Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Genetic diversity in local and commercial dry bean (*Phaseolus vulgaris*) accessions based on microsatellite markers

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Genet. Mol. Res. 10 (1): 140-149 (2011)
Received August 2, 2010
Accepted October 15, 2010
Published February 1, 2011
DOI 10.4238/vol10-1gmr993

ABSTRACT. Dry beans are considered to be a crop of great socio-economic importance, because they are an inexpensive source of nutrients and because their cultivation requires considerable manual labor. Studies of genetic diversity have been very important for genetic improvement programs, because they give parameters for the identification of genitors that can provide large heterosis effects and improved segregation in recombinants, increasing the probability of obtaining superior genotypes in the progeny. We evaluated the genetic diversity of 57 dry bean accessions, including 31 local accessions, propagated by small-scale farmers, 20 accessions supplied by the Brazilian Agricultural Research Agency, and six commercial accessions, using 16 microsatellite primers. Among these primers, 13 were found to be polymorphic, giving 29 polymorphic alleles. The largest number of alleles per locus was observed for primer BM141, which had four alleles. The polymorphic information content varied from 0.11 to 0.51, observed for loci BM212 and BM141, respectively. The lowest degree

of dissimilarity (0.0) was found between the accession Iapar 81 and the accessions E03, E04, E09, and E13 and between the accession pairs E08 with E16 and Iapar 31 with E06. The highest degree of dissimilarity was found between the accessions Carioca and E22 (1.0). Grouping analysis revealed four groups, according to the place of origin. This tendency was also found in the principal coordinate analysis. The local genotypes were found to have relatively high genetic diversity, while the EMBRAPA and commercial cultivars had a relatively narrow genetic basis.

Key words: Germplasm characterization; Fingerprint; SSR makers; Genetic resources

INTRODUCTION

The dry bean (*Phaseolus vulgaris* L.) is one of the most important components of the Brazilian diet, with a per capita consumption of over 17 kg/year (CONAB, 2006). It is recognized as an excellent source of protein, also containing large quantities of complex carbohydrates, fiber and isoflavonoids (Anderson et al., 1999), besides being an important source of iron, phosphorus, magnesium, and manganese, and to a lesser extent, of zinc and calcium (Broughton et al., 2003). Brazil is the world's largest producer of this legume; the States of Paraná and Minas Gerais have the largest plantings, accounting for 22.29 and 16.88%, respectively, of the country's production (FAO, 2009; IBGE, 2009). In the State of Espírito Santo, dry beans are considered to be the third most important agricultural product, cultivated in an area of approximately 21.6 thousand hectares, producing 17.7 thousand metric tons, with a productivity of 832 kg/ha in the 2008/2009 season (IBGE, 2009). It is mainly cultivated by subsistence farmers, who market excess production (Cabral et al., 2010).

Since small-scale farmers do not purchase commercial seed for planting, using seeds that they or their neighbors have produced themselves, there is a mix of varieties and natural crosses between individual plants (Bonett et al., 2006), which results in considerable genetic variability in the seed of these small producers. However, there is a tendency for loss of the genetic variability maintained by these family farms, due to the substitution of local varieties with commercial seed lines (Cardoso, 2009).

This situation underlines the importance of obtaining knowledge concerning genetic diversity of local cultivars compared to commercial and improved lines, to aid genetic improvement programs, allowing growers to make good use of existing variability and of cultivars adapted to the climatic conditions of specific regions (Loarce et al., 1996; Franco et al., 2001).

Among the tools used to estimate genetic diversity, molecular markers are especially important (Vieira et al., 2007; Gonçalves et al., 2009; Oliveira et al., 2010). Molecular markers permit us to make estimates of genetic diversity directly at the DNA level, reducing the interference of environmental variations. Also, many markers are available for each genome, there are high levels of polymorphism, and they are free of pleiotropic effects (Ferreira and Grattapaglia, 1998; Cardoso, 2009; Leal et al., 2010).

Simple-sequence repeats (SSR) or microsatellites are multiallelic and codominant and contain considerable genetic variation (Blair et al., 2003; Song et al., 2004). The high information content contained in SSR loci, together with their codominant expression, make SSRs

ideal for gene mapping and highly efficient for linkage studies, variety protection, marker-assisted selection, studies of diversity, etc. (Song et al., 2004; Leal et al., 2010).

Studies of genetic diversity have been of great importance for genetic improvement programs, because they provide information about identification parameters that have strong heterotic effects and segregate in recombinants, increasing the probability of obtaining superior genotypes in the progeny (Silva et al., 2008). Such studies also allow breeders to identify duplicates, thereby reducing the costs of maintaining germplasm banks (Oliveira, 2005).

Along this line, our objectives were to evaluate genetic diversity in 57 dry bean accessions, six of which were commercial lines and included for comparison, and to identify duplicates among the genotypes, using microsatellite molecular markers.

MATERIAL AND METHODS

Plant materials

The genetic material consisted of 57 accessions of dry beans, 20 of which were supplied by the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) - Trigo e Sorgo, 31 by local genotypes from the Fortaleza community in the municipality of Muqui, ES, and six by commercial cultivars: Carioca, Serrano, IAPAR 31, IAPAR 44, IAPAR 81, and Pérola (Table 1).

Table 1. Identification of genotype origin, growth characteristics (GC), commercial group (CG) and weight in grams of 100 seeds (W100).

Ident.	Origin	GC	CG	W100	Ident.	Origin	GC	CG	W100
Pérola	C	III	C	23.93	F33	L	I	R	36.38
F2	L	II	M	21.68	F34	L	I	J	46.02
F3	L	II	M	18.48	F35	L	II	P	15.74
F5	L	II	M	17.13	F36	L	II	O	38.99
F6	L	II	P	17.03	F37	L	II	P	18.20
F7	L	III	R	19.77	F38	L	II	P	15.07
F8	L	III	J	41.37	E01	E	II	P	16.68
F9	L	I	O	36.37	E02	E	III	C	22.87
F10	L	III	P	17.81	E03	E	II	P	19.15
F11	L	II	P	18.50	E04	E	II	P	19.24
F13	L	II	M	18.06	Iapar 31	C	II	O	23.23
F14	L	II	P	17.62	E06	E	II	P	22.27
F15	L	II	R	14.24	E07	E	II	P	21.59
F16	L	II	C	15.77	E08	E	II	P	20.13
F17	L	II	O	17.76	E09	E	II	P	16.89
F18	L	II	R	14.52	E10	E	II	P	26.39
F19	L	II	M	18.21	E11	E	II	P	21.06
F20	L	II	O	18.16	E12	E	II	C	22.36
F21	L	II	M	18.84	E13	E	II	P	20.63
F23	L	II	P	15.28	E14	E	II	P	20.21
F24	L	II	P	17.42	E15	E	II	P	19.61
F25	L	II	R	17.73	E16	E	II	P	21.39
F26	L	II	P	21.97	E17	E	II	P	21.23
Iapar 81	C	II	C	20.92	E18	E	II	P	20.78
F28	L	II	M	16.97	E19	E	III	C	21.17
Carioca	C	III	C	22.10	Iapar 44	C	II	P	19.25
Serrano	C	II	P	16.04	E21	E	II	P	28.09
F31	L	I	J	36.20	E22	E	I	O	36.38
F32	L	I	P	30.96					

Ident. = identification of genotype origin. Origin: L = local; E = Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA); C = commercial cultivars. GC: I = determinate bush; II = indeterminate bush; III = indeterminate prostrate. CG: C = Carioca; J = Jalo; M = Mulatinho; P = Black; R = Rosinha; O = others.

Molecular analysis

The 57 accessions were planted in labeled plastic cups, containing commercial substrate and kept in a greenhouse until the first trifoliate leaves appeared. DNA was extracted from leaf samples, using the protocol of Doyle and Doyle, with some modifications, proposed by Abdelnoor et al. (1995).

The DNA was quantified by electrophoresis on 0.8% agarose gel, compared to a DNA standard (lambda DNA vector - 25, 50 and 100 ng). When necessary, the DNA samples were diluted to 10 ng/µL.

Sixteen pairs of SSR primers for dry beans were selected (Gaitán-Solís et al., 2002; Oliveira, 2005; Blair et al., 2006). The selection criteria for choosing the primers were a high degree of polymorphism and a wide distribution of SSRs in the linkage groups in the consensus map for dry beans (Yu et al., 2000), to provide the largest possible coverage of the genome and linking of SSRs with agro-economically important characteristics (Table 2).

The amplification reactions of the primers were done in a final volume of 15 µL, containing $MgCl_2$ (2.4 mM), Tris-KCl, pH 8.3 (0.25 mM), dNTP (0.25 mM of each nucleotide), 0.6 µM of each primer, 1 U Taq-polymerase, and 30 ng DNA.

The amplifications were run in a thermocycler (Techne TC 412), under the following conditions: an initial step for 5 min at 94°C and 30 cycles of 1 min at 94°C, 1 min at 50°C and 2 min at 72°C, with a final extension at 72°C for 10 min. For the primers run at two annealing temperatures, only the cycles were altered: nine cycles at 94°C for 20 s, 58°C for 20 s and 72°C for 20 s, and then 25 more cycles at 94°C for 20 s, 60°C for 20 s and 72°C for 20 s.

The amplified fragments were separated by electrophoresis on a 3.0% agarose gel containing 0.02 µg/mL ethidium bromide, SB1X buffer (0.04% (v/v) NaOH and 0.25% (v/v) boric acid), at 110 V for approximately 3 h. After the run was terminated, the gels were photographed under ultraviolet light, using the photo-documentation system Biolocus L PIX.

Data analysis

The genetic dissimilarity values were estimated by the complement of the weighted index: $D_{ii'} = 1 - (\frac{1}{2}\sum_{j=1}^{L} p_j c_j)$, where L = total number of loci in the study; $p_j = \frac{a_j}{A}$ = weight associated with locus j, determined by a_j (total number of alleles at locus j) and A (total number of alleles studied), and c_j = number of alleles in common between pairs of accessions i and i'. They were then grouped hierarchically with the unweighted paired group method using arithmetic averages (UPGMA) and principal coordinate analysis (PCoA). The polymorphic information content (PIC) was also calculated: $1 - \sum_{i=1}^{a} p_i^2 - \sum_{i,j=1(i\neq j)}^{a}\sum^{a} 2p_i^2 p_j^2$, where a = number of loci studied and p_i and p_j = the allele frequencies of the loci. The calculation of the distance matrix and of PIC was done with the GENES program (Cruz, 2006), UPGMA grouping was done with the R program (www.r-project.org), and PCoA was performed with the GenALEX 6.0 program (Peakall and Smouse, 2006).

RESULTS AND DISCUSSION

Among the 16 SSR primers used to genotype the 57 accessions, 13 were polymorphic and 3 were monomorphic, with a total of 29 polymorphic alleles. The number of alleles per

locus varied from 2 to 4, with a mean of 2.23; primer SSR BM141 gave the largest number of alleles. Similar numbers of alleles per locus were found for beans by Campos et al. (2007), Hanai et al. (2007) and Cardoso (2009).

The PIC varied from 0.11 for locus BM212 to 0.51 for locus BM141, with a mean of 0.27 (Table 2). Similar results were obtained by Bertini et al. (2006), when they evaluated genetic diversity in cotton accessions with SSR markers. Benchimol et al. (2007) studied genetic diversity of dry beans with 87 SSR loci; they found PIC values from 0.05 to 0.83, with a mean of 0.45.

Table 2. Sequences of the SSR primers, annealing temperature (Tm), linkage group (LG), number of alleles (NA), and polymorphic information content (PIC).

Primer	Sequence 5'-3'	Tm	LG	NA	PIC
PV-ctt001 F	GAGGGTGTTTCACTATTGTCACTGC	48	B04	2	0.35
PV-ctt001 R	TTCATGGATGGTGGAGGAACAG				
PV-gccacc001 F	CGTTAGATCCCGCCCAATAGT	48	B02	2	0.31
PV-gccacc001 R	CCGTCCAGGAAGAGCGAGC				
X57022 F	AAG GAT GGG TTC CGT GCT TG	58/60	B04	2	0.28
X57022 R	CAC GGT ACA AAC CAT GCT ATC				
X74919 F	CCG TTG CCT GTA TTT CCC CAT	58/60	B05	2	0.20
X74919 R	CGT GTG AAG TCA TCT GGA GTG GTC				
BM141 F	TGA GA GGA ACA ATG GTG GC	55	B11	4	0.51
BM141 R	CTC ACA AAC CAC AAC GCA CC				
BM139 F	TTA GCA ATA CCG CCA TGA GAG	58/60	B02	3	0.21
BM139 R	ACT GTA GCT CAA ACA GGG CAC				
BMd10 F	GCTCACGTACGAGTTGAATCTCAG	50	B01	1	0.00
BMd10 R	ATCTGAGAGCAGCGACATGGTAG				
BM142 F	TTCCGCTGATTGGATATTAGAG	50	B02	1	0.00
BM142 R	AGCCCGTTCCTTCGTTTAG				
BM181 F	ATGCTGCGAGTTAATGATCG	50	B03	2	0.37
BM181 R	TGAGGAGCAAACAGATGAGG				
BM199 F	AAGGAGAATCAGAGAAGCCAAAAG	50	B04	1	0.00
BM199 R	TGAGGAATGGATGTAGCTCAGG				
BMd53 F	TGCTGACCAAGGAAATTCAG	50	B05	2	0.20
BMd53 R	GGAGGAGGCTTAAGCACAAA				
BM185 F	AAGGAGGTTTCTACCTAATTCC	50	B07	2	0.28
BM185 R	AAAGCAGGGATGTAGTTGC				
BM189 F	CTCCCACTCTCACCCTCACT	50	B08	2	0.33
BM189 R	GCGCCAAGTGAAACTAAGTAGA				
BMd54 F	GGCTCCACCATCGACTACTG	50	B09	2	0.12
BMd54 R	GAATGAGGGCGCTAAGATCA				
BM212 F	AGGAAGGGATCCAAAGTCACTC	50	B10	2	0.11
BM212 R	TGAACTTTCAGGTATTGATGAATGAAG				
PV ag001 F	CAATCCTCTCTCTCTCATTTCCAATC	50	B11	2	0.28
PV ag001 R	GACCTTGAAGTCGGTGTCGTTT				

The analysis of the dissimilarity frequencies among 1596 pair-wise combinations of the 57 common bean accessions (Figure 1) showed a non-uniform distribution, varying from 0.00 to 1.0, with a mean of 0.45, indicating wide genetic variability. Loarce et al. (1996) reported that breeding programs are based on the use of genetic variability within a species for the production and selection of new cultivars with high production potential.

Approximately 92% of observed total dissimilarity was concentrated within the interval 0.1-0.79. The class comprising the values from 0.2 to 0.29 gave the greatest frequency, comprising 17.89% of the total. Alzate-Marin et al. (2003) reported that 17 of 21 elite dry bean cultivars fell within the similarity distances 0.03-0.33.

The lowest degree of dissimilarity (0.0) was found between the accessions Iapar 81 and the accessions E03, E04, E09, and E13, where they were considered to be equal, which

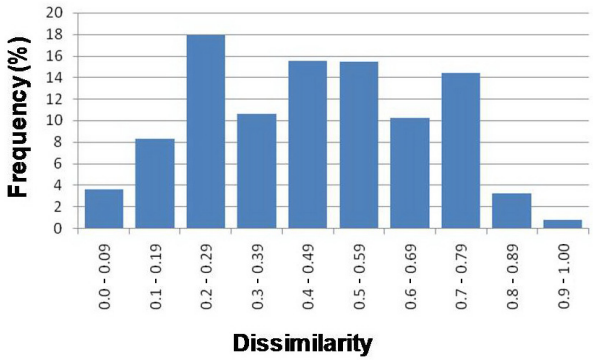

Figure 1. Frequency distribution of dissimilarity based on SSR markers for 57 dry bean genotypes in 10 classes.

also can be seen in the grouping analyses (Figure 2). The pairs E08 with E16 and Iapar 31 with E06 also showed zero dissimilarity. The highest degree of dissimilarity (1.0) was found between accessions Carioca and E22; comparisons of the latter cultivar with all the other genotypes also gave the highest mean dissimilarity (0.77), which demonstrates the high degree of divergence of this genotype with the rest of the accessions in our study.

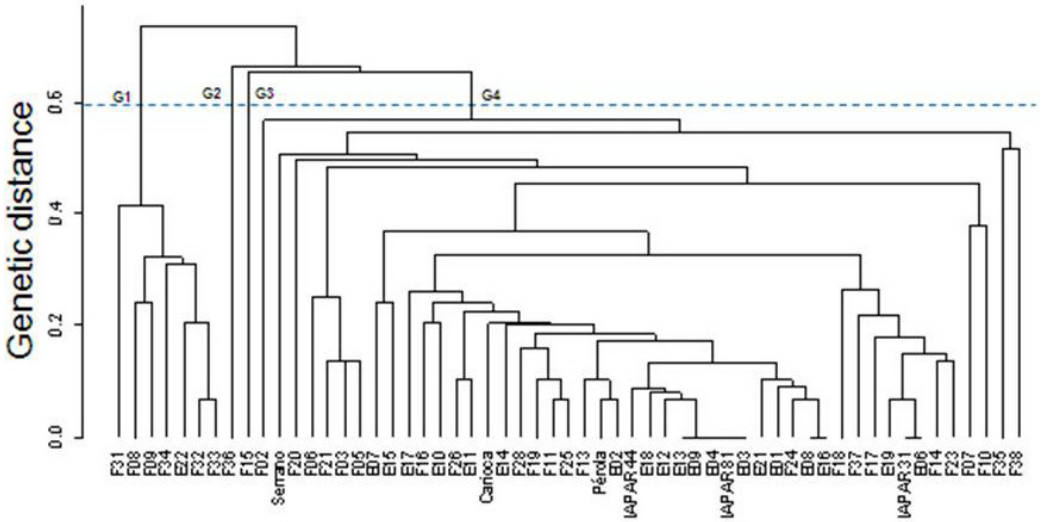

Figure 2. Dendrogram obtained using microsatellite markers with the complement of the weighted index, using the UPGMA method, of 57 dry bean genotypes. Blue line: cut-off point in the dendrogram at approximately 0.6.

The cophenetic correlation value was high and adequate (r = 0.92). This coefficient translated the product-moment relationship, calculated between the elements of the original matrix and those of the cophenetic matrix, resulting from the simplification provided by the grouping method, after construction of the dendrogram. Values of cophenetic correlation value above 0.8 indicated good representation among the distances (Bussad et al., 1990).

Using the cut-off point in Figure 2, four groups were formed. Group G1 included the accessions F31, F08, F09, F34, E22, F32, and F33; these accessions were considered to be of Andean origin, based on seed weight (Table 1). According to Gepts and Bliss (1986), dry bean genotypes with 100-seed weights less than 25 g normally belong to the Mesoamerican group and those with weights near or above 33 g, would be from the Andean group.

Lioi et al. (2005), evaluating the genetic diversity of 33 dry bean populations with SSR and amplified fragment length polymorphism (AFLP) markers, observed that both types of markers grouped the genotypes into Andean and Mesoamerican centers of origin. Benchimol et al. (2007), in a study of the genetic diversity of 20 dry bean genotypes, using SSR markers, observed that two large groups were formed, one containing the Andean-origin genotypes and the other the Mesoamerican-origin genotypes. These investigators demonstrated the capacity of SSR markers to separate genotypes by centers of origin, supporting the conclusions that we made in our study.

Accession F36, alone, formed group G2. This genotype had a 100-count seed weight above 33 g; but it did not group with the Andean-origin accessions. According to Alzate-Marin et al. (2003), some genotypes, even though they have large and heavy seeds, have a low percentage of Andean genes.

Group G3, consisting of the single accession F15, had the lowest weight per 100 seeds (14.24 g), which demonstrates genetic divergence from the other genotypes considered to be Mesoamerican (group G4).

Group G4 included all commercial cultivars (Serrano, Iapar 81, Iapar 31, Carioca, Pérola, and Iapar 44) and also nearly all cultivars from EMBRAPA, except for accession E22. This demonstrates the small degree of genetic variability among the commercial and EMBRAPA genotypes, and between these two groups. All genotypes within this group are considered to be of Mesoamerican origin, based on P100 (Table 1).

Based on the genetic dissimilarity analysis, we observed a tendency toward grouping of accessions with the same tendency for growth. Among the seven accessions of group G1, six have a determinate growth habit (type I); only accession F08 was classified as having indeterminate growth. In group G4, all accessions have indeterminate growth (types II and III). Masi et al. (2009), who studied genetic diversity of 73 dry bean genotypes, using 30 pairs of SSR primers and 393 AFLP markers, observed grouping of genotypes according to the growth patterns in both analyses.

We did not observe a tendency to group genotypes based on seed-coat color (commercial group of the grain).

The dissimilarity matrix among the commercial cultivars is given in Table 3. Dissimilarity varied from 0.07 to 0.55, with the lowest dissimilarity observed between Iapar 81 and Iapar 44 (0.07) and the highest degree between Serrano and three other cultivars, Pérola, Carioca and Iapar 44 (0.55). A high degree of similarity was found among the Iapar cultivars. Cultivar Serrano gave the highest dissimilarity compared to the other commercial cultivars.

Carvalho et al. (2008), using analyses made with random amplified polymorphic DNA (RAPD) markers, reported a low degree of dissimilarity among commercial dry bean cultivars. Emygdio et al. (2003), working with genetic diversity of dry bean cultivars in Rio Grande do Sul, based on RAPD markers, concluded that the commercial cultivars had a high degree of similarity with each other.

Table 3. Dissimilarity matrix of the commercial cultivars constructed using the complement of the weighted index based on the SSR markers.

	Pérola	Iapar 81	Carioca	Serrano	Iapar 31	Iapar 44
Pérola	0					
Iapar 81	0.14	0				
Carioca	0.21	0.14	0			
Serrano	0.55	0.48	0.55	0		
Iapar 31	0.28	0.14	0.28	0.34	0	
Iapar 44	0.21	0.07	0.21	0.55	0.21	0

The local genotypes were widely distributed in the dendrogram (Figure 2), which demonstrates wide genetic variability among those genotypes and compared to the EMBRAPA and commercial cultivars. Carvalho et al. (2008) stated that maintenance of the genetic diversity that is held by small-scale farmers can be of great value, as they probably contain alleles for local adaptations, disease resistance and tolerance to the principal edaphoclimatic adversities in the regions.

In the PCoA, the first two coordinates explained 78.03% of the total variation, with 58.21% explained by the first and 19.82% by the second (Figure 3). Somata et al. (2009), who evaluated the divergence between 39 lines of *Vigna uniculata*, using 48 pairs of SSR primers, observed that the first two principal components accounted for 21.74% of the variation, 14.18% by the first and 7.56% by the second. Benchimol et al. (2007) evaluated the divergence among 29 genotypes of dry beans using 87 SSR primer pairs; the three first principal components explained 45% of the total variation.

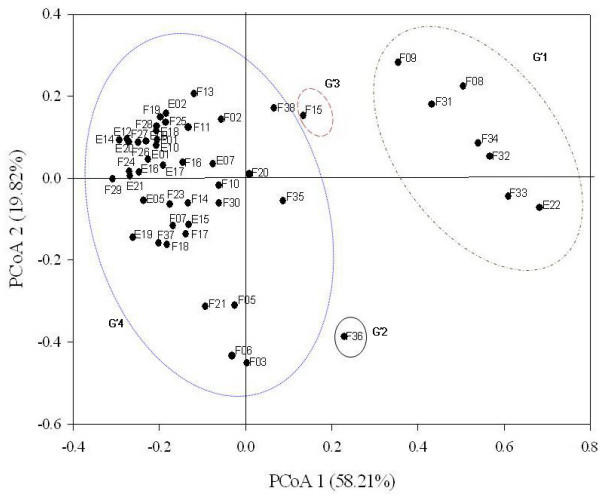

Figure 3. Principal coordinate analysis (PCoA) obtained from the complement of the weighted index based on SSR markers of 57 dry bean genotypes.

Based on the four groups formed, the genotypes classified as Andean were grouped as G'1 and G'2, and those classified as Mesoamerican origin in groups G'3 and G'4, the same groupings found with UPGMA analysis. Benchimol et al. (2007), in a study of the genetic diversity of dry beans using SSR markers, observed that the genotypes grouped according

to center of origin, in both groupings and principal coordinate analyses, confirming what we found in our study.

ACKNOWLEDGMENTS

Research supported by Fundação de Amparo à Pesquisa do Espírito Santo (FAPES). Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) awarded a Masters of Science scholarship to P.D.S. Cabral.

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