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Evaluation of Common Bean Accessions for Resistance to *Pythium ultimum*

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Abstract. **Common bean accessions were evaluated to identify white- or light-seeded beans with resistance to *Pythium ultimum* Trow. In total, 568 common bean (*Phaseolus vulgaris* L.) accessions were inoculated with a hyphal suspension of *P. ultimum* under greenhouse conditions. The bean accessions included represented the Andean–Middle American core collections (406 accessions) and 162 additional white- or cream-seeded accessions. The accessions were categorized into 12 groups according to seedcoat color. Accessions with light seedcoats exhibited higher levels of disease symptoms, with white-seeded bean accessions being the most susceptible class. No symptomless white-seeded accessions were identified. The most resistant white-seeded accessions were PI 430207, PI 527803, PI 290996, PI 299021, PI 194574, and PI 304110. Cream-seeded beans exhibited higher levels of resistance, with nine accessions rated as symptomless out of 188 cream and white accessions tested. Of 568 accessions, 48 tested were symptomless, whereas disease ratings of the other accessions ranged from resistant to highly susceptible.**

Pythium ultimum Trow is a major causal agent of seed decay and pre-emergence/post-emergence damping-off in beans *(Phaseolus vulgaris* L.) (Hendrix and Campbell, 1973; Pieczarka and Abawi, 1978a). Infected bean seeds or seedlings typically become discolored, chlorotic, and soft and decayed even if they germinate, and can often wilt or die within a few weeks (Pfender, 1991). The pathogen can survive in plant residues or soil in a quiescent state, resisting adverse conditions for extended time periods (Stanghellini, 1971a). Seed or seedling exudates from susceptible plants stimulate germination of sporangia and growth of the pathogen (Aghihotri and Vaartaja, 1967; Deacon and Donaldson, 1993; Ko, 1998; Nelson, 1990; Nelson and Craft, 1989; Ruttledge and Nelson, 1997) contributing to an increase of disease incidence. Host penetration and infection takes place rapidly (within 24 h) giving the pathogen a competitive advantage over secondary colonizers (Paulitz and Baker, 1988; Stanghellini and Hancock, 1971b). Rey at. (2001) observed that pathogenesis of *P. ultimum* in hydroponic tomato cultures was likely based on fungal toxins and hydrolytic enzymes. *Pythium ultimum* grows extremely well in cold, wet conditions (Ayers and Lumsden, 1975) especially if they are suboptimal for the parasitized organism. Even small underground infections may be enough to significantly reduce host productivity, and disease severity in beans is increased as temperature is decreased and soil water is increased (Pieczarka and Abnawi 1978b).

Pythium ultimum can be controlled using seed fungicide treatments; however, these are either cost-prohibitive or unavailable for organic and developing world agriculture. The use of bio-control agents, such as *Tricoderma spp.* and *Enterobacter cloacae* have not been effective (Lifshitz et al., 1984) or have had unpredictable results (van Dijk and Nelson, 1998) and limited practical use. Plowing to bury infected crop residue is believed to help reduce disease incidence in heavily infected soils in New York and Maryland (Abawi, 1991; Lewis et al., 1983) but had no effect in Georgia fields (Sumner et al., 1986).

Host plant resistance to *P. ultimum* is the most effective long-term solution to limiting damage caused by this pathogen. Previous studies have identified sources of resistance to *P. ultimum* (Dickson and Abawi, 1974; Dickson and Boettger, 1977; York et al., 1977) in wild accessions of common bean and documented the genetic control as quantitative. Both authors reported the discovery of a resistant off-white-seeded line and its genetic control was examined in inheritance studies. They observed that the association between seedcoat color and resistance could be broken. However, there were no pure white-seeded cultivars documented as resistant to the fungus.

Resistance has been associated with the phenolic compounds and reducing sugars present in the exudates of germinating seeds and seedlings but other organic substances may also have a major role in the resistance mechanism (Aghihotri and Vaartaja, 1967; Kraft, 1974,). Kraft (1974) discovered that seedling exudates from resistant and susceptible peas had similar amounts of phenols. White-seeded beans are particularly susceptible to infection by *P. ultimum* and identification of resistance to this disease would help reduce damage from damping-off in commercial and developing world agriculture. Resistance has previously been associated with several colored bean accessions (Dickson and Petzoldt, 1988) but introgression of resistance has been difficult due to an interaction and/or linkage drag with white seedcoat color. Resistance has been reported in colored beans and there is the possibility of bringing it into white-seeded beans as well as using light-colored beans as an alternative to the conventional white-seeded cultivars. Identification of a white or cream-colored bean with *P. ultimum* resistance could be beneficial to the snap bean market and reduce the need for seed fungicides if it could be introgressed into a commercial cultivar.

While incomplete resistance was identified in snap beans, an interaction also existed between white-seeded beans and susceptibility to *P. ultimum* (Deakin, 1974; Dickson and Boettger, 1977; Dickson and Petzoldt, 1988). White-seeded beans typically exhibit green seedcoat color until the seeds are full size, which then disappears by the time the seeds are dry. However, color-seeded beans begin modifying their pigmentation when seeds reach half of their size (Baggett and Kean, 1984). Snap beans with colored seeds had high levels of *P. ultimum* resistance; however, the color was considered unacceptable for processing.

Off-white-seeded bean accessions exhibited lower disease severity symptoms following *P. ultimum* infection than white-seeded accessions, but when processed, color was observed in the can (Dickson and Boettger, 1979). Pure white-seeded beans are considered essential (particularly for processed snap beans) to avoid colored liquor in the can during processing. Development of white-seeded beans with *P. ultimum* resistance could be achieved through identification of new white sources that are not associated with *P. ultimum* susceptibility, or using an alternative approach to controlling white seedcoat color.

Color in *P. vulgaris* is controlled by eight genes with complex epistatic interactions (Bassett, 2002; Praken, 1972). The *pp* genotype at the P locus is universally used to create white-seeded beans. Double recessive genes at the P locus (*pp* genotype) automatically transform seedcoat color into white (Feenstra, 1960; Prakken, 1970, 1972, 1975) and this approach is used in all commercial snap bean cultivars. However, deleterious genes or pleiotropic interactions are associated with the *pp* genotype that may be responsible for decreasing performance of these plants (Bassett, 1994; Deakin, 1974; Dickson and Petzoldt, 1988). The *pp* genotype is important for white seedcoat color in snap bean cultivars, however; alternative approaches can be used including recessive genes at the C-, D-, and J-loci *(cc dd jj)* (Praken, 1970, 1972, 1975). The alleles c^{cr} or c^{u} for cartridge buff seedcoat color or p^{gri} for gray-white seedcoat color have been suggested by Bassett (1994, 1996), who characterized the c^{u} *d j* genotype as a synonym of white-colored beans. The cultivar Early Wax also has white seedcoat color that is not controlled by the *pp* genotype or by the C-, D-, J-loci (Dickson and Petzoldt, 1988).

The purpose of this study was to evaluate the core collection of *P. vulgaris* to examine differences in seed color types when chal-

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Table 1. Mean disease severity ratings (MDSR) of bean seedcoat colors in the USDA *P. vulgaris* core collection

Seedcoat color	Accessions (no.)	MDSR
Tan	2	1.25 d[z]
Pink	4	1.78 dc
Purple	1	2.00 c
Yellow	18	2.06 c
Black	97	2.09 c
Red	47	2.13 c
Cream	8	2.27 cb
Variegated	172	2.29 cb
Brown	74	2.45 cb
Grey	4	2.88 b
Green	3	2.96 b
White	20	3.68 a

[z]Mean separation by Duncan's multiple range test ($p \leq 0.01$).

Table 2. Mean disease severity ratings (MDSR) of the most resistant white– and cream-seeded beans.

Accession	MDSR
White-seeded	
PI 430203	1.50 e[z]
PI 527803	1.75 de
PI 290996	2.38 c–e
PI 299021	2.50 b–e
PI 194574	2.50 b–e
PI 304110	2.63 b–d
PI 310718	2.75 a–d
PI 415899	2.75 a–d
PI 198033	2.88 a–d
PI 189407	2.88 a–d
PI 152322	2.88 a–d
PI 207553	3.13 a–c
PI 415955	3.13 a–c
PI 215762	3.25 a–c
PI 415944	3.25 a–c
PI 415963	3.25 a–c
PI 198026	3.31 a–c
PI 415949	3.31 a–c
PI 282014	3.38 a–c
PI 207206	3.50 a–c
Cream-seeded	
PI 282028	1.00 f
PI 527705	1.00 f
PI 313727	1.00 f
PI 313660	1.00 f
PI 441828	1.00 f
PI 282089	1.00 f
PI 352744	1.00 f
PI 353758	1.00 f
PI 313852	1.00 f
PI 325732	1.38 ef
PI 337095	1.38 ef
PI 150409	1.38 ef
PI 415942	1.38 ef
PI 352750	1.38 ef
PI 151026	1.38 ef
PI 299020	1.63 d–f
PI 352759	1.75 d–f
PI 352748	1.88 d–f
PI 207129	2.00 c–f
PI 313601	2.00 c–f

[z]Mean separation by column using Duncan's multiple range test ($p \leq 0.01$).

lenged with *P. ultimum*, and to evaluate white and cream-seeded accessions for resistance to the disease.

Materials and Methods

Plant material. The USDA *P. vulgaris* core collection (Western Regional Plant Introduction Station, Pullman, Wash.) consists of 406 plant introductions (PIs) representing both the Andean and Middle-American gene pools. All 406 core-collection accessions were evaluated for resistance to *P. ultimum* together with 162 accessions that were white- or light-seeded (Western Regional Plant Introduction Station). Eight seeds of each accession were planted in 128-cell styrofoam trays with resistant and susceptible controls (Speedling, Sun City, Fla.) in 'Cornell Mix' (Boodley and Sheldrake, 1982) with one seed per cell. Due to the importance of the seedcoat integrity for resistance to *P. ultimum* all seeds with cracks or malformations were eliminated before planting.

Inoculation. Seeds were germinated in greenhouses at 24 ºC/21 ºC (14-h photoperiod) under 1000-W metal halide lamps (300 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at Cornell University's New York State Agricultural Experiment Station (NYSAES), Geneva, on 1 to 22 Nov. 2001 and 12 Jan. to 1 Feb. 2002. *Pythium ultimum* isolate P4 was obtained from George Abawi (Dept. Plant Pathology, Cornell University) and 2 ml of the hyphal segment suspension (1.2×10^8 propagules) was used to inoculate the seeds of 568 accessions on 1 Nov. 2001 and 12 Jan. 2002.

The pathogen cultures were maintained by periodic transfers on Potato Dextrose Agar (PDA) plates (2%) and incubated in a dark room at 24 ºC. Long-term storage was achieved using 2% corn-meal agar (CMA) slants infected with mycelia and covered with sterile oil. The isolate was grown for 4 d at 20 ºC and blended in diH_2O for 30 s.

The final concentration was adjusted to 6×10^7 hyphal segments per mL and 2 mL of this suspension was directly pipetted on to each individual seed. To optimize the infection the soil was kept moist during the entire experiment and *P. ultimum* was re-isolated and quantified from the soil/infected plant tissue following the experiment on 2% water agar (WA) plates prepared with the antibiotics ampicilin (250 $\mu g \cdot \mu L^{-1}$) and rifampicin (10 $\mu g \cdot mL^{-1}$). *Pythium ultimum* hyphal segments were counted using dilution and plating in PDA.

Evaluation. Plants were evaluated based on symptoms of aerial and underground infection using a rating scale of 1 to 4, where 1 = no observed symptoms and 4 = inability to germinate or emerge. Mean disease severity ratings (MDSR) were calculated for all accessions tested and results were grouped according to color class, initially split into 95 color classes and then segregated into 12 major color classes (Table 1) to evaluate potential associations of seedcoat color with resistance to *P. ultimum*. Means grouping were calculated using Duncan's multiple range test (SAS, 1997), and accessions rating 2.0 or lower were considered to be resistant.

Results

Beans were categorized into 12 groups according to seedcoat color. These groups exhibited variable responses to *P. ultimum* infection, with the tan and pink color groups showing the lowest mean disease severity ratings (MDSR) of 1.25 and 1.78 respectively (Table 1). However, these color groupings represented only 6 of the 406 accessions comprising the core collection. The most susceptible color class of the core collection comprised the white-seeded types with a MDSR of 3.68. No white-seeded accessions from the core collection exhibited resistance in all accessions when challenged with *P. ultimum*. However, cream-seeded accessions showed higher levels of resistance. Additional accessions representing white and cream-colored seeds were tested separately (Table 2). Of 568 accessions tested, 48 showed no disease symptoms (MDSR = 1.0) including nine of 39 cream-colored accessions tested (PI 282028, PI 527705, PI 313660, PI 313727, PI 441828, PI 282089, PI 352744, PI 353758, and PI 313852). Resistance to *P. ultimum* was observed in accessions from both the Andean and Middle American collections. Only 7 of 39 cream-seeded accessions tested were susceptible. Of 107 white-colored accessions, 65 were susceptible, and no white-seeded accessions were identified that were immune to *P. ultimum*. Accessions PI 430202, PI 527803, PI 290996, PI 299021, PI 194574 and PI 304110 exhibited high levels of resistance relative to other white-seeded types. The full table of results for this work will be added to the USDA GRIN database (http://www.ars-grin.gov/npgs/holdings.html).

The yellow-seeded class had a MDSR of 1.0; however, this evaluation was based on a single accession (PI 313661). The cream-colored accession PI 313852 exhibited a high level of resistance to *P. ultimum* and set a high number of pods of a size which might allow efficient introgression to commercial cultivars.

Discussion

This study showed that disease severity ratings vary throughout all color groups of common bean, and resistance could be found for all seed colors. White was the only seedcoat color from which an accession could not be identified with zero infected plants. This suggests that susceptibility may not be a function of seedcoat color but of other internal mechanisms that might interact with the pigmentation process, level of water imbibed by seeds, speed of emergence and level of root exudation. Identification of a resistant white-seeded type could be used to improve snap bean seed quality and resistance, as attempts at introgressing *P. ultimum* resistance from color-seeded sources have been unacceptable. The introgression of resistance identified from the cream-seeded accessions in this study may enable an alternative approach to breeding *P. ultimum* resistant snap beans, as cream could be an acceptable color for processing.

Pigmentation of colored seeds occurs in an early stage and induces an undesirable off-color to the canned product, which is the reason why processors prefer white-seeded cultivars. Although high levels of resistance were not observed in white-seeded accessions, several light colored PIs exhibited resistance when infected with *P. ultimum*. Light-seeded beans (cream, yellow, or tan) may be accept-

able for both fresh-market and canning snap beans if the pigment of the seed or pods does not significantly discolor the brine. Canning trials with light-colored beans could help to better understand the impact of pigments in the brine color. Dean (1968) suggested that a green color was an improvement over white in processed products, an alternative to the conventional quality standards. A green-seeded resistant accession (PI 313473) was identified that could potentially be used to introgress resistance into commercial cultivars.

Breeding of snap beans with cream seeds might allow the development of commercial cultivars with higher levels of *P. ultimum* resistance and avoid deleterious traits associated with white seeds such as poor quality and susceptibility to pests (Dickson and Boettger, 1977). Higher yields can also be obtained with color-seeded cultivars (Deakin, 1974) as they are more resistant to abiotic stress (Dickson and Petzoldt, 1988) and require less fertilizers or pesticides.

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