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RAPD analysis of the genetic diversity among accessions of Fabaceous forages (*Poincianella* spp) from the Caatinga

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ABSTRACT. Among members of the Fabaceae family, native to the Brazilian Caatinga, the species *Poincianella pyramidalis* and *P. bracteosa* exhibit particular potential as forage for cattle, sheep and goats. With the aim of establishing genetic relationships within *Poincianella*, random amplified polymorphic DNA analysis was performed on eight accessions of *P. pyramidalis* and two accessions of *P. bracteosa*, originating from the semiarid zone of the state of Piauí, northeastern Brazil, and present in the germplasm bank of Embrapa Meio Norte (Teresina, Piauí, Brazil). Amplification reactions using 11 selected arbitrary sequence primers generated 167 fragments with an overall polymorphism of 70.38%. Five monomorphic loci were generated exclusively in *P. pyramidalis* accessions, while three unique monomorphic loci were associated with *P. bracteosa*, and these represented potential species-specific markers. The similarity coefficients between *Poincianella* accessions were low (mean value 0.59) but with a wide variation (range 0.443 to 0.748). The similarity

matrix and the dendrogram constructed using the unweighted pair group method allowed the separation of *Poincianella* accessions into two major clusters represented by the two distinct species, while the accessions of *P. pyramidalis* could be separated further into three subgroups. The high level of genetic diversity detected in the genus *Poincianella* could be used in future breeding programs to produce enhanced cultivars, although the variability could be better exploited if more specimens were collected from other locations within the semiarid region of northeastern Brazil.

Key words: Forage; Genetic diversity; Species-specific markers; RAPD; Plant improvement

INTRODUCTION

The availability of forage for livestock in the semiarid region of northeastern Brazil is severely limited because of sparse and irregular precipitation and high temperatures. The Caatinga vegetation, which is typical of this environment, is characterized primarily by small, thorny trees and grasses, and these constitute an important source of animal fodder. The Fabaceous trees *Poincianella pyramidalis* (Tul.) L.P. Queiroz (catingueira-verdadeira) and *P. bracteosa* (Tul.) L.P. Queiroz (catinga-de-porco) are native to the Caatinga and exhibit particular potential as forage crops. *P. pyramidalis* produces shoots even under conditions of low humidity and becomes green with the first rainfall of the wet season (Maia, 2004), while the leaves, flowers and pods that fall from *P. bracteosa* can be grazed on the ground by cattle and small ruminants such as sheep and goats (Costa et al., 2002).

A species lacking in sufficient genetic diversity may not have the capacity to respond to environmental changes, to compete with other plants or to provide an efficient defense against pests and predators. Therefore, for the purpose of plant breeding, it is essential that genetic variability be present between individuals of the same species, or between groups of interspecific crosses, or even between different species of the same genus. On this basis, knowledge regarding genetic diversity is crucial for a successful plant improvement program, particularly in the case of a non-domesticated plant such as *P. pyramidalis*.

The genetic variability within a species can be readily determined using hybridization-based or polymerase chain reaction (PCR)-based molecular markers (Esfahani et al., 2009; Gomes et al., 2011). Random amplified polymorphic DNA (RAPD) is a powerful PCR-based tool for genetic studies that offers various advantages over other procedures by being rapid, easy to perform and relatively inexpensive. Moreover, the technique employs a single primer to detect polymorphisms in all regions of the genome with no requirement for prior knowledge of nucleotide sequences in the genomic DNA (Welsh and McClelland, 1990; Reiter et al., 1992; Williams et al., 1990, 1993; Haymer and McInnis, 1994; Ferreira and Grattapaglia, 1998). For these reasons, RAPD markers have been used in phylogenetic analysis, fingerprinting and genome mapping (Smith, 1995; Smith and Register, 1998). RAPD markers have been used to assess genetic diversity in the Fabaceous forage species *Flemingia macrophylla* (Anderson et al., 2006), *Desmodium* sp. (Irshad et al., 2009), *Lablab purpureus* (Rai et al., 2010), and *Stylosanthes capitata* and *S. macrocephala* (Chiari et al., 2010). Information regarding the genetic diversity of *Poincianella* species is, however, somewhat scarce. The aim of the present

study was, therefore, to analyze accessions of *P. pyramidalis* and *P. bracteosa* available in the germplasm collection of Embrapa Meio-Norte using RAPD markers.

MATERIAL AND METHODS

Plant material and extraction of DNA

Eight accessions of *P. pyramidalis* (PP2-PP6, PP8-PP10) and two accessions of *P. bracteosa* (FG1 and FG2) were present in the germplasm collection of Embrapa Meio-Norte (Teresina, PI, Brazil), all of which originated from the municipality of São João do Piauí located 450 km south of Teresina within the Serra da Capivara National Park. Young healthy leaves were collected from the accessions and stored in the freezer at -20°C until required for analysis. Leaf samples (100 mg) were macerated separately in a Precellys® 24 tissue homogenizer/grinder (Bertin, Montigny-le-Bretonneux, France) together with extraction buffer from a DNeasy Plant kit (Qiagen, São Paulo, SP, Brazil) following the procedure recommended by the manufacturer. Aliquots of extracted DNA were subjected to electrophoresis on a 0.8% agarose gel in Tris-borate-EDTA (0.5X TBE) buffer and subsequently stained with GelRed™ (10,000X; Biotium, Hayward, CA, USA). Genomic DNA was quantified by comparison with 100 ng λ DNA standards, and DNA samples were stored at -20°C until required for RAPD analysis.

RAPD analysis

Samples of genomic DNA were amplified using 100 arbitrary sequence decamer primers obtained from Operon Technologies (Alameda, CA, USA), and the most suitable primers were selected for RAPD reactions. Eleven primers (Table 1) were chosen on the basis of resolution and high levels of polymorphism, and these were subsequently employed in the PCR amplification of DNA samples derived from the ten accessions of *Poincianella*. Amplification reactions were carried out in 0.2 mL microtubes using a Veriti 96 Well Thermal Cycler (Applied Biosystems, Foster City, CA, USA) with a reaction mixture comprising 20 µL 1X Invitrogen buffer (Life Technologies do Brasil, São Paulo, SP, Brazil), 3.0 mM $MgCl_2$, 0.25 mM dNTP, 1 U Taq DNA polymerase (Invitrogen), 0.2 µM primer, 1 µL DNA template (~15 ng), and ultrapure distilled water to a final volume of 20 µL. The PCR conditions were: initial denaturation for 1 min at 92°C, 45 cycles comprising denaturation for 1 min at 92°C, annealing for 1 min at 35°C and extension for 2 min at 72°C, and final extension for 5 min at 72°C. The resulting amplicons were separated by electrophoresis on 1.5% agarose gel in 0.5X TBE buffer at 110 V for approximately 3 h, stained with GelRed™, visualized under a UV transilluminator and subsequently photographed.

Phylogenetic analysis

The number of well-resolved and intense polymorphic bands generated by each primer was determined by visual inspection. Each band was considered to represent a single character, and a binary matrix was created in which 1 indicated the presence of the marker and 0 its absence. Genetic similarities between accessions of *Poincianella* species were estimated from Dice coefficients and the corresponding similarity matrix. A dendrogram was constructed using the unweighted pair group method with arithmetic average (UPGMA) clustering technique. The cophenetic correlation coefficient (*r*) was calculated from the similarity matrix and the

dendrogram. The bootstrap confidence index was also evaluated from the binary matrix of amplified fragments generating a dendrogram from 1000 permutations. The cut-off point was based on the mean genetic similarity (sg_m) calculated according to the equation $sg_m = \Sigma\, sg_{ij} / N$, in which sg_{ij} is the genetic similarity between pairs of individuals and N is the number of pairs obtained from 1000 permutations. Analyses were performed with the aid of the PAST version 1.34 software (Hammer et al., 2001).

Table 1. Numbers of amplified and polymorphic loci obtained following amplification of genomic DNA from 10 accessions of *Poincianella pyramidalis* and *P. bracteosa* using primers selected.

Primer	Nucleotide sequence	No. of amplified loci	No. of polymorphic loci
A08	5' GTG ACG TAG G 3'	22	22
A09	5' GGG TAA CGC C 3'	11	11
F02	5' GAG GAT CCC T 3'	17	16
M01	5' GTT GGT GGC T 3'	16	14
M04	5' GGC GGT TGT C 3'	10	9
M15	5' GAC CTA CCA C 3'	17	16
M19	5' CCT TCA GGC A 3'	14	14
M20	5' AGG TCT TGG G 3'	11	11
N06	5' GAG ACG CAC A 3'	16	16
N08	5' ACC TCA GCT C 3'	14	11
P06	5' GTG GGC TGA C 3'	19	15

RESULTS AND DISCUSSION

The 11 primers selected for RAPD analysis of the accessions of *Poincianella* species generated a total of 167 loci, with an average of 15.18 (range 10 to 22) loci per primer (Table 1). In comparison, RAPD analysis of the leguminous forage *Stylosanthes guianensis* performed by Chiari et al. (2006) generated an average of only 5.25 loci per primer. The loci generated by primers A08, N06, M19, A09, and M20 were exclusively polymorphic (with 22, 16, 14, 11, and 11 bands, respectively), while the loci generated by primer N08 showed the least polymorphism (78.57%). Figure 1 shows an example of the electrophoretic profile of RAPD amplifications of DNA samples from *P. pyramidalis* and *P. bracteosa* generated by primer A08. The sizes of the amplified fragments ranged between 396 and 4000 bp, a variation that is much greater than that established by Santos et al. (2012) for 13 accessions of *P. pyramidalis* (250-1000 bp) or those reported by Andersson et al. (2006, 2007) for *F. macrophylla* (440-2500 bp) and *Cratylia argentea* (320-2900 bp).

Monomorphic bands that were unique to *P. pyramidalis* accessions were generated by primers P06 (1550 bp), M20 (1400 and 1500 bp), M01 (2000 bp), and F02 (2200 bp), while primers A09 (500 bp), M15 (1600 bp) and N08 (3054 bp) produced monomorphic bands that were exclusive to *P. bracteosa*. Such unique bands not only distinguish the two species of *Poincianella* but also represent potential species-specific markers.

Of the 144 fragments produced by accessions of *P. pyramidalis* in the germplasm collection of Embrapa Meio Norte, 126 were polymorphic, indicating a polymorphism of 87.50%. This value is lower than the 94.6% polymorphism reported by Santos et al. (2012) for accessions of *P. pyramidalis* collected in the State of Sergipe, but greater than those reported for six species of *Acacia*, an economically important genus within the Fabaceae (Nanda et al., 2004). The level of polymorphism observed in the two accessions of *P. bracteosa* was 53.26% (49/92), a value that is higher than that found in accessions of *S. capitata* (39.13%) and *S. mac-*

rocephala (34.28%) as reported by Chiari et al. (2010).

The high level of polymorphism detected in accessions of *Poincianella* species is probably due to the self-incompatibility of plants of this genus. According to Leite and Machado (2009), *P. pyramidalis* forms fruits only when there is manual or natural cross-pollination. Additionally, these non-domesticated species still preserve their original genetic traits, since they have not been submitted to artificial selection. As stated by Clement (2001), the lower the degree of domestication of a species, the greater the level of genetic variability is detected.

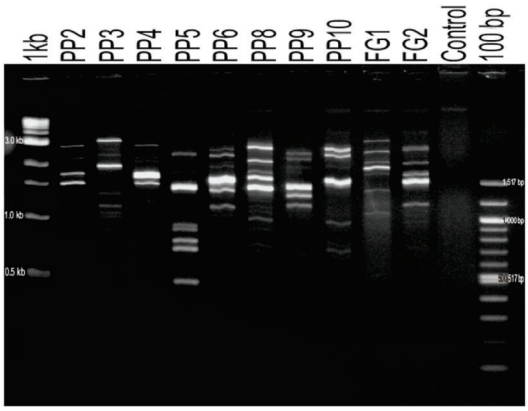

Figure 1. Electrophoretic profiles of RAPD amplifications of DNA samples from eight accessions of *Poincianella pyramidalis* (PP2-PP6, PP8-PP10) and two accessions of *P. bracteosa* (FG1 and FG2) generated by primer A08.

The genetic similarity matrix presented in Table 2 reveals that the coefficients between *Poincianella* accessions were low but varied widely. The mean similarity coefficient was 0.59 (range 0.443 to 0.748), a value that is higher than that reported by Croft et al. (1999) for species of *Lathyrus*. On the other hand, the mean similarity coefficient and the range of variation obtained in the present study were smaller than those reported by Santos et al. (2012), although these authors employed a larger number of *Poincianella* accessions in their RAPD analysis. It would appear, therefore, that accessions originating from the semiarid region of Piaui studied herein exhibit less similarity than those from Sergipe investigated by Santos et al. (2012).

The similarity coefficients within the accessions of *P. pyramidalis* ranged from 0.504 to 0.748, with accessions PP4 and PP8 being the most similar and PP2 and PP6 the most divergent. The two *P. bracteosa* accessions FG1 and FG2 showed moderate similarity (0.637), while the lowest similarity (0.443) was observed between accessions PP2 and FG2 of the different species. In contrast, Chiari et al. (2010) employed 26 primers in a RAPD analysis of two species of *Stylosanthes* and obtained high coefficients of similarity with small ranges of variation. For example, the coefficients in *S. capitata* varied from 0.741 to 0.913, while in *S. macrocephala* the range was between 0.724 and 0.924. Hence, the intraspecific variation in *Stylosanthes* was much less pronounced than that observed for *Poincianella* in the present study.

The cophenetic correlation (r = 0.87) confirmed good agreement between the Dice similarity matrix and the dendrogram constructed using the UPGMA method, thereby increasing the reliability of the interpretations related to genetic distances. Delimitation of the den-

drogram with a cut-off point of 0.59 revealed the genetic interrelationships between the ten *Poincianella* accessions and allowed the separation of the accessions into two major clusters (Figure 2) represented by the two distinct species, *P. bracteosa* (group I) and *P. pyramidalis* (group II). Additionally, the accessions of *P. pyramidalis* could be separated intro three subgroups, namely IIA (PP5 and PP10), IIB (PP4,PP8,PP9, and PP6), and IIC (PP3 and PP2).

Table 2. Genetic similarity matrix for eight accessions of *Poincianella pyramidalis* (PP2-PP6, PP8-PP10) and two accessions of *P. bracteosa* (FG1 and FG2) generated from Dice coefficients calculated on the basis of RAPD markers.

Accession	PP2	PP3	PP4	PP5	PP6	PP8	PP9	PP10	FG1
PP3	0.72000								
PP4	0.61905	0.63415							
PP5	0.59420	0.68148	0.58824						
PP6	0.50370	0.63636	0.64662	0.59310					
PP8	0.61654	0.66154	0.74809	0.55944	0.67143				
PP9	0.60870	0.69630	0.69118	0.67568	0.67586	0.72727			
PP10	0.57576	0.63566	0.58462	0.66197	0.51799	0.59854	0.64789		
FG1	0.55285	0.56667	0.51240	0.49624	0.55385	0.50000	0.54135	0.47244	
FG2	0.44286	0.54015	0.47826	0.49333	0.54422	0.49655	0.49333	0.44444	0.63704

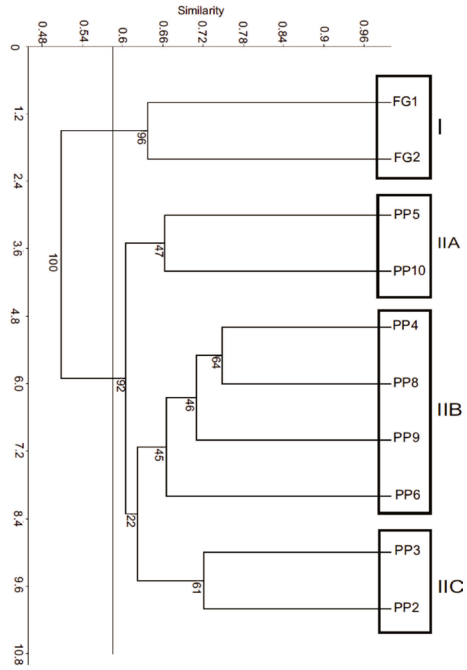

Figure 2. UPGMA dendrogram based on 11 RAPD polymorphic markers showing similarity relationships between eight accessions of *Poincianella pyramidalis* (PP2-PP6, PP8-PP10) and two accessions of *P. bracteosa* (FG1 and FG2).

It is important to emphasize that all accessions studied originated from the municipality of São João do Piauí. The distribution of the accessions into four clusters demonstrated the large genetic diversity present in the genus *Poincianella* and indicated that this variability

could be better exploited if more specimens were collected from other locations in the semi-arid region of northeastern Brazil.

The RAPD marker technique was shown to be an appropriate tool for establishing genetic relationships within the genus *Poincianella*, since it was possible to observe a high degree of polymorphism, especially in *P. pyramidalis* (87.50%). A large genetic diversity among the ten *Poincianella* accessions available in the germplasm collection of Embrapa Meio-Norte was detected, and this resource could be employed in future breeding programs to generate enhanced cultivars. The non-domesticated nature *Poincianella* spp was considered to be the main factor responsible for the extensive genetic variability observed among the accessions.

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