Molecular Characterization of Genotypes Selected from the Germplasm Bank of *Cajanus cajan* (L.) Millsp and Cross-species Amplification in Three Legume Species

Adna Cristina Barbosa de Sousa¹, Danilo Augusto Sforça¹, Tatiana de Campos¹, Rodolfo Godoy², Leticia Jungmann¹, Liana Jank³ and Anete Pereira de Souza⁴

Introduction

*Cajanus cajan* (L.) Millsp (2n = 22) is one of the important grain legume crops in the tropics and subtropics. The centre of diversity is in India (SMARTT, 1990), which accounts for more than 80% of the world’s *C. cajan* production. Its seed is primarily consumed as dhal (dried dehusked split cotyledons) and in Latin America the tender green seeds are used as canned peas. This species has wide adaptability to diverse climates and soils. Because of its multiple uses as food, fodder, fuel wood, rearing lac insects, hedges, windbreaks, soil conservation, roof thatches and green manure, it plays an important role in subsistence agriculture. However, the average world productivity of *C. cajan* is rather low (709 kg ha⁻¹) (FAO, 2004), indicating an urgent need for improving the genetic potential of the crop.

Microsatellite markers are of high interest in *C. cajan* breeding programs. They are quite effective to estimate genetic diversity and genetic relationships, as well as to predict the genetic value of selected candidates derived from intraspecific crosses and the performance of their hybrid progenies. Microsatellite markers are short tandem repeat sequence motifs consisting of repeat units of 1-6 base pairs (bp). They are highly polymorphic DNA markers with discrete loci and co-dominant alleles (AUTZ and SCHLOTTERER, 1994).

The present study reports the characterization of 67 microsatellite markers for investigated a genetic diversity of *C. cajan* and cross-species amplification in other legume species.

Materials and methods

A. Plant material

The set of 77 *C. cajan* genotypes was obtained from the Germplasm Bank of the Brazilian Agricultural Research Corporation (Embrapa) – Sudeste, São Carlos - São Paulo, Brazil. In addition, cross-amplification tests were applied to three legume species: *Phaseolus vulgaris* (CAL-143, IAC-UMA, BAT and JALO), *Phaseolus lunatus* (87-JP-12) and *Vigna sp* (F100in00), from the Instituto Agronômico de Campinas (IAC) – Campinas, São Paulo, Brazil.

B. DNA extraction and amplification of microsatellite loci

Genomic DNA was extracted from freeze-dried leaf samples following the CTAB method of Doyle and Doyle (1989), which accounts for more than 80% of the world’s *C. cajan* production. Its seed is primarily consumed as dhal (dried dehusked split cotyledons) and in Latin America the tender green seeds are used as canned peas. This species has wide adaptability to diverse climates and soils. Because of its multiple uses as food, fodder, fuel wood, rearing lac insects, hedges, windbreaks, soil conservation, roof thatches and green manure, it plays an important role in subsistence agriculture. However, the average world productivity of *C. cajan* is rather low (709 kg ha⁻¹) (FAO, 2004), indicating an urgent need for improving the genetic potential of the crop.

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B. DNA extraction and amplification of microsatellite loci

Genomic DNA was extracted from freeze-dried leaf samples following the CTAB method of Doyle and Doyle (1989). DNA samples were quantified by comparison with known quantities of λ phage uncut DNA on 1% agarose gel. The study used sixty-seven microsatellite loci were analyzed. PCR reactions were performed using the following conditions: 94°C for 1 min followed by 30 cycles of 94°C for 1 min, specific Ta for 1 min, 72°C for 1 min, and a final extension of 72°C for 5 min. Amplification products were verified by electrophoresis on 3% agarose gel containing 0.1 mg ethidium bromide/ml in 1X TBE buffer (89 mM Tris-borate, 2 mM EDTA, pH 8.0) and 6% denaturing polyacrylamide gel in 1X TBE buffer, using a 10 bp ladder (Invitrogen) as a standard size. The amplified fragments were visualized in 6.0% polyacrylamide gels silver stained according to Creste et al. (2001).

C. Data analysis

Genotype data was used to calculate the number of alleles at each locus, observed (H₀) and expected (Hₑ) heterozygosity using the GDA program (LEWIS and ZAYKIN, 2002). The polymorphism information content (PIC) values were calculated to provide an estimate of marker informativeness (CORDEIRO et al., 2003). To compare the efficiency of the markers in varietal identification, we estimated the discriminating power (D) of each locus (TESSIER et al., 1999).

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software TFPGA (Tools for Genetic Population Analysis) (MILLER, 1997) was used for calculating expected and observed heterozygosities, and to evaluate the Hardy-Weinberg equilibrium (HWE) using Bonferroni correction. All 77 genotypes were clustered with the Unweighted Pair Group Method using arithmetic average (UPGMA) in the SAHN procedure of the NTSYS-PC v2.1 (ROHFL, 2000). The Neighbor joining trees and the principal coordinates analysis plots were obtained with the software DARwin v. 5.0.157. The software STRUCTURE version 2.2 (Pritchard et al., 2000) was used to cluster individuals according to distinct allele frequencies sets.

Results and Discussion

Of the 67 microsatellite markers used, 16 (23.9%) were polymorphic. A total of 83 putative alleles were obtained with these 16 microsatellite polymorphic markers. The number of alleles per locus ranged from 2 to 12, with an average of 5.1 alleles per locus (Table 1). The PIC and D values ranged from 0.11 to 0.80 (average 0.44) and 0.23 to 0.91 (average 0.58), respectively. The highest PIC and D values were found in locus CZ681938a whith presented 8 alleles. The observed (H_0) and expected heterozygosity (H_e) values ranged from 0.01 to 0.59 (average 0.26) and 0.01 to 0.82 (average 0.44), respectively. Three loci (CZ681930, CZ681938b and CZ681983) showed a significant deviation from Hardy-Weinberg equilibrium (HWE) after Bonferroni correction (P: (5%) < 0.0039). No significant linkage disequilibrium was detected (P<0.01) using chi-squared test.

All polymorphic markers were tested for cross-amplification in Phaseolus vulgaris (CAL-143, IAC-UMA, BAT and JALO), Phaseolus lunatus (87-JP-12) and Vigna sp (Fradinho) (Table 2). Thirteen microsatellite loci successfully amplified all different species. Five loci (AJ310691, AJ312891, AJ312893, AJ312895 and CZ681930) were successfully amplified in all species. Non-specific amplification of CZ644531, CZ682017a and CZ682017b, was observed between species. The efficiency of heterologous amplification was 70%. However, this shows a considerable level of sequence, conservation within the primer regions flanking microsatellite loci. These results suggest that the microsatellites here reported the high potential for their use in comparative and phylogenetic studies.

Data from 16 polymorphic loci were used to construct a UPGMA dendrogram showing the genetic relationship among the 77 genotypes. This analysis revealed eleven distinct clusters (Figure 1). Based on the Rogers modified distance all genotypes were successfully differentiated. However, INPA and Fava-Larga were distinct. Genotypes of C. cajan from the same locations in Africa and Brazil did not consistently cluster together (data not shown). The eleven clusters obtained comprised the three different legume species. The genotypes JALO and Fradinho showed 100% similarity to each other. The average genetic similarity value between C. cajan genotypes and legume species (P. vulgaris, P. lunatus and Vigna sp) was 0.34%. The cophenicetic correlation observed for this clustering pattern was r = 0.71. The Bayesian algorithm used by STRUCTURE software allowed for the identification of four population clusters (K = 4). The phylogenetic (Neighbor-joining) tree, which was constructed based on the genetic distance matrix, was colored according to STRUCTURE results. Furthermore, a strong tendency of correspondence between the Bayesian clusters a neighbor-joining tree was observed (data not shown).

These markers satisfactorily assessed the genetic relationship among the 77 C. cajan genotypes. Knowledge of the genetic structure in this species is fundamental in elaborating of further breeding programs and germplasm conservation strategies.

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References


