Evaluation of Plant Introductions of *Phaseolus* spp. for Resistance to White Mold

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ABSTRACT

Hunter, J. E., Dickson, M. H., Boettger, M. A., and Cigna, J. A. 1982. Evaluation of plant introductions of *Phaseolus* spp. for resistance to white mold. Plant Disease 66:320-322.

Higher levels of resistance to white mold (*Sclerotinia sclerotiorum*) than previously known in *Phaseolus vulgaris* were detected under growth chamber conditions using a limited-term inoculation method. Fifty percent or higher survival occurred in only 13 of 310 lines from Europe, whereas 16 of 139 introductions with tolerance to root rot survived the white mold test. Most of these originated in Central or South America, although two lines from Turkey had a high level of resistance. A group of F_5 plants derived from *P. vulgaris* × *P. coccineus* crosses had a comparatively high survival ratio. The eight lines of *P. coccineus* subsp. *polyanthus* from Mexico that were tested all had a high level of resistance. A change in the limited-term inoculation method is described that makes it easier and faster to use for large-scale testing.

Recently we reported the development of a method, called limited-term inoculation (LTI), to evaluate beans (*Phaseolus* spp.) for resistance to white mold incited by *Sclerotinia sclerotiorum* (Lib.) de Bary (9). A major advantage of the method is that it is sensitive enough to detect partial resistance, which is important because high levels of resistance have not been found. The LTI method has the advantage of enabling the researcher to evaluate prebloom plants, whereas blossoms are normally required before infection occurs (1,2,10). Also, with this method resistance to disease is not confused with escape from disease, which can occur because of plant morphology (5,12).

Accepted for publication 22 June 1981.

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0191-2917/82/04032003/$03.00/0

Because of the limitations of other methods of evaluating germ plasm for resistance to white mold, only a small number of lines have been screened previously (3). We report herein the results of a series of tests using the LTI method to evaluate a diverse collection of germ plasm of *Phaseolus* spp., especially *P. vulgaris* L., for resistance to white mold.

MATERIALS AND METHODS

Inoculum for the LTI method consists of pieces of an organic substrate colonized by the fungus. A small piece of celery, prepared as described previously (9), was used in all tests except one. In this instance, canned snap beans were used as the organic substrate to determine the incidence of white mold in lines previously reported to have a low incidence of disease under field conditions. The snap bean inoculum was prepared by spraying a suspension of ascospores (4,000/ml) of *S. sclerotiorum* onto a single layer of autoclaved snap beans. Two size 303 cans were used per baking pan (23 × 33 cm), which was covered with aluminum foil. Inoculated beans were incubated at 22 C for 4 days to allow colonization of the tissue by the fungus. The soft, colonized celery pieces or snap beans were crushed and mixed, and then a mass of the tissue was wrapped completely around the stem above the cotyledonary node of 3-wk-old plants (9).

Plants inoculated with celery were left for about 48 hr in a growth chamber at 21 C and 95–98% relative humidity. Those inoculated with the canned snap beans were left in a greenhouse mist chamber at 18–20 C for about 44 hr. In both cases, the plants were rated as having collapsed or upright stems when they were removed from the chambers. The inoculum was removed from those with upright stems and the plants left for 5 days on a greenhouse bench, at which time the number of plants surviving was recorded. Five plants were grown per pot for each line. Lines in which any plants survived 7 days after inoculation were retested.

Plants used in these studies were grown for 2 wk under regular greenhouse light at 20–24 C. During the third week, they were grown under metal halide lights at 18–20 C. These conditions result in hardy plants that more nearly resemble those grown under field conditions. The first trifoliolate leaf was fully expanded at the time of inoculation.

The following *P. vulgaris* material was tested by the LTI method: 310 accessions of the plant introduction (PI) collection from western Europe, including Russia but excluding the eastern Baltic countries and Turkey; 139 PI accessions from South and Central America, Turkey, Ethiopia, and Kenya that previously had shown tolerance to root rot incited by *Pythium ultimum* Trow and *Fusarium solani* (Mart.) Appel and Wollenw. f. sp. *phaseoli* Snyd. & Hans. (7); and several cultivars or lines that have been reported to have tolerance to white mold or to escape from disease because of plant morphology (4–6,11). A total of 809 F_5 plants derived from *P. vulgaris* × *P. coccineus* L. 'Kelvedon Marvel' crosses was also tested. Finally, eight lines of *P. coccineus* subsp. *polyanthus* (Greenman) Maréchal, Mascherpa, and Stanier from Mexico were tested by the LTI method.

P. vulgaris 'Bush Blue Lake 47' and 'Black Valentine' and *P. coccineus* 'Kelvedon Marvel' were included in each test as susceptible, slightly resistant, and moderately resistant checks, respectively. These ratings are based on previous studies (9). The PI lines listed in Table 1, each of which had a combined test score of ≥50% survival, were compared with the check lines using a nonparametric statistical test; ie, the proportion of healthy and diseased plants for each PI line was compared with each check cultivar in a 2×2 contingency table using the chi square test.

RESULTS AND DISCUSSION

Based upon data accumulated from all of the tests, the ratio of the number of plants surviving/number inoculated for the susceptible, slightly resistant, and moderately resistant check lines was 2/162, 31/165, and 84/100, respectively. To be statistically equivalent ($P = 0.01$) to Bush Blue Lake 47, Black Valentine, or Kelvedon Marvel, the combined score of the two tests for each PI line (Table 1) had to be ≤1, ≤5, or ≥6, respectively. These data indicate that conditions were ideal for detecting moderate or higher levels of resistance.

The 310 lines of *P. vulgaris* from Europe were generally susceptible. Using the combined results of two tests (five plants per line per test), 50% or higher survival occurred in only 13 lines. Among the 139 introductions with tolerance to root rot, 50% survival occurred in 16 lines (Table 1). All but three of these more resistant lines were from Central or South America. However, PI 169787, a bush bean from Turkey, was one of the best in this group (Table 1). Some of the lines from both groups with 50% survival were retested with snap bean inoculum, and similar results were obtained.

Lines or cultivars of *P. vulgaris* previously reported to have a low incidence of white mold in the field were highly susceptible when inoculated by the LTI method (Table 2). In some cases, this might have been because the LTI inoculation method was too severe to detect low levels of resistance that were responsible for the better field performance. However, for other lines, better field performance probably can be attributed to morphological characteristics that favor escape from rather than resistance to white mold. This might occur because of a drier microclimate in or near the canopy of lines with a more open, upright habit of growth (5,12).

The F_5 plants derived from the interspecific crosses had a much higher survival rate than any other group (199/809). Most of these possessed characteristics of *P. vulgaris*: ie, they exhibited epigeal germination and they were self-pollinating, although many were partially sterile.

Table 1. Plant introductions of *Phaseolus vulgaris* in which 50% or more of the plants survived following inoculation with *Sclerotinia sclerotiorum*[a]

Identification code	Country of origin	Test[b] 1	2
Plant introductions from Europe			
PI 415965	Italy	3	3
PI 281596	Italy	4	2
PI 281978	Italy	3	3
PI 255960	Italy	2	4
PI 415994	Russia	2	3
PI 226865	Russia	2	5
PI 285693	Poland	3	4
PI 264789	France	1	4
PI 271999	Spain	3	4
PI 189567	Netherlands	3	5
PI 287536	Netherlands	3	4
PI 180753	Germany	3	4
PI 180732	Germany	4	2
Plant introductions with root rot tolerance			
PI 204717	Turkey	4	3
PI 169787	Turkey	4	5
PI 325737	Mexico	2	3
PI 313761*[c]	Mexico	2	4
PI 312045	Mexico	2	3
PI 313329*	Mexico	4	3
PI 282078	Chile	5	5
PI 282042	Chile	4	4
PI 307469*	Brazil	3	4
PI 310649*	Guatemala	3	3
PI 310744*	Guatemala	4	2
PI 310805*	Guatemala	5	1
PI 310557	Honduras	3	2
PI 310583*	Honduras	3	4
PI 308901*	Costa Rica	2	3
PI 197226	Costa Rica	2	3

[a] A total of 310 lines from Europe and 139 with root rot tolerance (mostly from Central and South America) were evaluated. A mass of autoclaved celery that had been colonized by the fungus was wrapped around the stem of 3-wk-old, greenhouse-grown plants. After incubation in a moist environment, the plants were scored for collapsed or upright stems. The 50% survival figure is based on the average of two tests.

[b] Number of plants surviving in one pot containing five plants. Data were recorded 5 days after removal of inoculum and transfer of plants to greenhouse. Check cultivars used were Bush Blue Lake 47 and Black Valentine (both *P. vulgaris*) and Kelvedon Marvel (*P. coccineus*), which have no, slight, and moderate resistance, respectively. Survival ratios were 2/162, 31/165, and 84/100, respectively. To be statistically equivalent ($P = 0.01$) to one of the three check cultivars, combined score of two tests for each PI line had to be ≤1, ≤5, or ≥6, respectively.

[c] * = Short-day flower plants.

The eight lines of *P. coccineus* subsp. *polyanthus* from Mexico had a high level of resistance. In two LTI tests, 17/18 and 10/13 plants of PI 417603 survived (Table 2); in a single test, 5/5 plants of each of three lines survived. The most promising of these lines will be crossed with *P. vulgaris* and the progeny tested for resistance.

These tests demonstrated that some lines had more resistance than previously observed in *P. vulgaris*. However, even the best of these lines could be killed if the inoculum were left on the stem in a humid chamber for a longer period. Therefore, superior lines need to be tested under field conditions to determine if the resistance observed by the LTI method is adequate to control natural infection. The level of resistance in pods also needs to be compared with that in stems, but a method that will provide uniform results has not been developed.

The best lines include various plant types: PI 415994 and 169787 are bush types and PI 204717 is a semivine. Some lines have large stems and some have small ones: eg, PI 417603, the *P. coccineus* subsp. *polyanthus* line with a

Table 2. Incidence of white mold among bean lines (*Phaseolus* spp.) previously reported to have a low incidence of disease under field conditions as compared with lines of known levels of resistance as determined by limited-term inoculation method[a]

Pedigree	Test[b] 1	2
Field "resistant" lines		
Charlevoix	1/10[c]	0/13
Aurora	0/10	2/13
Knight	1/10	0/15
Black Turtle Soup (BTS)	0/10	0/11
BTS selection T39	0/10	0/15
Rico 23	···	0/15
Venezuela 350	0/10	0/15
Comparison lines[d]		
Bush Blue Lake 47	0/12	2/10
Black Valentine	1/14	10/10
Kelvedon Marvel	14/14	9/10
6985	8/10	3/10
9224	15/35	18/43
PI 417603	18/19	10/13

[a] An organic substrate that had been autoclaved and colonized by *Sclerotinia sclerotiorum* was wrapped around the stem above the cotyledonary node of 3-wk-old, greenhouse-grown plants. After incubation in a moist environment, the plants were scored for collapsed or upright stems.

[b] Test 1 was conducted in 1980 using colonized celery pieces. Test 2 was conducted in 1981 using colonized canned snap beans.

[c] Number of plants surviving/number inoculated.

[d] *P. vulgaris* 'Bush Blue Lake 47' and 'Black Valentine' and *P. coccineus* 'Kelvedon Marvel' were used as susceptible, slightly resistant, and moderately resistant checks, respectively. The others are lines with relatively high levels of resistance; 6985 is a small-stem *P. vulgaris*, 9224 is *P. vulgaris* derived from *P. coccineus*, and PI 417603 is *P. coccineus* subsp. *polyanthus*.

high level of resistance, has a very small stem; resistance is thus probably not of a structural nature. However, PI 417603 seedlings are small and initially slow to develop, probably because the seeds are very small. Therefore, the plants will succumb in the LTI test unless inoculation is delayed until the first trifoliolate leaf has expanded.

The uniformity of the results in Table 2 indicated the reliability of the LTI method, regardless of whether pieces of celery petiole or canned snap bean were used as the organic substrate for the fungus. Preparation of canned snap bean inoculum was, however, much faster. Although we inoculated the canned snap beans by spraying them with ascospores, it might be possible to use ground mycelium if a supply of ascospores is not available. The use of colonized canned snap beans for inoculum and a greenhouse mist chamber for incubation made the LTI method feasible for large-scale testing.

In conclusion, these tests demonstrated higher levels of resistance than realized previously, at least in the stem, in *P. vulgaris* and compatible species. In addition, the tests indicated that the LTI method can be used to determine whether lines that have less disease in the field have a high level of resistance. The extreme susceptibility in our LTI tests of cultivars and lines previously reported to have less disease in the field suggests that breeding for disease escape—eg, plants with an open canopy—should be actively pursued. Another advantage of an open canopy is that when fungicides must be used they will be more likely to penetrate the canopy and cover the blossoms, which is necessary to achieve maximum control (8). Thus, breeding for both resistance and escape mechanisms should produce cultivars with the best prospect for minimizing the incidence of white mold.

ACKNOWLEDGMENT

We thank R. C. Seem for his assistance with the statistical analysis of the data.

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