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Seven Diverse *Phaseolus* Germplasm Lines Derived through Interspecific Hybridization of Common Bean and Tepary Bean

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The Ohio Agricultural Research and Development Center announces the release of seven diverse Ohio *Phaseolus* germplasm (OPG) lines developed through interspecific hybridization between distinct common bean (*P. vulgaris* L.) parents and a subspecies cross between cultivated (*P. acutifolius* A. Gray var. *latifolius* Freeman) and wild (*P. acutifolius* var. *tenuifolius* A. Gray) tepary bean. The backcross lines were selected from populations that arose from crosses between a self-sterile interspecific hybrid and both small- and large-seeded recurrent common bean parents.

The tepary bean is a traditional crop produced primarily in semi-arid regions of the southwestern U.S. and northern Mexico (Pratt and Nabhan, 1988). It is a potential germplasm donor of highly desirable traits for improvement of common bean (Pratt, 1983; Pratt and Nabhan, 1985; Singh, 2001; Thomaset al., 1983). Tepary bean displays resistance to multiple pathogens and pests causing serious economic damage to common bean, as well as drought, salinity and heat stress tolerance (Singh, 2001). Deleterious traits in tepary bean that may pose drawbacks to successful integration of desirable traits include susceptibility to bean common mosaic virus (BCMV) (Pratt and Nabhan, 1985) and to white mold [causal agent *Sclerotinia sclerotiorum* (Lib.) de Bary] (personal observation). Tepary beans do not germinate well during cold weather (Thomas et al., 1983) may contain unusually high levels of lectins, and sometimes require prolonged cooking times (Kabbara et al., 1987). Indeterminacy, short-day photoperiodicity of Central American accessions (Pratt and Erickson, 1989), seed size smaller than most small-seeded varieties of common bean, and a slight tendency of pods to shatter prematurely (Pratt and Nabhan, 1985) may also present additional problems in selection of elite cultivars for particular environments or markets.

Pronounced reproductive barriers separate common and tepary beans, and embryo rescue is necessary to obtain interspecific hybrids (Sabja et al., 1990; Waines et al., 1988). The successful recovery of backcrosses from sterile hybrids also presents major challenges and different avenues for recovery of fertile backcrosses have been explored (Mejía-Jimenez et al., 1994; Pratt and Gordon, 1994; Thomas and Waines, 1984; Waines et al., 1988). Gene introgression into backcross populations using common bean as the recurrent parent has been studied by Pratt and Gordon (1994) and through congruity backcrossing by Mejia-Jimenez et al. (1994). Common bacterial blight [causal agent *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye = *X. axonopodis* pv. *phaseoli* (Smith)Vauterin et al.] resistance has been transferred from tepary to common bean (Scott and Michaels, 1992; Singh, 2001) but the transfer of more complex traits has not been reported.

The possibility of linkage drag caused by deleterious donor genes or chromosomal segments, or differential transmission of *P. vulgaris* alleles, are potential barriers to successful introgression of useful genes from related species, such as tepary bean, in the tertiary gene pool of common bean. Use of tepary bean germplasm resources might be increased if it could be demonstrated that desirable lines could be obtained from interspecific backcross populations. Instead of introgressing a single desirable trait from tepary bean into common bean, we wished to determine if it would be possible to select BC_2 and BC_3 populations (common bean recurrent parent) with high yield potential. An additional objective was to determine if linkages to negative traits associated with tepary bean would hinder successful selection. It is hoped that the findings and the germplasm resulting from this project will contribute to the long-term improvement and broadening of the gene-base of common bean.

Origin

Interspecific hybridization between diverse common bean genotypes and tepary bean was performed as described in Pratt et al. (1985). Backcrosses between self-sterile interspecific hybrids and *P. vulgaris* recurrent parents were undertaken as described by Pratt et al. (1985) and Pratt and Gordon (1994). The backcross lines arose from the interspecific hybrid designated F_1-3. It was a result of a cross between a *P. vulgaris* hybrid ('Sanilac'/Puebla152Bl) and an individual F_2 plant obtained from the cross between a cultivated and a wild tepary accession (PI 440790/MBAC). Additional backcrosses were made to diverse *P. vulgaris* parents (Pratt and Gordon, 1994; Pratt et al., 1985). Fifty seed, or as many as available, from eight S_0BC_2 and one S_0BC_3 ($S_0 = F_2$) populations were planted in the greenhouse and selected for fertility (production of at least 10 mature seed per plant). In 1992, about 300 $S_{0:1}$ progenies obtained from eight BC_2 populations, and one BC_3 population, were space-planted in single-row plots at the Ohio Agricultural Research and Development Center (OARDC) near Wooster. Selections were made in unreplicated nurseries within and across lines using the pedigree method through the S_3BC_2 and BC_3 generations. Selections were based on acceptable maturity, upright or short vine habit, and production of pods and seeds that remained sound until a delayed harvest was conducted in late October or early November. Replicated performance evaluations of 20 selected lines were initiated in 1995. Further selection of lines was conducted based on yield and the criteria mentioned previously. Bulked seed from selected lines were sorted based on seed characteristics and several color variant sister lines of S_4BC_2 and BC_3 lines were formed. Additional replicated performance evaluations and selections were conducted at one site in 1997 and at two sites in 2000. All tests conducted through 2000 were performed in Wayne County, Ohio in bordered plots with 76-cm row spacing except the 2000 test at Muddy Fork Farm which was planted in unbordered two-row plots with 30-cm row spacing. A random complete block experimental design was used in all replicated field evaluations. Selected S_6BC_2 and BC_3 lines were increased in the greenhouse in 2001.

In 2002 and 2003 evaluations of the responses to infection by *X. campestris* pv. *phaseoli*, causal agent of common bacterial blight, and agronomic characteristics, of the advanced BC lines were determined in Ontario, Canada. Four local strains, two of isolates *X. campestris* pv. *phaseoli* fuscans no. 12 and no. 118, and two of nonfuscans no. 18 and no. 98, were grown on yeast salts–agar (Dye 1968) for 48 h at 25 °C. A sterile distilled water suspension of bacterial

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Table 1. Origin of accessions used in interspecific *Phaseolus* hybridization and backcrossing program.

Accession	Origin	Market class
P. vulgaris		
GO-4115	C.I.A.T. accession	Black (small seed)
'Jamapa'	Mexico (Costa Rican accession)	Black (small seed)
'Masterpiece'	United Kingdom	Fresh market
'Pinquito'	U.S. (Calif.)	Brown specialty
Puebla 152Bl	Mexico	Black (large sd.)
'Sanilac'	U.S. (MI)	White Navy
UI-114	U.S. (Idaho)	Pinto
P. acutifolius		
PI 440790	Mexico (Sonora)	White (cultivated)
P. acut. var. acutifolius		
MBAC	U.S. (Arizona)	Mottled (wild)

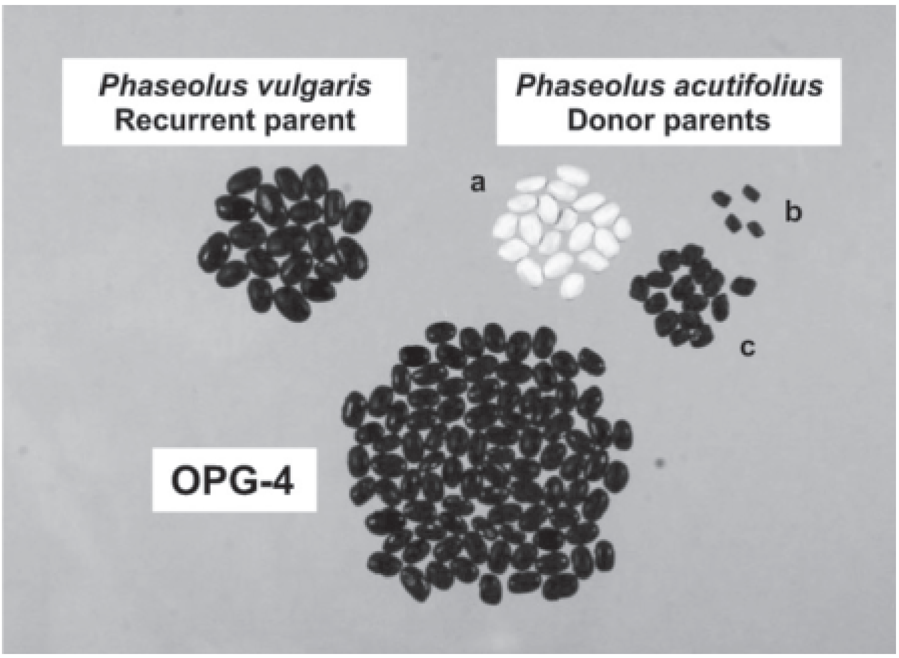

Fig. 1. Seed samples of the BC3 line OPG-4 derived from interspecific hybridization of *Phaseolus vulgaris* and a *P. acutifolius* subspecies cross. Also shown in the upper portion of the figure are the last *P. vulgaris* recurrent parent ('Jamapa'), the *P. acutifolius* donor parents (**a**) PI 440790 (cultivated) and (**b**) MBAC (wild), and (**c**) F2:3 seed obtained by self-pollination of the plant (F2-K2A) used as the parent in the original interspecific cross.

growth was made, adjusting concentration to 15 Klett units against a red filter in a Klett-Summerson colorimeter. Suspensions of the four strains were mixed in equal volume and 7 L were added to 190 L of water in a spray tank. The inoculum, containing 10^6 colony forming units/mL, was applied to 3-week-old plants at about 1333 kPa to ensure visible water-soaking injury using a spray boom. Disease severity assessments were made 3 and 6 weeks after inoculation of unreplicated single-row field plots. In 2003, agronomic characteristics of the advanced BC lines also were determined in an unreplicated field plot in Michigan through the courtesy of James Kelly.

Following the evaluations conducted over several years, seven advanced lines were selected for release. The selected lines OPG-1 and OPG-2 were selected from the BC_2 population (2-4), which had CIAT Go4115 as the first recurrent parent and 'Jamapa' as the second recurrent parent. The other five backcross lines were selected from the BC_3 population (1-2-1), which had Pinquito/Pinto UI-114)//'Masterpiece' as the first recurrent parent; 'Sanilac'/Puebla152Bl as the second recurrent parent; and 'Jamapa' as the third recurrent parent) (Table 1) (Figs. 1, 2, and 3).

Description and Performance

The yield of all released OPG lines was not significantly different ($P = 0.05$) from check cultivar 'Jamapa' in a combined ANOVA (Table. 2). Most lines displayed average lodging resistance and again no significant differences were detected between the parental check 'Jamapa' and any of the germplasm lines (Table 2). The lines displayed considerable variation in architecture, from upright bush (Type I) to prostrate vine (Type III) and this variation also was observed within some lines (Table 2). The lines also displayed varying degrees of indeterminacy, and some variation was observed for vine length among the indeterminate types (Type II), so this group was split into a and b subclassifications that were semi-determinate and indeterminate vining, respectively (Table

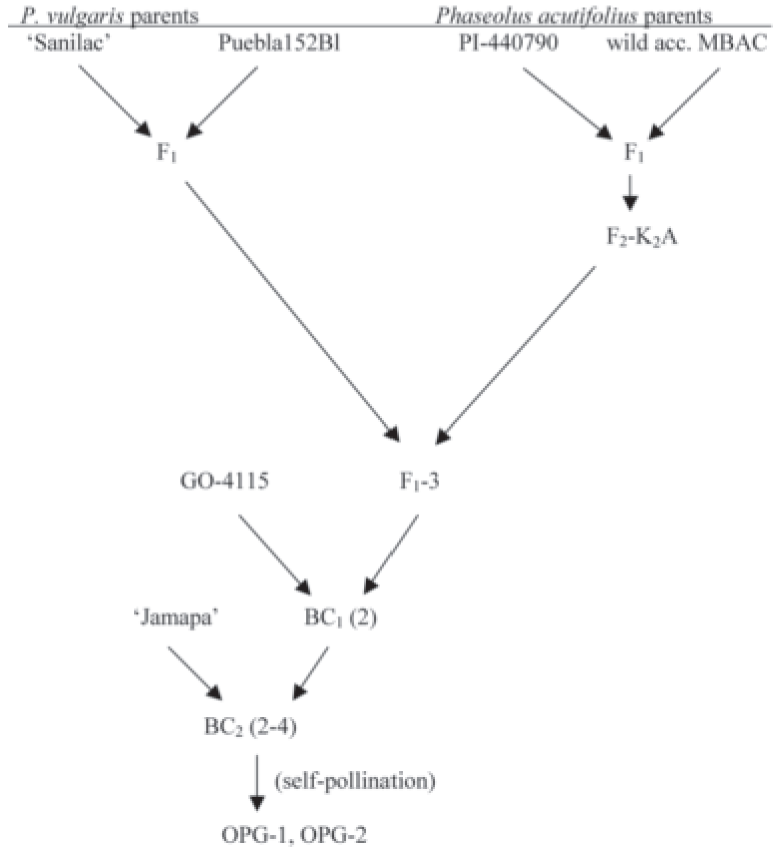

Fig. 2. Pedigree of Phaseolus germplasm lines OPG-1and 2.

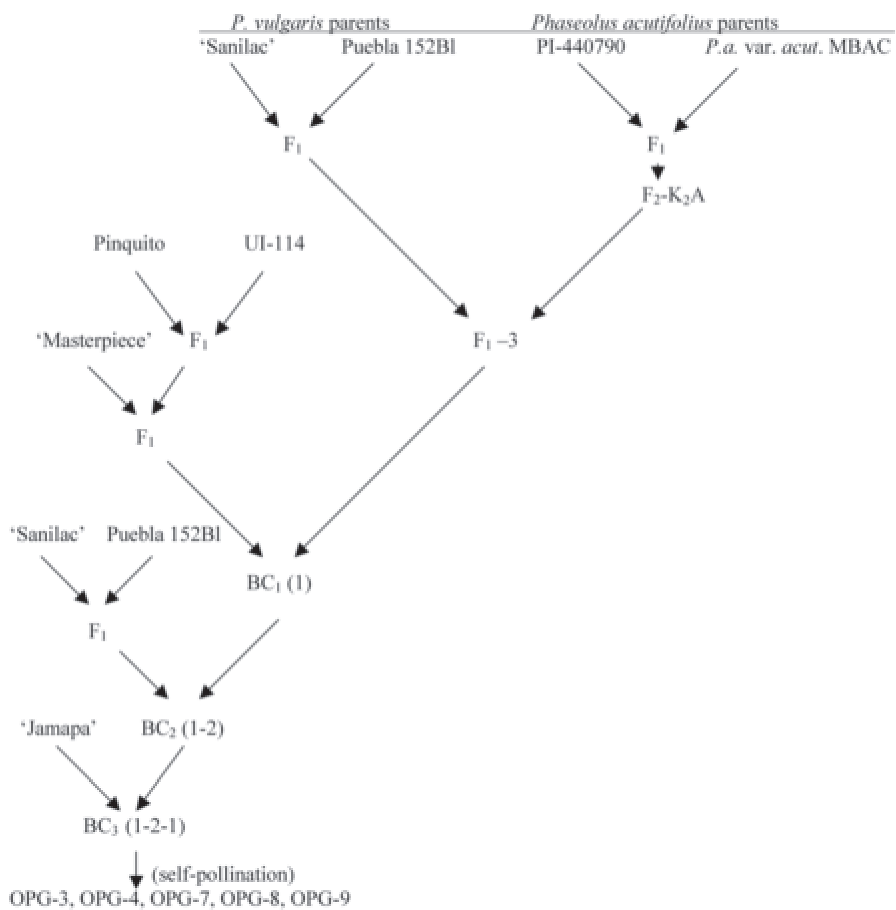

Fig. 3. Pedigree of Phaseolus germplasm lines OPG-3,4 and OPG-7,8, and 9.

2). The maturity of some lines (OPG 2, 7, and 9) is suitable for the Great Lakes region whereas others are too late-maturing and may be better suited elsewhere (Table 2).

The seed colors and patterns displayed by the lines are highly diverse—ranging from white, light to dark brown, pinto-like variegation, pink and red, to black and mottled. (Table 2). Seed coat color variation is apparent in some of the lines (especially OPG-3) because it has not been possible to fix the predominant color type. Most seed weights are comparable to those of the small-seeded to medium weight common beans (Table 3). Water imbibition and cookability of seeds stored at room temperature for one year was determined according to the methods of Jackson and Varianno-Marston (1981). All lines displayed water imbibition and cookability properties similar to those of 'Jamapa' and commercially available checks, with the exception of OPG-1, which was somewhat slower to imbibe water.

It has been confirmed that the tepary bean parents used in this study demonstrated highly resistant/immune responses when screened using a mixture of several *X. campestris* pv. *phaseoli* isolates from southern Ontario. Evaluations conducted in Ontario during two seasons using several local isolates revealed that some of the lines display resistance to infection by *X. campestris* pv. *phaseoli*, but that resistance is segregating within lines (Table 2). Susceptible plants were present in the partially inbred line obtained from the F_2 tepary parent used to produce the interspecific hybrids, suggesting PI 440790 and MBAC were not allelic for CBB resistance. Both segregation within accessions and multiple alleles for resistance in tepary bean has been previously demonstrated by Urrea et al. (1999). Directed selection for resistance in the lines may provide additional genes for pyramiding resistance in commercial common bean cultivars (Singh, 2001).

The lines were planted for seed increase and purification in 2001. Seed harvested from these plots were grown in the greenhouse during the winter of 2001–02 to confirm plant health and produce seed in a disease free environment.

Availability

Samples of seed may be obtained for a fee from Richard C. Pratt, Department of Horticulture and Crop Science, OSU/OARDC, 1680 Madison Ave. Wooster, OH 44691. One sample will be provided without charge to non-profit breeders and researchers. A material transfer agreement (MTA) will provide guidelines for use of the germplasm.

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Table 2. Yield and agronomic characteristics of seven dry bean germplasm lines and a varietal check cultivar grown over 5 locations in Ohio (1995–2000), two seasons in Ontario, Canada (2002–2003) and during one season in Michigan (2003).

Entry	Mean yield (Mg ha^{-1})[z]	Mean lodging score (1–9)[y]	Arch type[x]	Mean days to maturity[w]	Mean common bacterial blight response(s)[v]
OPB-1	2.72	6.0	IIaIII	104	MS/S
OPG-2	2.72	4.7	IIb	99	S
OPB-3	3.05	4.6	IIb/III	103	MR/S
OPG-4	2.92	5.2	I/III	101	R/S
OPB-7	2.72	5.0	III	98	MR/S
OPG-8	2.50	4.9	IIb/III	100	R/S
OPG-9	3.13	5.2	III	97	S
Jamapa	2.57	3.2	IIa	96	S
Dresden	---	---	---	---	S
Xan159	---	---	---	---	R/MR
HR67	---	---	---	---	R
PI-440790					I
$F_{2:6}$-A K2					MS

[z]Yield data were not collected in Ontario. All yield plots were replicated except the Michigan plot of 2003. ANOVA for genotype main effect on yield was not significant at $P = 0.05$ level. Mean grouping for alpha = 0.10.

[y]Lodging data (1 = erect while 9 = 100% lodged) were obtained from two trials in Ohio and one in Michigan. ANOVA for genotype main effect on lodging was not significant at $P = 0.05$ level.

[x]Architectural type (Type I = determinate, upright growth habit; Type IIa = indeterminate, upright growth habit, short vines; Type IIb = indeterminate, upright growth habit, terminal guide of varying length and climbing ability; Type III = indeterminate, prostrate vining habit) and days to maturity data were not collected in Ohio.

[w]Days to maturity = days from planning to the time all plants in plot have reached harvest maturity

[v]Common bacterial blight ratings reflect the responses to artificial inoculation observed for each line during 2002 and 2003 seasons in Ontario. I – Immune, R = resistant, MR = moderately resistant, MS = moderately susceptible, and S = susceptible. Controls were planted only in 2003 season.

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Table 3. Seed characteristics and cookability of seven diverse Ohio *Phaseolus* germplasm lines.

		Cookability			
Line	Color or class	Imbibition (% wt)[z]	Firmness (g resistance)[y]	Visual quality[x]	100 Seed wt (g)[w]
OPG-1	Black (small seed)	86	Fully cooked	1.5	20.2 bcd[v]
OPG-2	Black (small seed)	99	Fully cooked	1.3	18.7 d
OPG-3	Mixed	103	Fully cooked	1.4	23.9 ab
OPG-4	Black, black mottled	---	---	1.4	18.4 cd
OPG-7	Brown mottled	97	197	1.0	22.6 bc
OPG-8	Brown	117	147	1.0	27.8 a
OPG-9	Brown mottled	93	159	---	27.8 a
Jamapa	Black (small seed)	97	Fully cooked	1.8	20.0 bcd
Raven	Black (small seed)	98	Fully cooked	---	18.0
Black Hawk	Black (small seed)	109	Fully cooked	---	13.4
Commercial Navy	White Navy	102	97	---	26.3
Commercial Black	Black (small seed)	105	195	---	20.7
PI 440790	White, light green	---	---	1.0	13.4
$F_{2:6}$-A K2	Black	---	---	1.0	8.7

[z]Imbibtion was performed for 60 min using one sample of 100 seed.

[y]Firmness (cookability) evaluations were conducted using 15 samples with one seed per sample. Time to cook was determined using a bean cooker described by Jackson and Varianno–Marston (1981). Following 1 h of cooking, firmness tests were performed with an Instron penetrometer.

[x]Visual evaluations of seed quality on a 1 to 5 scale (1 is the best) were made from one plot observed in Ontario during 2003.

[w]100 seed weight data of OPG lines, Jamapa, and the tepary parents are the mean of values obtained from two locations in Ohio and one in Michigan. Only replicated samples of the OPG lines and the recurrent parent (Jamapa) were tested using Fisher's LSD mean comparisons test. Means within a column followed by the same letter are not significantly different. All other 100 seed weight values were obtained in the cookability study only.