Genetic Diversity of Snap Bean Cultivars Determined Using Randomly Amplified Polymorphic DNA (RAPD) Markers

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Abstract: DNA extractions from 77 snap bean and 2 dry bean cultivars were evaluated for molecular polymorphisms. In total, 100 10-mer oligonucleotide primers were evaluated, and 31 primers that amplified clear and repeatable polymorphisms among bean cultivars were selected. These primers amplified a total of 49 polymorphisms between the cultivars and were used to differentiate the cultivars and evaluate the genetic diversity between them. All cultivars were clustered according to genetic similarities using GenStat 5.0 software, and groupings of pod types were observed when cultivars were separated based on a dissimilarity index. The RAPD polymorphisms will be useful for cultivar determination, seed purity testing and estimation of genetic distances.

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Common bean (*Phaseolus vulgaris* L.) is one of the most widely cultivated legumes worldwide. They are harvested fresh as snap beans or as dry bean crops. Most snap beans and some dry beans originate from Andean

(Chile, Peru, Nueva Granada) areas of domestication, while most dry bean types have a Middle America (Jalisco, Durango, Meso-America) area of domestication (Gepts et al., 1986). These types can be determined based on phaseolin seed protein when separated by SDS polyacrylamide gel electrophoresis (Koenig et al., 1990). Common beans of Andean origin are characterized as T-, C-, H- and A-types, while beans of Middle America origin are S- and B-types (Gepts et al., 1986; Singh et al., 1991). Snap beans are marketed under different classes that include large sieve green beans, yellow/golden wax beans, flat podded romano beans and small sieve whole beans. The genetic diversity of common beans has previously been investigated, but has focused primarily on dry bean market classes (Beebe et al., 1995; Metais et al., 2000; Skroch et al., 1998). Investigation of genetic similarities among cultivars within or between market classes can help to determine variety identification (Graham et al., 1994), variability between gene pools (Haley et al., 1994; Johns et al., 1997), pedigree (Alzate-Marin et al., 1996), and choice of parents to maximize genetic distance (Machado et al., 2000).

Genetic diversity between common bean cultivars has been estimated using several types of molecular markers including RAPD, restriction fragment length polymorphisms

Table 1. Source and pod type of common bean cultivars used to determine genetic diversity

Cultivar	Source	Type	Cultivar	Source	Type
Acclaim	Seminis	gb ²	Moncayo	Seminis	rb
Amboto	Syngenta	gb	Marseille	Harris Moran	wb
Aurora	Zwaan	db	Masai	Syngenta	wb
Baby Bop	Seminis	wb	Medinah	Syngenta	wb
Beany Baby	Seminis	wb	Mercury	Syngenta	gb
Bench Mark	Syngenta	gb	Minuette	Harris Moran	gb
Benton	Syngenta	gb	Mirada	Syngenta	gb
Blue Crop	Harris Moran	gb	Nicelo	Seminis	gb
Bonanza	Seminis	gb	Normandie	Harris Moran	wb
Brio	Seminis	gb	Opus	Seminis	gb
Calgreen	Syngenta	gb	Pix	Seminis	wb
Capricorn	Syngenta	gb	Podsquid	Seminis	gb
Castano	Syngenta	gb	Probe	Harris Moran	gb
Celtic	Syngenta	wb	Producer	Harris Moran	gb
Charon	Syngenta	gb	Prosperity	Harris Moran	gb
Conella	Syngenta	gb	Rhapsody	Harris Moran	gb
Crest	Seminis	gb	Roma II	Syngenta	rb
Dandy	Syngenta	wb	Romano	Harris Moran	gb
Daytona	Harris Moran	gb	Seafarer	Michigan St.U	db
Derby	Harris Moran	gb	Seville	Syngenta	gb
Earlybird	Maffet Seed	gb	Shade	Harris Moran	gb
Envy	Harris Moran	gb	Slenderette	Syngenta	wb
Espada	Harris Moran	gb	Slenderwhite	Syngenta	wb
Flevoro	Seminis	wb	Slimgym	Syngenta	wb
Flo	Seminis	gb	Sonata	Harris Moran	gb
Fury	Seminis	gb	Sprite	Seminis	gb
Gator Green	Harris Moran	gb	Stretch	Seminis	gb
Gold Mine	Seminis	wx	Summit	Syngenta	gb
Golden Rod Wax	Harris Moran	wx	Sunburst	Seminis	wx
Hercules	Seminis	gb	Symphony	Harris Moran	gb
Hialeah	Harris Moran	gb	Tapia	Seminis	gb
Hystyle	Harris Moran	gb	Tenderlake	Harris Moran	gb
Igloo	Pure Line	gb	Teseo	Syngenta	gb
Indy Gold	Syngenta	wx	Titan	Seminis	gb
Jade	Syngenta	gb	Trueblue	Harris Moran	gb
Labrador	Seminis	gb	Valiant	Syngenta	gb
Laureat	Seminis	wb	Vitagreen	Syngenta	gb
Leon	Syngenta	gb	Yukon	Pop Vriend	wx
Legion	Seminis	gb	Zeus	Seminis	gb
Majestic	Syngenta	wx, wb			

²wb = Whole bean, wx = wax bean, rb = romano bean, db = dry bean, gb = green bean.

(RFLPs), sequence characterized amplified regions (SCARs), amplified fragment length polymorphisms (AFLPs), and simple sequence repeats (SSRs)/microsatellites (Bartish et al., 2000; Bautista et al., 2002; Gaitan-Solis et al., 2002; Lu et al., 1997; Steiger et al., 2002). Molecular markers can generate important information for germplasm clarification, cultivar identity, pedigree relationships and seed purity. The use of RAPD markers (Davis and Myers, 2002; Skroch et al., 1992;) is preferable as the relative cost is low and the availability of SCARs, SSRs, and expressed sequence tags (ESTs) for *P. vulgaris* is limited.

The evaluation of RAPD marker polymorphisms among snap bean cultivars is important for determining dissimilarities between the different classes of snap bean, and characterizing genetic variability among snap bean cultivars in general. In this study, we evaluated RAPD marker data to characterize snap bean cultivars with respect to genetic similarity and provide a molecular basis for variety identification of snap beans.

Materials and Methods

Germplasm. 77 snap bean cultivars and two dry bean cultivars ('Aurora' and 'Seafarer') were included in this study (Table 1). The cultivars included two romano bean cultivars, six wax bean cultivars, two dry bean cultivars, and 14 whole bean cultivars. The remaining 55 cultivars were considered to be large sieve, green bean types. All beans were planted in 32-cell (125-cm³) Styrofoam trays ('Speedling', Sun City, Fla.) in 'Cornell Mix' (Boodley and Sheldrake, 1982) with one seed per cell on 1 July 2002. Plants were grown to the first trifoliate, which was removed for genomic DNA extraction 14 d after planting. The cultivars were grown in a greenhouse at 23/20 °C day/night with a 14-h photoperiod under 1000-W metal halide lamps (300 µmol·m⁻²·s⁻¹).

DNA procedures. DNA was extracted and bulked from the terminal leaflets of the first trifoliate leaf of four plants for each cultivar (Doyle and Doyle, 1990). DNA pellets were resuspended in 500 µL of T.E. (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) and stored at -20 °C. Random fragments of DNA were amplified using single, 10-mer, oligonucleotide primers (Biotechnology Lab., University of British Columbia, Vancouver, B.C.), in a modified polymerase chain reaction (PCR) buffer (0.5 M Tris pH 8.3, 10 mM MgCl₂, 10 mM Tartrazine, 14% w/v Ficoll). RAPD polymorphisms were generated using standard PCR procedures (Williams et al., 1990) for 45 cycles in a gradient thermal-cycler (Brinkman instruments Inc., Westbury, N.Y.). Each cycle consisted of 60 s at 94 °C, 60 s at 35 °C, and 90 s at 72 °C. Fragments were separated by gel electrophoresis on a 1.5% agarose gel at 100V for 1 h in 1× Tris-Acetic Acid-EDTA (TAE) buffer. Gel banding patterns were visualized under ultraviolet light after staining with ethidium bromide (4 ng·mL⁻¹) using a Gel Doc 2000 system and pictures were digitally recorded with Quantity One software (Bio-Rad Laboratories, Hercules, Calif.). Ge-

netic differences were scored based on the presence or absence of polymorphisms generated by 31 primers for the 79 cultivars tested. The 49 polymorphisms scored represented RAPD bands that were clear and repeatable.

Data analysis. Genetic similarities were estimated from the genotypic data using a simple matching coefficient as described by (Sneath and Sokal, 1973). The 79 cultivars were clustered based on a matrix of genetic similarities using the clustering algorithm with GenStat 5.0 software. The dissimilarity index measured the mean genetic distance between a cluster and all other clusters.

Results

In total, 49 polymorphisms were identified based on amplification of the DNAs of the 79

common bean cultivars (Table 2). The range of frequencies of these polymorphisms varied from 5% to 92.5% and provided molecular information for determining genetic similarities of the cultivars. A cluster analysis was performed based on the 49 polymorphic loci and used to generate a dendrogram (Fig. 1), which formed six major clusters, one of which (cluster [3]) contained a single variety 'Sprite'. The dendrogram separated the two S-type phaseolin dry bean cultivars from the Mesoamerican gene pool ['Aurora' (small white) and 'Seafarer' (navy bean)] at the 0.75 dissimilarity index (cluster [1]). Most small sieve whole bean cultivars were separated at the 0.62 dissimilarity index (cluster [2]). The two romano beans shared the same cluster along with four large sieve cultivars. The majority of large sieve cultivars were clustered together (clusters [4], [5], and [6]). Certain

Table 2. Estimated size and frequency of polymorphisms generated by primers between the 79 common bean varieties.

Primer no.	Frequency (%)	Polymorphism length (bp)
² UBC 101	28.8	825
UBC 102	48.8	850
UBC 104 a	82.5	825
UBC 104 b	87.5	650
UBC 106	85.0	500
UBC 110 a	12.5	1500
UBC 110 b	76.3	610
UBC 111	82.5	425
UBC 112 a	16.3	1650
UBC 112 b	56.3	1500
UBC 114	83.8	1400
UBC 119	86.3	110
UBC 120 a	53.8	1625
UBC 120 b	23.8	1500
UBC 132	30.0	1600
UBC 134 a	82.5	1700
UBC 134 b	5.0	600
UBC 137	78.8	1300
UBC 145 a	53.8	1400
UBC 145 b	70.0	750
UBC 146 a	78.8	1400
UBC 146 b	57.5	1100
UBC 146 c	26.3	500
UBC 147	47.5	1050
UBC 148 a	81.3	2000
UBC 148 b	57.5	1650
UBC 150 a	15.0	1250
UBC 150 b	55.0	1050
UBC 150 c	17.5	675
UBC 151	48.8	725
UBC 154	38.8	1500
UBC 156	92.5	1100
UBC 157 a	50.0	800
UBC 157 b	12.5	550
UBC 158	51.3	1500
UBC 165 a	45.0	1400
UBC 165 b	46.3	1200
UBC 165 c	28.8	825
UBC 174	78.8	850
UBC 175	86.3	1350
UBC 177	26.3	1450
UBC 185 a	87.5	1625
UBC 185 b	5.0	1150
UBC 186 a	27.5	2000
UBC 186 b	62.5	1690
UBC 186 c	91.3	750
UBC 194 a	81.3	650
UBC 194 b	10.0	550
UBC 199	43.8	1450

²UBC = University of British Columbia.

Fig. 1. Dendrogram showing genetic dissimilarities of 79 common bean varieties and formation of six clusters; wb = whole bean, wx = wax bean, db = dry bean, rb = romano bean, se = Seminis vegetable seeds, hm = Harris Moran vegetable seeds, sy = Syngenta vegetable seeds, pls = Pure Line seeds, pv = Pop Vriend.

classes had high levels of genetic similarity, such as, the wax beans 'Yukon', 'Sunburst', and 'Goldmine'. The only cultivars that could not be separated based on the 49 polymorphisms were 'Podsquad' and 'Opus', which are both marketed by Seminis vegetable seeds and have similar horticultural characteristics.

Discussion

Snap beans are common beans developed primarily from the Andean (T-type) gene pool (Gepts and Bliss, 1985). This study evaluated genetic variation between cultivars and classes of snap beans. The two dry bean cultivars that were included in this study were clearly separated from the snap bean cultivars based on genetic dissimilarity. The study was also able to differentiate the majority of the small-sieve genotypes into a single cluster that contained only one larger-sieve type ('Capricorn'). It is likely that these cultivars share a common pedigree that is more closely associated with the two dry bean cultivars than the majority of snap bean cultivars. This suggests that many of the small-sieve cultivars have a higher genetic similarity to the dry bean cultivars developed out of the Middle American gene pool, and may be based partly on hybridizations to germplasm from this gene pool. Determination of phaseolin type of these whole bean cultivars would be informative in determining whether they contain Middle American material in their backgrounds. The six wax beans evaluated were not separated from the majority of snap bean cultivars, suggesting that this phenotype does not involve a high degree of genetic dissimilarity. However, the wax beans were all closely related, with 'Yukon', 'Sunburst' and 'Goldmine' forming a tight cluster, and 'IndyGold' and 'Golden Rod' showing very close similarity. These associations are probably related to pedigree as are the associations of many of the snap beans in this study. The small-sieve wax bean 'Majestic' was less closely related and was clearly identified from all other cultivars tested with a genetic dissimilarity of 0.28.

These results identified common molecular polymorphisms in different classes of snap beans that highlight similarities in their breeding pedigrees. The results also provide information to enable wide crosses of cultivars while maintaining the intended pod type. The

use of RAPD markers in this study has demonstrated the benefits of molecular markers in identifying genetic diversity with the snap bean market class. The information documented can also be used in determining similarity of cultivars developed by different breeders/seed companies, in determining seed purity of seed lots and differentiating cultivars if errors have been made in distribution.

This research will help breeders when choosing parents for use in crosses, and will help to exploit the genetic variation that exists in snap bean germplasm. The evaluation of plant introductions at germplasm repositories using these markers would also help in determining duplications and the extent of genetic diversity represented in the common bean core collection.

Literature cited

- Alzate-Marin, A.L., G.S. Baia, S.M. Filho, T.J. de Paula, Jr., C.S. Sedyama, E.G. de Barros, and M.A. Moreira. 1996. Use of RAPD-PCR to identify true hybrid plants from crosses between closely related progenitors. *Braz. J. Genet.* 19:621-623.
- Bartish, I.V., L.P. Garkava, K. Rumpunen, and H. Nybom. 2000. Phylogenetic relationships and differentiation among and within populations of *Chaenomeles* Lindl. (*Rosaceae*) estimated with RAPDs and isozymes. *Theor. Appl. Genet.* 101:554-563.
- Bautista, R., R. Crespillo, F.M. Canovas, and N.G. Claros. 2002. Identification of olive-tree cultivars with SCAR markers. *Euphytica* 129:33-41.
- Beebe, S.E., I. Ochoa, P. Skroch, J. Nienhuis, and J. Tivang. 1995. Genetic diversity among common bean breeding lines developed for Cental America. *Crop Sci.* 35:1178-1183.
- Boodley, J.W. and R. Sheldrake, Jr. 1982. Cornell peat-lite mixes for commercial plant growing. *N.Y. Agr. Expt. Sta. Agr. Info. Bul.* 43.
- Davis, J. and J.R. Myers. 2002. Phylogenetic analysis of snap beans using RAPD markers. *Annu. Rpt. Bean Impr. Coop.* 45:16-17.
- Doyle, J. and J. Doyle. 1990. Isolation of plant DNA from fresh tissue. *Focus* 12:13-15.
- Gaitan-Solis, E., M.C. Duque, K.J. Edwards, and J. Tohme. 2002. Microsatellite repeats in common bean (*Phaseolus vulgaris*): isolation, characterization and cross-species amplification in *Phaseolus* spp. *Crop Sci.* 42:2128-2136.
- Gepts, P. and F.A. Bliss. 1985. F₁ hybrid weakness in the common bean: Differential geographic origin suggests two gene pools in cultivated bean germplasm. *J. Hered.* 76:447-450.
- Gepts, P., T.C. Osborn, K. Rashka, and F.A. Bliss. 1986. Phaseolin protein variability in wild forms and landraces of the common bean (*Phaseolus vulgaris*): Evidence for multiple centers of domestication. *Econ. Bot.* 40:451-468.
- Graham, G.C., R.J. Henry, and R.J. Redden. 1994. Identification of navy bean cultivars using random amplification of polymorphic DNA. *Austral. J. Expt. Agr.* 34:1173-1176.
- Haley, S.D., P.N. Miklas, L. Afanador, and J.D. Kelly. 1994. Random amplified polymorphic DNA (RAPD) marker variability between and within gene pools of common bean. *J. Amer. Soc. Hort. Sci.* 119:122-125.
- Johns, M. A., P. W. Skroch, J. Nienhuis, P. Hinrichsen, G. Bascur, and C. Munoz-Schick. 1997. Gene pool classification of common bean landraces from Chile based on RAPD and morphological data. *Crop Sci.* 37:607-613.
- Koenig, R., S. P. Singh, and P. Gewpts. 1990. Novel phaseolin types in wild and cultivated common bean (*Phaseolus vulgaris* Fabaceae). *Econ. Bot.* 44:44-50.
- Lu, J., M.R. Knox, M.J. Ambrose, J.K. Brown and T.H. Ellis. 1997. Comparative analysis of genetic diversity in pea assessed by RFLP- and PCR-based methods. *Theor. Appl. Genet.* 93:1103-1111.
- Machado, G.F., J.B. Dos Santos, G.H. Nunes, and J.M. Duarte. 2000. Efficiency of genetic distance based on RAPD markers for choosing parents of common bean. *J. Genet. Breed.* 5:251-258.
- Metais, I., C. Aubry, B. Hamon, and R. Jalouzot. 2000. Description and analysis of genetic diversity between commercial bean lines (*Phaseolus vulgaris* L.). *Theor. Appl. Genet.* 101:1207-1214.
- Singh, S.P., R. Nodari, and P. Gepts. 1991. Genetic diversity in cultivated common bean. I. Allozymes. *Crop Sci.* 31:19-23.
- Skroch, P.W., J. Tiang, and J. Nienhuis. 1992. Analysis of genetic relationships using RAPD marker data, p. 26-30. In: Applications of RAPD technology to plant breeding. Joint Plant Breeding Symp. Ser., CSSA, ASHS, AGA, 1 Nov. 1992, Minneapolis, Minn.
- Skroch, P. W., J. Nienhuis, S. Beebe, J. Tohme, and F. Pedraza. 1998. Comparison of Mexican common bean (*Phaseolus vulgaris* L.) core and reserve germplasm collections. *Crop Sci.* 38:488-496.
- Sneath, P.H.A. and R.R. Sokal. 1973. Numerical taxonomy. W.H. Freeman Co., San Francisco.
- Steiger, D.L., C. Nagai, P.H. Moore, C.W. Morden, R.V. Osgood, and R. Ming. 2002. AFLP analysis of genetic diversity within and among *Coffea arabica* cultivars. *Theor. Appl. Genet.* 261:627-634.
- Williams, S., A. Kubelik, K. Livak, J. Rafalski, and S. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nuc. Acids Res.* 18:6531-6535.