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Random amplified polymorphic DNA variation within and among bean landrace mixtures (*Phaseolus vulgaris* L.) from Tanzania

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Summary

Genetic characterization of 51 individual pure lines from 13 landraces of three common bean (*Phaseolus vulgaris* L.) mixtures from the southern highlands of Tanzania was undertaken using random amplified polymorphic DNA (RAPD) analysis. A dendrogram generated by cluster analysis from data derived from fragments amplified by 12 random 10-base primers divided the bean individuals onto two main branches with less than 60% genetic similarity. Branches A and B subdivided into two and four clusters, respectively. Mixture 2, comprising three landraces, was the most uniform, most plants appearing on cluster 4 of branch B. Three of the four landraces of mixture 1 appeared on cluster 3 of branch B while the fourth landrace appeared on major branch A. Mixture 3 showed the greatest genetic variation with components appearing on both major branches. The clear separation of the 13 landraces onto two main branches of the dendrogram together with phenotypic characters, notably variation in bean size, suggests that the two groups might represent two distinct gene pools of *P. vulgaris*.

Introduction

The common bean *Phaseolus vulgaris* L. is the most important legume grown for food in eastern and southern Africa, providing 40% of the protein intake of the population (Allen, 1983). The predominant production system for the common bean in this region is subsistence agriculture which is characterised by diversity, both within and between crops. Such systems promote diversity of diet and income source, stability of production, reduced insect and disease damage, intensification of production with limited resources and minimization of risk (Clawson, 1985; Smithson & Lenné, 1996).

Farmers in eastern and southern Africa commonly grow and manage landraces of beans (genetically diverse, discretely recognisable phenotypes (based on seed colour and size) in which deliberate human selection has been imposed on natural selection pressures usually over considerable time) and, usually as varietal mixtures of beans (mixtures of landraces alone or with improved varieties) (Wood & Lenné, 1997). These mixtures show diversity of seed colours, patterns, shapes and sizes (Martin & Adams, 1987). The number of landraces in a mixture may vary from 2–30, however, usually three varieties account for 50–90% of each mixture (Voss, 1992). Differing morphological characters are used by farmers as markers for taste, texture, yield, storage characters, resistance to environmental stresses, use and maturity time (Wood & Lenné, 1993).

A study by Teverson et al. (1994) assessed the degrees of resistance to major bean diseases by individual phenotypes of three mixtures of bean landraces from the southern highlands of Tanzania. The results obtained show that considerable variation for resistance to five major diseases exists within and between these various landraces and that the mixtures are a rich source of both single and multiple disease resistances. They emphasised the importance of identifying and quantifying the inherent contribution of these landraces to reducing disease on-farm before attempting

to improve traditional mixtures for disease resistance (Davis & Panse, 1992; Teverson et al., 1994).

Although disease resistance is one of the important parameters targeted by plant breeders in bean improvement, especially in eastern and southern Africa, it is clear that a comprehensive evaluation of the genetic diversity that exists both within landraces, within mixtures and between mixtures is required in order that improvement is not achieved at the expense of sacrificing existing genetic diversity (Teverson et al., 1994). In the past, it has been possible to characterise such genetic diversity using a combination of both morphological and agronomic traits. However, the effectiveness of such an approach is limited by the fact that it requires accurate information on the amount of diversity present within the gene pools, as well as of the special distribution of diversity in relation to ecogeographic factors (Waugh et al., 1992). Modern advances in biochemistry and molecular biology have, however, allowed the development of rapid, sensitive and specific screening methods with which to study the genetic diversity of, and relatedness between, individuals.

In the case of bean, considerable effort has been directed at analysis of variation in electrophoretic patterns of seed proteins, especially phaseolin (Gepts, 1993). Sprecher (1988) successfully used additional isozymes to characterize landraces of common bean from Malawi. In recent years, molecular techniques including restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNA (RAPDs) analyses have been used to characterize variability in *Phaseolus* spp. Such studies have included the genetic variation among seven *Phaseolus* taxa and their phylogenetic relationship (Schmit et al., 1993), phylogenetic relationships among taxa belonging to the *Phaseolus vulgaris* complex (Llaca et al., 1994) and the ecogeographic distribution of *Phaseolus* spp. in Bolivia (Freyre et al., 1996). Two studies have been carried out on the genetic relationships among cultivars and landraces of lima bean (*Phaseolus lunatus*) from the Americas and the Caribbean (Nienhuis et al., 1995; Fofana et al., 1997). Studies of mitochondrial DNA polymorphisms in Malawian common bean lines divided the lines into two groups of small- and large-seeded beans corresponding to the Mesoamerican and Andean gene pools (Khairallah et al., 1990).

The aim of this project was to study, using RAPDs, the genetic diversity of different bean phenotypes selected on the basis of seed characteristics and which were landrace components of representative mixtures grown in the southern highlands of Tanzania.

Materials and methods

Plant material

Three bean mixtures were used in this study. They were selected as being representative of the diversity present among mixtures collected from farmers in the southern highlands of Tanzania in 1991 (Teverson et al., 1994). The three mixtures were made up of 7–15 landrace phenotypes based on seed colour and size (Teverson et al., 1994); only those which comprised more than 7% of the original mixtures, based on 2000 seeds, were selected for study. Pure stands of each of 13 phenotypes from the three mixtures were grown at Horticultural Research International, Wellesbourne, Warwick, CV35 9EF and pure lines of each phenotype were produced. Seeds from five of the pure lines were collected at random (J. Taylor, pers. commun., 1995). Phenotype numbers and seed characteristics of 51 pure lines which were available for this study are given in Table 1.

Three seeds of each individual pure line from each chosen phenotype of the three mixtures were sown in 20 cm pots in John Innes No. 3 compost and grown to the 4–5 leaf stage under glasshouse conditions.

DNA extraction

Three leaves were harvested from each plant and immediately frozen in liquid nitrogen, pulverized and lyophilized. The freeze-dried material was ground to a fine powder and stored at $-20\,^{\circ}C$. The leaf material from the three plants of each individual of each pure line of a landrace served as replicates.

DNA was extracted from the powdered leaf material using CTAB buffer according to the method described in Sreenivasaprasad et al. (1996), modified from Torres et al. (1993). DNA was quantified by ethidium bromide fluorescence on a UV transilluminator with known quantities of λ DNA (Sambrook et al., 1989).

RAPD analysis

After initial screening of one bean phenotype from each of the three mixtures (5058/1, 5067/1 and 5077/1) with RAPD primer kits A, B and F (20

Table 1. Landrace component numbers and characteristics of farmers' bean mixtures collected from southern Tanzania in September 1991*

Landrace component	Individuals	Colour/name	Growth habit	% of mix	Ten seed weight/g
Mixture 1: Origin: Mbimba					
5058	1, 2, 3, 4	small red/T3	climber	30.1	1.7
5059	1, 2, 3, 5, 6	golden brown/unknown	bush	22.7	2.9
5060	2, 6	red speckled/Kabanima	bush	18.8	3.3
5061	1, 2, 3, 4, 5	red, pink speckled/Nambalala	climber	13.5	3.1
Mixture 2: Origin: Sumbawanga					
5067	1, 2, 3, 4, 5	yellow, dark hilum/Kalimwa	climber	35.3	3.0
5068	1, 2, 4, 5	white speckled purple/Namwere	climber	26.3	3.5
5069	1, 2, 3, 4, 5	yellow long/Sumbawanga	climber	16.2	3.5
Mixture 3: Origin: Masebe, Nr Tukuyu					
5077	2, 3, 4, 5, 6	pale yellow, dark hilum/Big Kaloko	climber	20.6	4.1
5078	1, 5	pink/unknown	climber	13.9	3.0
5079	1, 2, 3, 4, 5	orange/Kaloko	climber**	10.4	3.6
5080	1, 4, 5	white/Kabaja	climber	8.6	2.4
5081	1, 2, 3, 4	pink/red speckled/unknown	climber	7.8	3.9
5082	1, 2	cream (yellow tinge)/unknown	climber	7.4	3.3

* Information obtained from Teverson et al. (1994) and D. Teverson (unpublished, 1996).
** Several seed selections from this landrace showed bush growth habit.

primers in each) supplied by Operon Technologies, CA, USA, 12 primers A11 (CAATCGCCGT), A14 (TCTGTGCTGG), B5 (TGCGCCCTTC), B6 (TGCTCTGCCC), B7 (GGTGACGCAG), B10 (CTGCTGGGAC), B15 (GGAGGGTGTT), F1 (ACGGATCCTG), F4 (GGTGATCAGG), F8 (GGGATATCGG), F9 (CCAAGCTTCC) and F10 (GGAAGCTTGG) were chosen for their ability to produce either a simple or complex pattern (Welsh & McClelland, 1990; Williams et al., 1990). Reaction mixtures contained 5 μl of appropriately diluted genomic DNA (20–30 ng), 5 μl of *Taq* 10 $\times$ buffer, 8 μl of 100 μM of deoxynucleotide triphosphate (dNTP) mix, 5 μl of primer (15 μg ml^{-1}), 0.25 μl of *Taq* DNA polymerase (5 U μl^{-1}) and sterile distilled water (up to a total volume of 50 μl). Amplification conditions comprised 45 cycles in a thermal cycler (Perkin Elmer Cetus). Each cycle consisted of 1.1 min at 94 °C, 2 min at 30 °C and 2.5 min at 72 °C, followed by a final extension time of 7 min at 72 °C. PCR products (20 μl) were visualised in 1.4% (w/v) agarose gels stained with ethidium bromide (0.4 μg ml^{-1}).

For data analysis, each amplified fragment with all 12 primers was treated as a separate character. DNA fragments of the same size were assumed to represent the same genetic locus and scored as either present or absent. The cluster analysis of the data was done based on a similarity matrix derived from the formula: number of shared characters/total number of characters. The dendrogram was generated by the 'group average' method using Jaccard's Index on the program GENSTAT 5 (Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, UK). Bootstrap analysis of the RAPD data was carried out using the computer package CONSENSE, PHYLIP (Felsenstein, 1993).

Results and discussion

Three replicate DNA samples from the 51 pure lines were assessed for polymorphism using random 10-base primers. All 12 primers amplified reproducible fragments from the DNA of each bean individual and a total of 62 major bands were visualized across the 51 individuals investigated. No variation was observed among the replicates of any of the pure lines. Among the 62 fragments detected only six were monomorphic; the remaining 56 were polymorphic in at least one pairwise comparison between individuals.

A dendrogram (Figure 1), generated by cluster analysis from data derived from fragments amplified by all 12 random primers, divided bean individuals on to two main branches with c. 60% genetic similarity. Branch A subdivided into two major groupings,

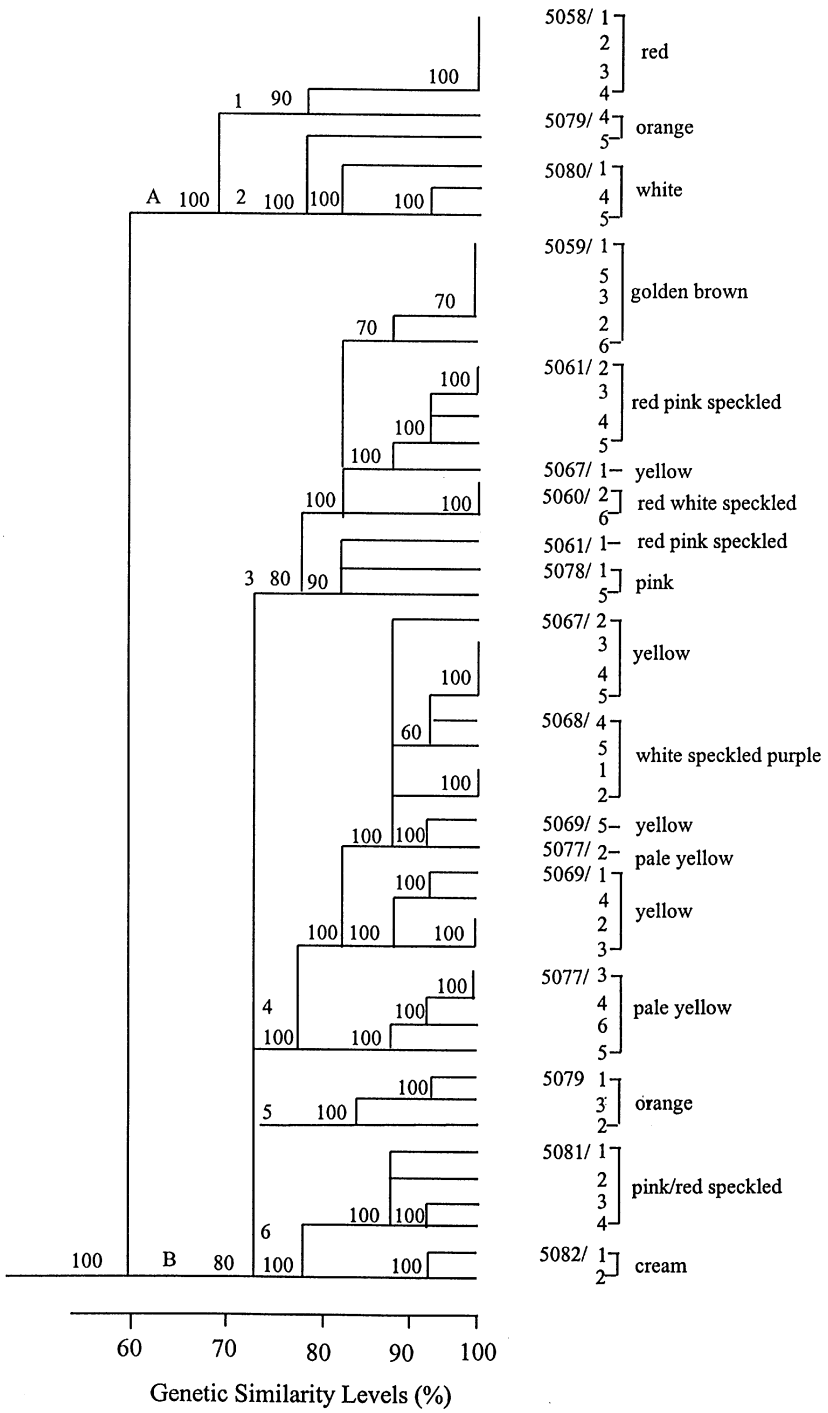

Figure 1. Clustering of individuals of *P. vulgaris* from pairwise comparison of RAPDs by the group average method. The values at the branch points are statistical values representing the strength of the relationship between branches using bootstrap analysis (see Table 1 for details of components and individuals).

clusters 1 and 2, and branch B subdivided into four major groupings, clusters 3, 4, 5 and 6. The groupings were supported by bootstrapping. The composition of these 6 groupings is highly heterogeneous but some distinct clustering consistent with established phenotypic classifications and geographic origins can be discerned.

Level of similarity among landraces within and across mixtures

Generally, the level of similarity, distinguished by morphological characters (mainly seed colour and size), was greater among individual plants within landraces than among landraces across mixtures (D. Teverson, unpublished, 1996).

With the exceptions of landraces 5078, 5082, 5080 from mixture 3 and 5060 from mixture 1, four to six plants of each landrace were analyzed using the 12 RAPD primers. In the majority of cases, most plants within a landrace showed at least 80% genetic similarity and many showed 90% or more genetic similarity, e.g. 5058, 5059, and 5061 from mixture 1 and 5077 from mixture 3. It was notable, however, that individual plants within other landraces, although morphologically indistinguishable by seed phenotype, were dissimilar genetically. The most notable case was landrace 5079 where plants 1, 2 and 3 which occurred on major branch B, cluster 5 and showed 80–90% genetic similarity, whereas plants 4 and 5 occurred on major branch A, cluster 2 and showed only 59% genetic similarity to plants 1, 2 and 3. It is known that this landrace shows variability in plant architecture with several seed selections producing plants of bush type although the majority are climbing growth type (D. Teverson, unpublished, 1996).

Of the three mixtures analysed, mixture 2 was the most uniform; all but two plants of landraces 5067, 5068 and 5069 occurred on cluster 4 (Figure 1) and showed 90% or more genetic similarity in spite of two distinct seed colour types (yellow and white with purple speckles) being represented. One could speculate on the possibility of one being derived as a colour segregant from the other especially if the components of the mixture had been grown together for some time.

In mixture 1, three of the four landraces 5059, 5060 and 5061 showed 85% or more genetic similarity and appear on cluster 3 (Figure 1). Landrace 5058 is very different from the other three appearing on major branch A along with two landrace components (5079/4 and 5, 5080) from mixture 3. Landrace 5058 being < 70% similar to 5079 and 5080 was not closely related and may have been collected by the farmer while travelling to some distant location.

Landrace phenotypes of mixture 3 showed the greatest genetic variability of the three mixtures. Landraces of this mixture occur on both major branches of the dendrogram (Figure 1). Despite occurring in clusters with landraces of the other two mixtures they appear too genetically dissimilar (< 80%) to be regarded as having similar origins. Beans in mixture 3, in fact, appear to have diverse origins.

There is a clear separation of the 13 landraces onto two main branches of the dendrogram (Figure 1) showing less than 60% genetic similarity. This separation together with phenotypic characters suggests that the two groups may represent two distinct gene pools of *P. vulgaris* which were first described by Evans (1976). This study is not, however, sufficiently detailed to confirm that they represent the two major Mesoamerican and Andean gene pools recognised from isozyme studies (Debouck et al., 1993) and DNA analysis using RFLPs (Khairallah et al., 1990; Becerra Velasquez & Gepts, 1994). However, landraces 5058 (red) and 5080 (white) are small beans, with 10 seed weights of 1.7 g and 2.4 g, respectively (Table 1). In contrast, the 10 seed weights of all other landrace components ranged from 2.9–4.1 g (Table 1). One of the characteristics typical of the Mesoamerican gene pool is small seeded beans (Evans, 1976; Gepts et al., 1986). It is interesting to speculate that landrace 5079 is a mixture of orange beans representing two distinct gene pools. Louette & Smale (1996) reported that maize farmers in a traditional community in Mexico classified seed obtained from outside the community as that of local landraces according to phenotype characteristics. It is possible that traditional bean farmers in Tanzania have mixed different sources of seed of the same colour. New seed would then be identified by the name of the local variety. Overtime a landrace could be developed from seed sources of quite diverse origins.

It is also noteworthy that the majority of the landraces of the three bean mixtures from the southern highlands of Tanzania were relatively large seeded (Table 1). In her analysis of bean landraces from Malawi, Sprecher (1988) also noted that the majority of landraces were large seeded and belonged to the Andean gene pool. There is growing evidence that beans from the Andean gene pool dominate the landrace population in this region of Africa.

Genetic similarity versus disease resistance

Resistance profiles in the bean mixtures to diseases such as angular leaf spot, anthracnose, haloblight, common bacterial blight and bean common mosaic virus, which have been extensively studied over recent years (Teverson et al., 1994), could be linked to some genetic groupings. The most uniform mixture in terms of reaction to major diseases is mixture 2 where landraces 5067, 5068 and 5069, which were found to be genetically similar (> 85%), are uniformly susceptible to angular leaf spot but resistant to rust, haloblight and common bacterial blight (Teverson et al., 1994). It is also interesting to note that a unique combination of resistance to angular leaf spot and susceptibility to rust was shown by landraces 5058, 5080 and 5079 (Teverson et al., 1994) two of the five components of which clustered together on major branch A and separated from the other landraces by at least 40% genetic dissimilarity.

Although a relatively small number of bean landraces were analysed in this study, the data indicates the value of including molecular markers as one of a range of parameters to measure diversity among bean mixtures especially for decision making for collection and conservation of valuable genetic resources.

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