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Genetic diversity in the Brazilian species of *Adesmia* DC (Leguminosae) as assessed by RAPD

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Abstract

The 17 Brazilian species of *Adesmia* DC were analysed, using 20 primers, with regard to their randomly amplified polymorphic DNA (RAPD) patterns. From a total of 357 individuals analysed, the 20 primers produced 2249 fragments with molecular sizes ranging from 200 to 2700 bp, 56% of which were polymorphic. Average intra-population genetic similarity, estimated by Jaccard's coefficient, ranged from 0.35 in *A. araujoi* to 0.80 in *A. punctata*. Mean intra-specific genetic similarity varied greatly among species, ranging from 0.19 for *A. tristis* to 0.89 for *A. arillata*. Mean genetic similarity among the species, estimated by Dice's coefficient, was 0.56. RAPD markers were efficient at separating all the accessions analysed. The results obtained generally agreed with the partition of genetic variability expected according to the mode of reproduction.

Keywords: *Adesmia*; forage species; genetic variability; RAPD

Introduction

In many regions of the world where the economy is based mainly on agriculture and animal production, the search for native forage, as alternatives and complements to traditionally cultivated species and native pastures, is a growing strategy. In Rio Grande do Sul, the southernmost state of Brazil (27–34°S, 49–58°W), animal production depends heavily on native pastures, which have around 400 grass and 150 legume species (Boldrini, 1997), many of them potentially good forage, but still unexplored. A multidisciplinary approach is highly recommended to gain better knowledge of this native ecosystem. Agronomically, more widespread use of many of these species is restricted by their concentrated summer production, leading to a lack of forage during winter, therefore causing animal weight loss during the cold season. The alternative of diet supplementation is not an economically viable option for all farmers. Therefore, the search for native winter-growing species is a goal being pursued by researchers and plant breeders.

Among the native winter-growing legumes, adapted to the soils and climate conditions of the region, the species of the genus *Adesmia* DC have caught the attention of researchers. The exclusively South American genus *Adesmia* comprises around 230 species, 17 of which are described for Brazil, restricted to the southern region (Miotto and Leitão Filho, 1993). The Brazilian species are mostly diploid ($2n = 20$), with regular meiotic behaviour and generally high pollen fertility (Tedesco *et al.*, 2002); most of them are allogamous or preferentially allogamous (Tedesco *et al.*, 1998, 2000). Data are available pointing to the good forage potential of some of these species in Uruguay and Brazil (Coll and Zarza, 1992; Scheffer-Basso *et al.*, 2001), species which could be improved by genetic breeding.

Germplasm characterization is a first essential step for any plant breeding project. Besides the cytogenetic and

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reproductive studies already done on *Adesmia* (Tedesco *et al.*, 1998, 2000, 2002), it is necessary to evaluate the overall genetic variability, which can be done efficiently with molecular markers such as randomly amplified polymorphic DNA (RAPD) markers.

RAPD markers have been extensively used in characterizing germplasm and assessing genetic variability in a wide range of wild and cultivated plants, such as *Leucaena* (Harris, 1995), *Triticum* (Sun *et al.*, 1998), *Lathyrus* (Croft *et al.*, 1999; Chtourou-Ghorbel *et al.*, 2002), *Hordeum* (De Bustos *et al.*, 1998), *Ilex* (Gauer and Cavalli-Molina, 2000), *Capsicum* (Rodriguez *et al.*, 1999; Buso *et al.*, 2001), *Medicago* (Brummer *et al.*, 1995; Mengoni *et al.*, 2000) and *Trifolium* (Gustine *et al.*, 2002), among many others. The restrictions on the use of these markers, due to their dominant nature and frequent lack of repeatability, are mostly overcome by the combination of relatively low price, safety (non-radioactive) and amount of information generated by the technique, compared to other molecular markers.

In this paper we report the assessment on intra- and inter-specific variability in the 17 Brazilian species of *Adesmia*, as revealed by RAPD.

Material and methods

Plant material

Seeds from 29 accessions of 16 *Adesmia* species from several collection places in southern Brazil, and one accession from Uruguay, were obtained from CENARGEN (Centro Nacional de Pesquisa de Recursos Genéticos e Biotecnologia) and from different field collections. For *A. paranensis*, leaf material from six different herbarium vouchers was used (Table 1). These 17 species belong to four taxonomic series: *Muricatae* (*A. muricata*), *Subnudae* (*A. riograndensis* and *A. securigerifolia*), *Bicolores* (*A. bicolor*, *A. incana*, *A. latifolia* and *A. punctata*) and *Psoraleoides*. After mechanical scarification with sandpaper, seeds were germinated in Petri dishes with moist filter paper. Plantlets were transferred to 4.5 kg capacity pots with garden soil when around 3 cm high and kept in a greenhouse. With the exception of *A. paranensis*, 4–15 individuals per each accession were employed for the RAPD assays.

DNA extraction and RAPD assay

DNA was extracted from freshly harvested young leaflets from plants growing in the greenhouse and from the herbarium vouchers of *A. paranensis*, using a modified small-scale CTAB procedure (Doyle and Doyle, 1987).

DNA amplification was performed following Williams *et al.* (1990), with small modifications. Twenty primers (OPA 1 to OPA 20) of the kit OPA (Operon Technologies Inc., USA) were used.

Data analysis

For each primer, the consistent amplified fragments were recorded as present (1) or absent (0) and data were transformed in a binary matrix, representing the RAPD phenotype of each individual. Faint bands were not scored.

For the generation of similarity matrixes, Dice's coefficient was used for inter-specific comparisons and Jaccard's coefficient for intra-population and inter-population analysis. The different coefficients were chosen considering their peculiarities: Jaccard's does not value shared absences (0), and therefore is better at comparing populations within species. On the other hand, Dice's, despite also not valuing shared absences, emphasizes positive concordance, therefore being more suitable for comparing different items in which the probability of common bands is smaller (Alfenas *et al.*, 1998). The resulting similarity matrix was utilized to construct a dendrogram by the UPGMA (unweighted pair-group method of arithmetic averages) cluster analysis method. Data analysis was carried out using the NTSYS PC computer package (Rohlf, 2001).

Results

RAPD variation within accessions

From a total of 357 individuals analysed, the 20 primers produced 2249 fragments, with molecular sizes ranging from 200 to 2700 bp, 1254 of which were polymorphic, showing a total of 56% polymorphism. The average number of fragments per primer ranged from 0.7 (in *A. paranensis* 30.1 and 30. 3) to 10.7 (in *A. bicolor* 03) (Table 2). Among the amplified fragments some were identified as individual-specific, such as the 400 bp fragment (OPA 14) in individual 12 of *A. incana* 08, and others were identified as accession-specific, such as the 2200 bp fragment (OPA 16) in *A. rocinbensis* 21.

Average intra-population genetic similarity, estimated by Jaccard's coefficient in the species for which more than one accession was analysed, ranged from 0.35 in *A. araujoi* 02 to 0.80 in *A. punctata* 17 (Table 3). In the three species for which only one accession was analysed, intra-population similarity coefficients were 0.76 for *A. sulina* 24, 0.49 for *A. vallsii* 28 and 0.60 for *A. reitziana* 29. For *A. paranensis*, the intra-population comparison cannot be applied, as actually six individuals (30.1, 30.2, 30.3, 30.4, 30.5, 30.6) and not six populations

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Table 1. List of the Brazilian *Adesmia* species and accessions used in this study for assessment of genetic diversity by RAPD

Accession	Species	Collector's number	Place of collection
01	*A. araujoi*	V10730	Soledade, RS
02	*A. araujoi*		Capingui, RS
03	*A. bicolor*	D. Real 559	Uruguay
04	*A. bicolor*		Bagé, RS
05	*A. ciliata*	STS 1966	São José dos Ausentes, RS
06	*A. ciliata*	STS 1968	São Francisco de Paula, RS
07	*A. incana*	V 9636	Santana do Livramento, RS
08	*A. incana*		Caçapava do Sul, RS
09	*A. latifolia*	Te 003	Mariluz, RS
10	*A. latifolia*	ZM 1777	Tramandaí, RS
11	*A. muricata*	V 10289	Caçapava do Sul, RS
12	*A. muricata*	V9570	Canguçú, RS
13	*A. arillata*	V 11318	Guarapuava, PR
14	*A. arillata*	V11426	Abelardo Luz, SC
15	*A. psoraleoides*	V 8003	Bom Jardim da Serra, SC
16	*A. psoraleoides*	EEL 57	Lages, SC
17	*A. punctata*	V6885	Vacaria, RS
18	*A. punctata*	V10812	Vacaria, RS
19	*A. riograndensis*	V9590	Santana da Boa Vista, RS
20	*A. riograndensis*		Lages, SC[a]
21	*A. rocinhensis*	V10805	Bom Jesus, RS
22	*A. rocinhensis*	V 11507	Palmas, PR
23	*A. securigerifolia*	V 10283	Dom Pedrito, RS
24	*A. sulina*	V11316	Guarapuava, SC
25	*A. tristis*	STS 1969	São Francisco de Paula, RS
26	*A. tristis*		São Joaquim, SC
27	*A. securigerifolia*	V 6978	Bagé, RS
28	*A. vallsii*	V11439	Palmas, PR
29	*A. reitziana*		Urubici, SC
30.1	*A. paranensis*	V11246	Castro, PR
30.2	*A. paranensis*	V10607	Água Doce, PR
30.3	*A. paranensis*	V12864	Santiago, RS
30.4	*A. paranensis*	STS 1605	Palmeira, PR
30.5	*A. paranensis*	V11485	Água Doce, PR
30.6	*A. paranensis*	ZM 1236	São Francisco de Assis, RS

PR, Paraná State; RS, Rio Grande do Sul State; SC, Santa Catarina State.
[a] Seed obtained from EMPASC (Empresa de Pesquisa Agropecuária de Santa Catarina), Lages, original collection site unknown.

were analysed. In this case, the similarity index reflects the similarity between six individuals from six different places of collection.

Intra-specific RAPD variation

The RAPD intra-specific variability was analysed in 13 of the 17 Brazilian *Adesmia* species, i.e. those for which more than one accession was available: *A. araujoi*, *A. bicolor*, *A. ciliata*, *A. incana*, *A. latifolia*, *A. muricata*, *A. arillata*, *A. psoraleoides*, *A. punctata*, *A. riograndensis*, *A. rocinhensis*, *A. securigerifolia* and *A. tristis*. For this kind of analysis, fragments with the same molecular weight and common to a pair of accessions were scored. A total of 395 fragments were considered, ranging from 11 in the accessions of *A. tristis* to 57 in the accessions of *A. bicolor* (Table 4). Mean intra-specific genetic similarity varied greatly among species, ranging from 0.19 for *A. tristis* to 0.89 for *A. arillata* (Table 3).

The 147 fragments common to all species were used to generate the dendrogram in Fig. 1. The mean genetic similarity (0.56) was considered as the cutting point. For some species both accessions of the same species grouped together at the 0.75 level of similarity, such as *A. incana* 07 and 08, *A. latifolia* 09 and 10, *A. tristis* 25 and 26 and *A. securigerifolia* 23 and 27; others grouped at the 0.70 level of similarity, such as *A. ciliata* 05 and 06, *A. bicolor* 03 and 04 and *A. rocinhensis* 21 and 22; but in other cases, accessions of different species grouped together, such as *A. psoraleoides* 15 and *A. riograndensis* 19, and *A. psoraleoides* 16 and *A. sulina* 24. Surprisingly, accessions 14 of *A. arillata* and 18 of *A. punctata* grouped at the 0.80 level of similarity.

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Table 2. Number of primers and RAPD fragments analysed, mean number of fragments per primer and fragment size in each accession of the Brazilian species of *Adesmia* examined

Accession	Number of primers analysed	Number of fragments analysed	Mean number of fragments per primer	Fragment size in bp (min–max)
01	19	102	5.3	300–2400
02	20	117	5.8	236–2300
03	17	183	10.7	283–2700
04	17	134	7.8	280–2072
05	20	115	5.7	345–2300
06	20	80	4.0	300–2182
07	20	99	4.9	327–2457
08	19	99	5.2	310–2462
09	20	106	5.3	370–2700
10	20	102	5.1	300–2300
11	18	76	4.2	287–2300
12	11	50	4.5	452–2100
13	10	27	2.7	320–1781
14	15	61	4.0	240–2300
15	11	33	3.0	400–2300
16	19	69	3.6	200–2400
17	19	96	5.0	300–1800
18	20	69	3.4	300–2400
19	12	58	4.8	250–2160
20	13	67	5.1	340–2600
21	18	60	3.3	200–2200
22	12	58	4.8	220–2400
23	12	51	4.2	300–1784
24	7	31	4.4	300–2072
25	18	77	4.2	260–2400
26	10	38	3.8	200–2300
27	10	47	4.7	260–1800
28	13	42	3.2	400–1500
29	10	63	6.3	200–1900
30.1	7	5	0.7	853–1941
30.2	7	7	1.0	600–2500
30.3	7	5	0.7	300–1325
30.4	7	8	1.1	213–1941
30.5	7	6	0.8	213–1941
30.6	7	8	1.1	500–2116

Discussion

RAPD markers were efficient at separating all the accessions analysed. The accessions were all individualized, that is, no individual of a given accession mixed with individuals of other accessions and no pair of accessions presented a similarity index of 1.0. The identification of some individual-specific and accession-specific bands may be useful for obtaining a genetic fingerprint for *Adesmia*. The surprising result of a close similarity between accessions 14 of *A. arillata* and 18 of *A. punctata* may be spurious. As use of the wrong sample during DNA extraction or RAPD assay is very unlikely, an alternative explanation could be mislabelling of the original seed sub-sample. Further support for this is the fact that accessions 17 and 18 of *A. punctata* came from exactly the same collection site (Miotto and Leitão Filho, 1993) and should be expected to be much more similar to each other than to accession 14 of *A. arillata*.

RAPD markers are presently considered not to be reliable for analysing species relationships, mainly due to the validity of the assumptions, such as independence of fragment amplification, origin and repeatability of each band, and homology of bands of the same molecular weight, that are implicitly accepted when RAPD data are used in establishing species relationships (Harris, 1995; Gillies and Abbott, 1998). Therefore the grouping of accessions in the dendrogram of Fig. 1 does not reflect species relationships.

The results obtained in the intra-population as well as in the inter-population analysis generally agreed with the partition of genetic variability expected according to the

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Table 3. Mean intra-population and mean intra-specific genetic similarity as assessed by RAPD in the Brazilian species and accessions of *Adesmia* (Jaccard's coefficient)[a]

	A. araujoi		*A. bicolor*		*A. ciliata*		*A. incana*		*A. latifolia*		*A. muricata*		*A. arillata*		*A. psoraleoides*		*A. punctata*		*A. riograndensis*		*A. rocinhensis*		*A. tristis*		*A. securigerifolia*	
	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	25	26	23	27
01	0.56																									
02	**0.75**	0.35																								
03			0.50																							
04			**0.54**	0.51																						
05					0.59																					
06					**0.47**	0.63																				
07							0.75																			
08							**0.82**	0.72																		
09									0.60																	
10									**0.68**	0.58																
11											0.70															
12											**0.38**	0.62														
13													0.73													
14													**0.89**	0.71												
15															0.72											
16															**0.75**	0.73										
17																	0.80									
18																	**0.76**	0.70								
19																			0.65							
20																			**0.44**	0.62						
21																					0.72					
22																					**0.71**	0.64				
25																							0.75			
26																							**0.19**	0.40		
23																									0.74	
27																									**0.31**	0.58

[a] Plain type, intra-population indexes; bold type, intra-specific indexes.

Table 4. Intra-specific comparison between *Adesmia* accessions: number of primers and RAPD fragments analysed, fragment size and similarity index

Species	Accessions	No. of primers analysed	No. of fragments analysed	Fragment size (bp)	Mean inter-population similarity (Jaccard)
A. araujoi	01, 02	18	44	300–1500	0.75
A. bicolor	03, 04	19	57	300–1800	0.54
A. ciliata	05, 06	20	47	340–1900	0.47
A. incana	07, 08	16	49	370–2300	0.82
A. latifolia	09, 10	17	45	360–1500	0.68
A. muricata	11, 12	9	14	600–1200	0.38
A. arillata	13, 14	9	14	600–1800	0.89
A. psoraleoides	15, 16	9	17	500–1500	0.75
A. punctata	17, 18	18	42	300–1650	0.76
A. riograndensis	19, 20	9	15	380–1300	0.44
A. rocinhensis	21, 22	12	28	500–2400	0.71
A. securigerifolia	23, 27	5	12	300–1500	0.31
A. tristis	25, 26	5	11	300–1400	0.19

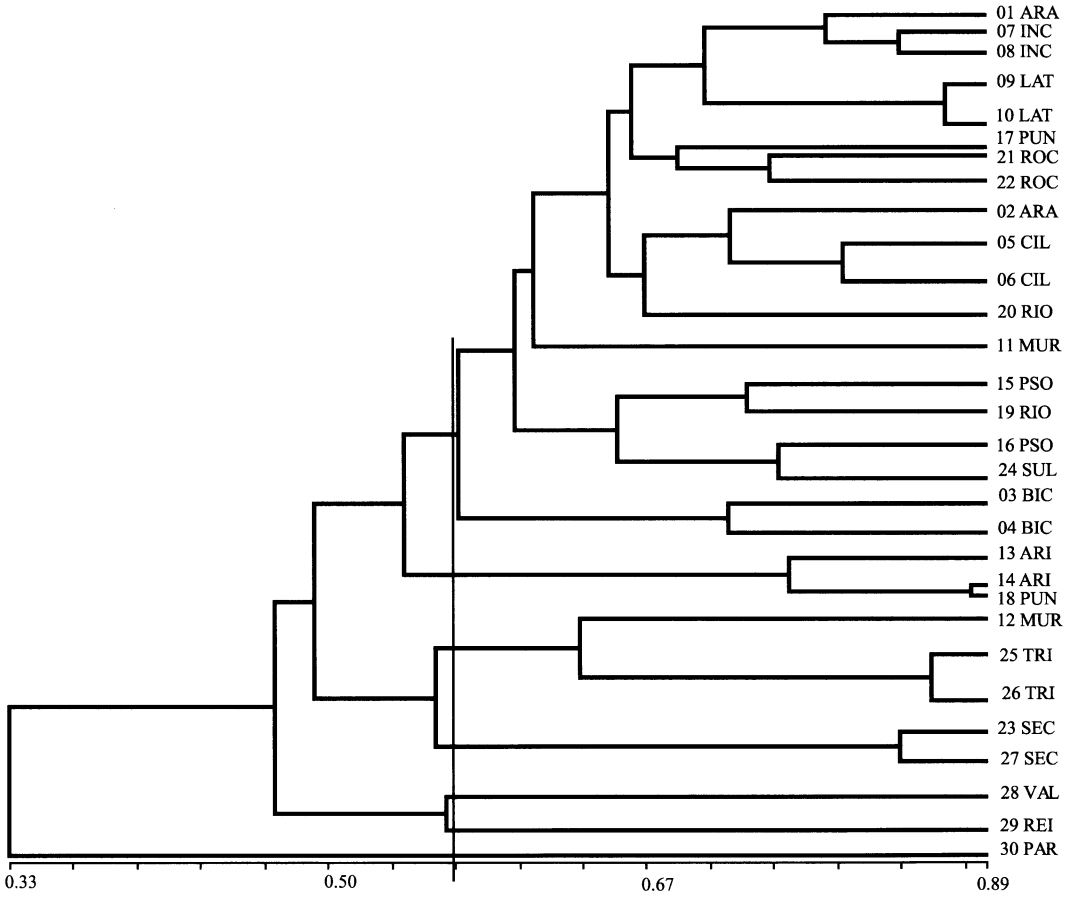

Fig. 1. Dendrogram constructed from RAPD-based genetic distances (Dice) of the Brazilian *Adesmia* species and accessions analysed. The thin vertical line indicates the cut point (mean genetic similarity). ARA, *A. araujoi*; ARI, *A. arillata*; BIC, *A. bicolor*; CIL, *A. ciliata*; INC, *A. incana*; LAT, *A. latifolia*; MUR, *A. muricata*; PAR, *A. paranensis*; PSO, *A. psoraleoides*; PUN, *A. punctata*; REI, *A. reitziana*; RIO, *A. riograndensis*; ROC, *A. rocinhensis*; SEC, *A. securigerifolia*; SUL, *A. sulina*; TRI, *A. tristis*; VAL, *A. vallsii*.

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mode of reproduction, allogamous species normally presenting lower intra-population and higher intra-specific similarities (higher intra-population and lower intra-specific variability), whereas autogamous species tend to have higher intra-population similarities and lower intra-specific similarities (higher intra-specific and lower intra-population variability). Total or partial allogamy seems to be the most common mode of reproduction in *Adesmia* species. According to Tedesco *et al.* (1998, 2000), *A. latifolia*, *A. muricata*, *A. punctata*, *A. tristis* and *A. bicolor* are allogamous or preferentially allogamous, *A. securigerifolia*, *A. riograndensis* and maybe *A. incana* are preferentially autogamous, whereas *A. araujoi*, *A. arillata*, *A ciliata*, *A. psoraleoides*, *A. reitziana*, *A. rocinhensis*, *A. sulina* and *A. vallsii* may be preferentially allogamous.

The relatively high mean level of intra-accession polymorphism observed in *Adesmia* (average of 56% considering all the primers and species) is normally expected in cross-pollinating species. In other allogamous species, such as *Trifolium pratense* (Campos-de-Quiroz and Ortega-Klose, 2001) and *Lolium perenne* (Kölliker *et al.*, 1999), percentages of polymorphic RAPD loci were 80% and 64%, respectively, therefore higher than the 56% found for *Adesmia*. However, those authors analysed only one species. On the other hand, in the present work we have analysed 17 species with varying modes of reproduction and as the overall estimate of polymorphic loci included all the species, the polymorphism value was probably decreased by the autogamous and versatile species. Autogamous species are expected to be less polymorphic. For example, in *Lathyrus sativus*, an autogamous species, 50% of the RAPD fragments were shown to be polymorphic (Croft *et al.*, 1999). The assessment of isozymic polymorphism in 10 *Lathyrus* species disclosed high levels of diversity for allogamous species and low levels of diversity for the autogamous ones (Bem Brahim *et al.*, 2002).

As expected, some of the allogamous or preferentially allogamous *Adesmia* species presented lower intra-population than intra-specific similarities, for example *A. latifolia*, *A. psoraleoides* and *A. bicolor*, whereas the preferentially autogamous species such as *A. securigerifolia* and *A. riograndensis* presented higher intra-population than intra-specific similarities (Table 3). In several other allogamous species, such as *Medicago sativa* (Yu and Pauls, 1993; Crochemore *et al.*, 1996), *Lolium perenne* (Sweeney and Danneberger, 1994) and *Trifolium pratense* (Kongkiatngam *et al.*, 1995), intra-population variability assessed by DNA markers was higher than intra-specific variability. In *Ilex paraguariensis* (Gauer and Cavalli-Molina, 2000), 85% of the RAPD diversity was detected within populations. On the other hand, for the autogamous *Oryza glumaepatula*, RAPD intra-population variability accounted for 11% of the total variance (Buso *et al.*, 1998). The results for some *Adesmia* species did not fit those expected with regard to their mode of reproduction, as for example in the allogamous *A. tristis* and in the autogamous *A. incana*. This could be due to varying degrees of reproductive versatility or to different genetic structures within the populations. In *Hordeum*, De Bustos *et al.* (1998) found a good correlation between the level of intra-specific RAPD variation and the reproductive system, with the exception of the autogamous *H. marinum* ssp. *gussoneanum* which showed an exceptionally low inter-populational variation.

In conclusion, RAPD markers were efficient in the characterization of all the *Adesmia* accessions studied, allowing the assessment of genetic variation in the 17 Brazilian species of *Adesmia*. In general, the partition of genetic variability was in agreement with the mode of reproduction of the species, allogamous species presenting higher intra-population variability and lower inter-population variability, the opposite being true for autogamous species. The data reported here are important contributions to a better knowledge of the Brazilian *Adesmia* species and may be important for biodiversity management as well for the planning of genetic breeding programmes.

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