected for interspecific hybridization with susceptible pinto bean cultivars.

Experiment # 3

New Interspecific Crosses: Resistant *P. coccineus* and Susceptible Pinto Bean Cultivars

The number of F_1 seeds produced by hand emasculation and pollination for the two single crosses and first and second backcrosses of white mold susceptible pinto Othello and UI 320, with resistant *P. coccineus* accessions PI 433246 and PI 439534, respectively, are given in Table 3. The F_1 seed of these crosses and backcrosses will be grown in greenhouse in winter of 2003-2004. Resulting progenies will be allowed to inbreed for one or more additional generations before screening in the field for sensitivity to photoperiod followed by field and greenhouse screenings for white mold resistance.

Table 3. New interspecific crosses made between white mold re and PI 439534 and susceptible pinto bean cultivars Othello and

Interspecific crosses	No. of F_1 seeds p
Othello/PI 433246	182
Othello//Othello/PI 433246	163
Othello///Othello//Othello/PI 433246	191
UI 320/PI 439534	267
UI 320//UI 320/PI 439534	204
UI 320///UI 320//UI 320/PI 439534	187

Experiment # 4

Seed from pinto cultivars Othello and UI 320, P. coccineus accessions PI 433246 and PI 439534, and their F1 , F2 , and the two first backcrosses will be planted in greenhouse for screening for white mold reaction using the straw test at Fort Collins, Colorado in FY 2004. The frequency of resistance and susceptible genotypes in segregating populations will be subjected to the chi square test to determine the inheritance of resistance to white mold in the interspecific progenies.

Experiment # 5

Pyramiding White Mold Resistance From Across *Phaseolus* Species

Four hundred nineteen F_2 seeds were produced for the inter-gene pool double-cross MO 162/I 9365-25//Ex-Rico 23/G 122 in FY 2003 to pyramid white mold resistance from across *Phaseolus* species. The F_1 along with the four parents will be screened for white mold reaction in the greenhouse. Selected resistant plants will be used to make a multiple-parent cross with another white mold resistant common bean such as NY6020-4 and/or AN 57.

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