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Forage peanut (*Arachis* spp.) genetic evaluation and selection

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Abstract

Forage peanut improvement for use in grass–legume mixtures is expected to have a great impact on the sustainability of Brazilian livestock production. Eighteen cloned *Arachis* spp. ecotypes were evaluated under clipping in a Brazilian Cerrado region and results analysed using a mixed model methodology. The objective was to estimate genetic and phenotypic parameters and to select the best ecotypes based on selection index applied on their predicted genotypic value. The traits of total dry-matter (DM) and leaf DM yield presented moderate ($0{\cdot}30 < h_g^2 < 0{\cdot}50$) to high (>0·50) broad-sense heritability, in contrast to the low genetic variability in nutritional quality-associated traits. Ecotypes of *Arachis* spp. contained average crude protein concentrations of 224 g kg^{-1} DM in leaves and 138 g kg^{-1} DM in stems, supporting the potential role of these species to overcome the low protein content in Cerrado pastures. The correlations between yield traits and traits associated with low nutritional value in leaves were consistently significant and positive. Genetic correlations among all the yield traits evaluated during the rainy or dry seasons were significant and positive. The ecotypes were ranked based on selection index. The next step is to validate long-term selection of grass–*Arachis* in combination with pastures under competition and adjusted grazing in the Cerrado region.

Keywords: *Arachis pintoi*, *Arachis repens*, dry-matter yield, heritability, repeatability, nutritional value, seasonal production, Cerrado

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Introduction

In Brazil, livestock production systems based on grassland face the challenges of producing more meat to supply both increasing internal demands and economically important exports and, most of all, to achieve this objective without expanding the area of pasture land. As livestock production is influenced by both the nutritive value and the voluntary intake of forage (Lüscher *et al.*, 2014), the adoption of strategies to improve the low nutritional quality of grass forages in Brazil would have a great impact in relation to livestock sustainability. In this context, the planting of adapted forage legumes is expected to improve the nutritional value of the forage supply, as well as contributing to the nitrogen input of low-fertility tropical soils (Shelton, 2005).

Germplasm of tropical forage legumes has been collected since the 1950s, and this has resulted in more than 17 000 accessions of 20 genera (Shelton, 2005) stored in germplasm banks. Forage legume species have been evaluated for their adaptability and production since collections began (Miles, 2001; Shelton, 2005), and the forage peanut (*Arachis pintoi* Krapovickas & Gregory) has become one of the most commonly used legumes in mixed swards in the northern region of Brazil (Andrade *et al.*, 2012). Among the tropical herbaceous legumes already evaluated in Brazil, none has such a high persistence and success as that shown by cultivars of *A. pintoi*. In northern Brazil, Acre State has the largest area planted with forage peanut (*A. pintoi* cv. Belmonte) (Shelton, 2005; Valentim and Andrade, 2005), and in this area, it has persisted over the last 25 years (Andrade *et al.*, 2012).

Arachis pintoi, *A. repens* Handro and their hybrids present several features related to persistence in the pasture sward, and they differ from those of other forage legumes (Menezes *et al.*, 2012) in their tolerance to adverse management conditions, resistance to grazing and stoloniferous growth (having a strongly rooted stolon and well-protected meristem growing points). *Arachis* persistence is also related to the presence of buried seed reserves (Assis *et al.*, 2008, 2013).

Arachis spp. cultivars have been obtained based on the evaluation and phenotypic selection of natural ecotypes (Assis *et al.*, 2013) and clonally commercialized as either stolon or rhizome (Valentim and Andrade, 2005), but this has limited their planting in large areas (Andrade *et al.*, 2015). The method of selection in wild ecotypes promoted their contribution to the identification of ecotypes for use in cultivars, but the increasing global demand for animal products in sustainable system requires that forage breeding be more efficient than in the past. Recently, forage peanut breeding has been performed on the clonal evaluation of ecotypes in one locality and the establishment of both intra- and interspecific controlled crossing among the best ecotypes (Assis *et al.*, 2008). Performing genotypic selection on elite, less variable material followed by clonal evaluations in multiple places in Brazil provides information about the regional adaptability and potential production, as well as information about genotype × environment interaction (Falconer and Mackay, 1996), and the need for particular genotypes for particular environments. Regional experiments on forage peanut breeding are generally conducted in monocultures, in which the ecotypes and hybrids are measured for dry-matter (DM) yield over multiple clippings, soil coverage, regrowth ability, diseases and insect attacks (Annicchiarico *et al.*, 2014). Identification of locally adapted ecotypes also provides information to guide breeders' decisions and strategies to make continuing improvements to the species.

At the start of a breeding programme, estimates of genetic parameters for agronomic traits in populations are helpful to breeders for determining the effectiveness of over time selection (Rose *et al.*, 2008) and for defining the best selection methods (Resende *et al.*, 2013) for local or multiple cultivar-growing locations by considering the ecotypes according to their environment interactions. Based on this premise, our objectives in this work were to estimate genetic and phenotypic parameters as well as to predict genotypic values with the aim of selecting the best of the *Arachis* spp. ecotypes that were evaluated in Campo Grande, Mato Grosso do Sul State, Brazil.

Materials and methods

Arachis spp. breeding, ecotype evaluation

This experiment was conducted at Embrapa Beef Cattle (20°44′S, 54°72′W; 530 m a.s.l.) near Campo Grande, MS, Brazil. The soil is classified as a Haplic Ferralsol (Rhodic) (FAO, 2006). An initial characterization of the surface soil (0–20 cm) indicated a soil pH of 5·2 and Mehlich-1-extractable P, K, Ca, Mg and Al values of 3, 39, 480, 134 and 35 mg kg^{-1} respectively. Dolomitic lime [$(CaMg)(CO_3)_2$] was applied to the experimental area at 2 t ha^{-1} before planting, in 2007. The field area was also fertilized with 50 kg ha^{-1} of P_2O_5, 50 kg ha^{-1} of K_2O and 5 kg ha^{-1} of micronutrients. There was no external source of N fertilization. Rainfall and temperature data are summarized in Figure 1, in terms of average monthly values during the experimental years and the 1967–2013 historical averages.

Eighteen ecotypes (Table 1) from an experiment established in December 2006 were evaluated under regular harvest by clipping from 2007 to 2009, and the superior ecotypes were selected. The planting material was obtained from Embrapa Acre (Rio Branco, AC, North Region, Brazil), which evaluated the *Arachis* germplasm and selected the best ecotypes for adaptability and stability over multiple local evaluations. Stolon ramets sized 20–25 cm were packed in humidified paper, placed in plastic bags and planted as soon as they were received. The stolons were planted at 2 m × 2 m plots, with two stolons each at 50 cm apart and approximately 5 cm deep. Irrigation was applied during periods of low rainfall in the first year. No irrigation was provided thereafter.

The experimental layout was a randomized complete block design with six replications. The fresh yield was recorded from a 1 m^2 quadrat at the centre of each plot by clipping to ground level, and from each sampled quadrat, a 200-g subsample of fresh herbage was separated into its components (leaf, stem and dead leaves/stems), then dried at 60°C and weighed. The total dry-matter (TDM) yield (leaf + stem + dead leaves and stems), leaf dry-matter yield (LDM) and stem dry-matter yield (SDM) were estimated on the basis of the total fresh weight and subsample weight and recorded across six harvests on the following dates: 10 December 2007, 27 February. 2008, 15 May 2008, 1 September 2008, 22 January 2009 and 28 September 2009. There are two primary seasons in this region of the Brazilian Cerrado: a dry season, which usually lasts from May to September (mean monthly rainfall 49 mm), and a rainy season, which lasts from October to April (mean monthly rainfall 150 mm) (Figure 1). Based on this information, the second, third and fifth clippings were analysed as rainy-season data and the fourth and sixth clippings were analysed as dry-season data. The first clipping occurred during the establishment phase so it was not included in the analysis.

Dried leaf and stem samples from the second and fifth clippings were ground in a Wiley-type mill with a 1-mm mesh sieve and analysed to determine the crude protein (CP) content by the Kjedahl method

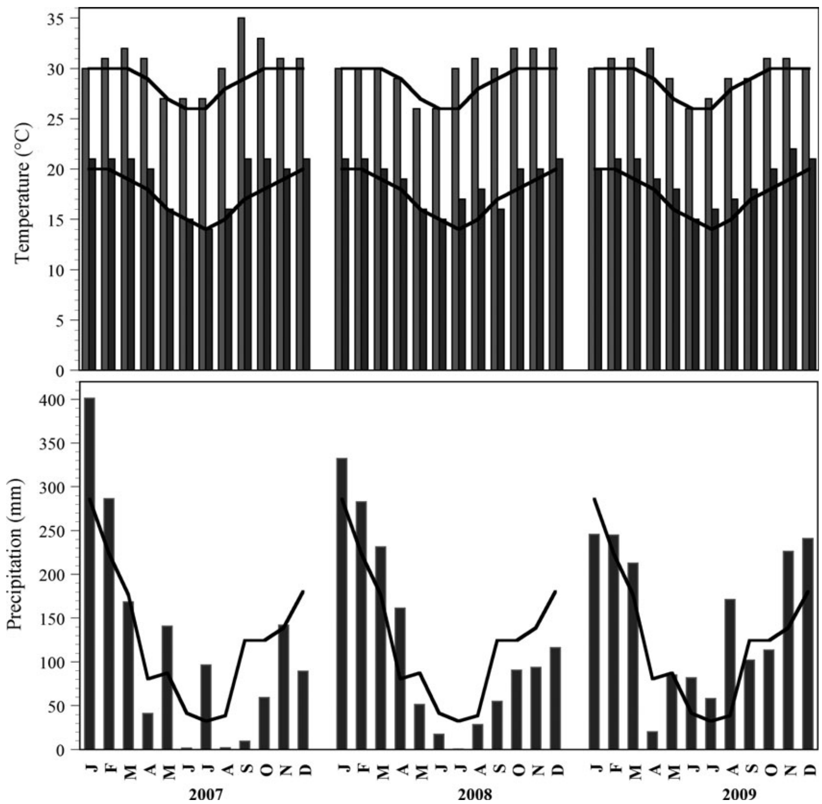

Figure 1 Average maximum and minimum monthly temperature and precipitation, which are indicated by the columns, at Embrapa Beef Cattle, Campo Grande, Brazil, during the experimental years (2007, 2008 and 2009), and the 46-year average monthly parameters are indicated by lines (the original data are available at http://bancodedados.cptec.inpe.br/).

and the contents of neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (Lig), according to van Soest (1994).

The analyses were performed under the following mixed linear model: $y = Xm + Zg + Wp + Ti + e$, in which y is the measure of the trait being analysed; m is a vector of fixed effects (i.e. a combination of the clipping number and replication); g is a vector of random genotypic effects from ecotypes; p is a vector of the random permanent environment; i is a vector of the random ecotype × clipping interaction; and e is the random residual effect. The elements X, Z, W and T are the incidence matrices for each vector described here respectively. The following parameters were estimated (Falconer and Mackay, 1996; Resende, 2007): (i) broad-sense heritability (h_g^2), $h_g^2 = \sigma_g^2 + \sigma_p^2 + \sigma_i^2 + \sigma_e^2$; (ii) repeatability, $\rho = (\sigma_g^2 + \sigma_p^2)/(\sigma_g^2 + \sigma_p^2 + \sigma_i^2 + \sigma_e^2)$; (iii) genotypic correlation among different clippings, r_{gm} and (iv) average ecotype broad-sense heritability, $h_{mg}^2 = (\sigma_g^2)/[\sigma_g^2 + (\sigma_i^2/c) + (\sigma_p^2/r) + (\sigma_e^2/cr)]$, in which c and r are the numbers of clippings and replications respectively. A genetic correlation between traits x and y was estimated as $y_{g_{xy}} = \frac{\text{cov}_{xy}}{\sigma_x \sigma_y}$. Deviance statistics were used to test the genotypic effect hypothesis. Adjustment to different models for random genotypic effect to the data was tested using Wilks' likelihood ratio test (LRT) (Dobson, 1990; Resende *et al.*, 2006). All statistical analyses were performed with Selegen REML-BLUP software (Resende, 2007).

The evaluated forage peanut ecotypes were ranked on the basis of their genotypic values as obtained through univariate analysis for the target traits, by considering all clippings (except the first). We also applied the Mulamba–Mock selection index (Mulamba and Mock, 1978) to improve the most important traits associated with animal performance under grazing. The selection index proposed by Mulamba and Mock (1978) is based on classification or ordering of the ecotypes for multiple traits. The principle of the Mulamba and Mock index is the transformation of the breeding values of the genotypes for each trait into rankings, according to the interest of the breeder, that is in the sense of increasing or decreasing the phenotypic expression. In our work, the ecotypes were ranked

Table 1 Identification of forage peanut ecotypes.

Ecotype ID (BRA code)	Collection site	Species
014931	Aracuai, MG	*A. pintoi*
033260	Sertanópolis, PR	*A. repens*
039799	São Simão, SP	*A. pintoi*
035068	Not available	*A. pintoi* × *A. repens* hybrid
035041	Not available	*A. pintoi* × *A. pintoi* hybrid
035033	Not available	*A. pintoi* × *A. pintoi* hybrid
030333	Formosa, GO	*A. pintoi*
039187	São Gonçalo dos Campos, BA	*A. pintoi*
014991	Presidente Juscelino, MG	*A. pintoi*
035114	Brasília, DF	*A. pintoi*
032352	Buenópolis, MG	*A. repens*
034436	Itaucu, GO	*A. repens*
032379	Buenópolis, MG	*A. repens*
032409	Engenheiro Navarro, MG	*A. pintoi*
037036	Rio Pardo, RS	*A. pintoi* cv. Alqueire-1
013251	Belmonte, BA	*A. pintoi*
031828	Itabuna, BA	*A. pintoi* cv. Belmonte
040550	Rio Branco, AC	*A. pintoi* cv. BRS Mandobi

BRA code: code from Embrapa.

based on the selection index by considering the TDM yield during the rainy season, the TDM yield during the dry season, stem NDF and stem CP. The best ecotypes in the index were selected for future evaluation under competition with grass and animal grazing.

Results

Genetic parameters

Highly significant genetic variation ($P < 0{\cdot}01$) was detected among the *Arachis* ecotypes for total, leaf and stem DM yield mostly during the rainy season or annually (Table 2). The expressed genetic variability was more pronounced during the rainy season, relative to the dry season, for almost all traits. Genetic variation was not expressed for the stem DM yield during the dry season. The broad-sense heritability ranged from moderate ($0{\cdot}30 < h_g^2 < 0{\cdot}50$) to high (>0·50) for total and leaf DM yield. The average ecotype broad-sense heritability (h_{mg}^2) was high for all evaluated traits as well as the genotypic correlation among clippings (r_{gm}), which indicates that the ecotype rank barely changed across different clippings. The genotypic value for total DM yield ranged from 0·59 t ha^{-1} per clipping (BRA035033) to 3·10 t ha^{-1} per clipping (BRA039799), meaning that there were opportunities for selection in this generation and in the next steps of forage peanut breeding, such as promoting controlled crosses to amplify variability and generate new alleles/ecotype combinations. Total biomass production ranged from 2·67 t ha^{-1} year (BRA035033) to 13·09 t ha^{-1} year (BRA039799) under frequent clippings. During the establishment year, the accumulated total biomass of ecotypes ranged from 0·13 t ha^{-1} year (BRA034436) to 3·17 t ha^{-1} year (BRA039799) as a result of the first clipping evaluation.

The rainy-season DM yield accounted for 0·70 of the annual production for all traits during the first year of evaluation, but during the second year, it accounted for 0·45–0·50 of all evaluated agronomic traits. Moreover, more dead leaves and stems appeared during the dry season than during the rainy season (data not shown), which explains the differences in the total DM yield and LDM yield plus SDM yield. *Arachis pintoi* presented a greater proportion of leaf relative to stem during the dry season (65% on average, compared with 43% during the rainy season), but this finding was observed only during the first year. The SDM yield proportion ranged from 0·33 to 0·41 of the total DM yield during the rainy season, and 0·33 to 0·34 during the dry season, based on *A. pintoi* ecotypes genotypic value in each season, per clipping. The LDM yield accounted for 0·55–0·66 of the total DM yield during the rainy season and 0·55–0·57 during the dry season. The four evaluated ecotypes of *A. repens* presented a greater proportion of the LDM yield during the dry season than that of *A. pintoi*, achieving 0·60 in relation to the total DM yield.

Repeatability was moderate when measured on an individual basis for all evaluated traits, except for the stem DM yield low value during the dry season (Table 2). Based on the observed repeatability, the number of clippings needed to obtain 90% accuracy in the selection was estimated for a maximum of two clippings for TDM and LDM yield. A low broad-sense heritability magnitude and a moderate-to-low repeatability that were estimated for the SDM yield will require more than five clippings to obtain high accuracy upon selection.

The genetic variability for the traits associated with leaf nutritional quality was low or absent in the ecotypes evaluated (Table 3). Genetic variability was present for the same traits that were evaluated in the stems, and it has been considered for the selection based on the index. The heritability and repeatability were of low magnitude for the NDF in leaves and for

Table 2 Genetic and phenotypic parameters estimated for the total dry-matter yield, leaf dry-matter yield and stem yield, in g m^{-2} per clipping, for *Arachis* spp. ecotypes that were evaluated under clippings and separated during rainy and dry seasons, and the total annual production.

	Total dry-matter yield			Leaf dry-matter yield			Stem dry-matter yield		
Parameter	Rainy season	Dry season	Annual	Rainy season	Dry season	Annual	Rainy season	Dry season	Annual
h^2_g	0·49	0·36	0·44	0·59	0·37	0·48	0·29	0·15	0·27
ρ	0·64	0·50	0·59	0·70	0·48	0·61	0·43	0·25	0·47
r_{gm}	0·88	0·95	0·90	0·90	0·91	0·85	0·71	0·49	0·78
h^2_{mg}	0·89	0·84	0·90	0·92	0·83	0·91	0·76	0·52	0·80
General mean	167·80	163·88	165·71	96·32	91·62	94·12	66·31	54·69	61·53
Minimum	59·01	70·46	59·75	31·98	30·10	27·58	29·69	25·83	28·54
Maximum	310·02	259·45	291·98	202·11	146·54	179·74	103·65	85·22	98·71
Number of clippings†	2	2	2	1	2	2	5	>10	6
LRT (genotypic effect)	36·60**	15·37**	58·39**	44·95**	13·63**	67·17**	14·90**	2·33^{ns}	26·36**

h^2_g, individual-plot broad-sense heritability; ρ, repeatability; r_{gm}, genotypic correlation among clippings; h^2_{mg}, genotypic mean broad-sense heritability; LRT, likelihood ratio test.Significant at **, 1% of probability by χ^2 test with 1 df. †Number of clippings to achieve 90% of accuracy in selection.

Table 3 Genetic and phenotypic parameters estimated for the forage quality traits for crude protein (CP, in g kg^{-1} DM), neutral detergent fibre (NDF, in g kg^{-1} DM), acid detergent fibre (ADF, in g kg^{-1} DM) and lignin (Lig, in g kg^{-1} DM) and then evaluated in *Arachis* spp. leaves and stems during the two rainy seasons.

	Leaf				Stem			
Parameter	CP	NDF	ADF	Lig	CP	NDF	ADF	Lig
h^2_g	0·14	0·32	0·21	0·003	0·28	0·34	0·28	0·33
ρ	0·14	0·33	0·23	0·011	0·45	0·37	0·29	0·36
r_{gm}	0·31	0·98	0·37	0·01	0·75	0·99	0·99	0·99
h^2_{mg}	0·37	0·76	0·47	0·014	0·65	0·77	0·73	0·77
General mean	224·4	423·3	252·2	59·3	138·2	483·1	390·8	89·4
First-year mean	225·1	459·6	258·2	64·0	128·2	476·9	384·9	78·1
Second-year mean	223·5	396·7	247·7	55·7	145·7	488·5	395·0	97·9
Minimum	209·9	406·3	229·0	55·3	128·4	467·8	375·9	82·8
Maximum	246·3	442·5	273·6	64·0	147·4	508·9	411·9	96·7
CV_g (%)	7·40	4·32	9·25	10·26	6·18	4·43	4·06	9·66
CV_e (%)	8·29	6·50	7·14	14·91	17·95	2·97	3·12	6·98
Number of clippings†	>10	3	>10	>10	7	3	4	3
LRT (genotypic effect)	0·80 ^{ns}	6·31*	1·55 ^{ns}	0 ^{ns}	3·94*	9·21**	7·21**	10·06**

h^2_g, individual-plot broad-sense heritability; ρ, repeatability; r_{gm}, genotypic correlation among clippings; h^2_{mg}, genotypic mean broad-sense heritability; CV_g, genetic coefficient of variation; CV_e, experimental coefficient of variation; ns, non-significant.Significant at *, 5% and **, 1% using the χ^2 test with 1 df. †Number of clippings to achieve 90% accuracy in selection.

the CP, NDF, ADF and lignin in stems, which would require 3–7 clippings to achieve a selection accuracy greater than 90%.

The genetic correlation coefficients were significant, of high magnitude and positive for all the yield traits studied (Table 4). This association was not found among the nutritional quality traits. The correlations between yield traits and traits that were associated with low nutritional value (ADF, NDF and lignin) in leaves were consistently significant and positive. By contrast, there were low correlations among yield traits and low nutritional value traits in the stems. The CP content in the stems also presented positive and significant correlations with the yield traits, except for the SDM yield during the dry season.

Table 4 Correlation coefficients among agronomic and nutritional quality traits that were estimated for 18 ecotypes of *Arachis* spp.

Traits	Annual LDM	Annual SDM	Rainy TDM	Rainy LDM	Rainy SDM	Dry TDM	Dry LDM	Dry SDM	Stem CP	Stem NDF	Stem ADF	Stem Lig	Leaf CP	Leaf NDF	Leaf ADF	Leaf Lig
Annual TDM	0·99**	0·95**	0·99**	0·97**	0·95**	0·97**	0·96**	0·86**	0·56**	−0·16	−0·11	−0·08	0·31	0·48*	0·54*	0·45*
Annual LDM		0·91**	0·99**	0·99**	0·91**	0·95**	0·94**	0·82**	0·60**	−0·19	−0·15	−0·12	0·28	0·47*	0·53*	0·43*
Annual SDM			0·90**	0·85**	0·98**	0·97**	0·95**	0·94**	0·41*	−0·02	0·01	0·03	0·44*	0·51*	0·49*	0·42*
Rainy TDM				0·99**	0·93**	0·91**	0·91**	0·78**	0·59**	−0·22	−0·15	−0·15	0·26	0·43*	0·53*	0·43*
Rainy LDM					0·87**	0·88**	0·88**	0·74**	0·63**	−0·26	−0·20	−0·20	0·20	0·41*	0·53*	0·41*
Rainy SDM						0·93**	0·92**	0·85**	0·41*	−0·04	0·01	0·00	0·41*	0·46*	0·50*	0·42*
Dry TDM							0·99**	0·94**	0·48*	−0·04	−0·03	0·04	0·39	0·53*	0·50*	0·45*
Dry LDM								0·91**	0·49*	−0·01	−0·02	0·05	0·40*	0·53*	0·48*	0·43*
Dry SDM									0·37	0·02	0·01	0·07	0·44*	0·52*	0·42*	0·38
Stem CP										−0·38	−0·34	−0·13	0·09	0·06	0·09	0·47*
Stem NDF											0·93**	0·79**	0·57**	0·09	−0·06	0·00
Stem ADF												0·82**	0·61**	0·06	−0·02	0·04
Stem Lig													0·57**	−0·11	−0·13	0·14
Leaf CP														0·09	0·03	0·23
Leaf NDF															0·83**	0·65**
Leaf ADF																0·67**

TDM, total dry-matter yield; LDM, leaf dry-matter yield; SDM, stem dry-matter yield; during rainy and dry season, and CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; and Lig, lignin in stems and leaves.Significant at the *, 0·05 and **, 0·01 probability levels, respectively.

Arachis spp. ecotypes selection

All information on genetic analyses were taken into account in the index composition for the identification of ecotypes to guide future controlled crosses in breeding programmes and to move forwards towards other steps in the evaluation. Thus, 33% of the evaluated ecotypes were selected with genotypic values based on the selection index as follows: BRA039187, 8·13; BRA039799, 7·95; cultivar Belmonte, 7·35; BRA014991, 7·02; BRA032379, 6·97, and BRA040550 cultivar BRS Mandobi, 6·77. Only the 032379 ecotype represented *A. repens.* No interspecific hybrids were selected. A ranking correlation among selected ecotypes in Campo Grande, MS, presented a low magnitude (0·44) with ecotypes selected on the basis of total DM yield and coverage ability as evaluated by Assis *et al.* (2008) in Rio Branco, Acre State. However, besides the different rank, the best four ecotypes in both places were the same. The first clipping ecotypes ranking, as based on DM yield genotypic values predicted, was compared with the annual selection (Figure 2). The performance of ecotypes did not change in either case.

Discussion

The improvement of *Arachis* spp. in the context of this experiment specifies the target selection of the ecotypes most adapted to the Cerrado region in indicating or producing a new cultivar. This work also identifies ecotypes of high productivity and nutritional quality through promoting interspecific or intraspecific crossings of the species evaluated as a strategy to continue breeding.

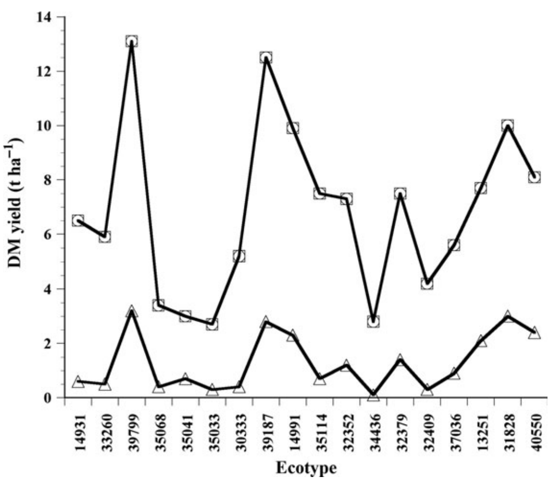

Figure 2 Dry-matter (DM) yield genotypic values in Arachis ecotypes predicted on annual basis and in the first clipping.

Both targets were investigated, given that two commercial cultivars (cvs. Belmonte and Mandobi) were selected from among the six ecotypes with the best performance. Two ecotypes, BRA039187 and BRA039799, presented a 43% greater average annual DM yield than the cultivars Belmonte and Mandobi. This result shows the importance of the evaluation of multiple ecotypes in multiple locales when aiming to form correct conclusions regarding the adaptation and potential use of a forage legume for a particular locality. Additionally, the importance of evaluating natural ecotypes with the aim of obtaining cultivars and genetic gains in a short time in wild species has seldom been explored (Miles, 2001). Nevertheless, a breeding programme aiming to achieve genetic gains over generations is indispensable.

The three interspecific hybrids were not among the selected ecotypes; however, this information must be considered relatively unimportant, given their low representation among the genetic material evaluated. The potential of interspecific hybridization in the breeding of the genus is inconclusive based on these data. Half-sib and full-sib progeny tests will be important in future work in order to draw conclusions about the best use of intraspecific and interspecific hybridization in *Arachis* breeding.

Considering the biomass yield traits evaluated, selection is favoured by the presence of genetic variation. Broad-sense heritability for DM yield presented a magnitude similar to that obtained for *Medicago sativa* L. ($h_g^2 = 0.43$ – Annicchiarico, 2015); however, the magnitude was lower than that obtained by Assis *et al.* (2008) for the same ecotypes evaluated in the northern Brazilian region. The relative amount of additive and non-additive genetic variation is still unknown for *Arachis* species. Information on the distribution of genetic variances associated with the elucidation of mating type in this species is important for making decisions about the best breeding method to be adopted (Resende *et al.*, 2013).

Unlike annual crop species, perennial forages are usually field-evaluated over successive clippings and sometimes for years. This long period of testing introduces the estimation of new genetic parameters that can be useful in breeding, such as repeatability (Falconer and Mackay, 1996). Repeatability estimates in the traits associated with productivity in forages are generally classified as having a moderate-to-high magnitude, as obtained in *Elymus repens* (Casler *et al.*, 1998), *Eragrostis curvula* (Renzo *et al.*, 2000), *Medicago sativa* (Ferreira *et al.*, 1999), *Trifolium repens* (Jahufer and Gawer, 2000), *Secale cereale* L. (Webb *et al.*, 2013), *Panicum maximum* (Resende *et al.*, 2004) and *Pennisetum* spp. (Cavalcante *et al.*, 2012). Interesting evidence was found for the high-magnitude correlation between the ecotype rankings in both nutritional analyses to NDF in leaves and in NDF, ADF and lignin in stems. In this case, even with low-magnitude repeatability, the ecotype ranking will not vary between assessments over different seasons of the year for each of these traits, which reduces labour and costs.

Information about the number of clippings for high accuracy allows a significant increase in the selection efficiency, and it improves the gain per unit of time. However, the number of annual clippings needed to obtain a high coefficient of determination, or, more appropriately, a greater selective accuracy, may vary among species. Differences in magnitude between individual broad-sense heritability and repeatability in all agronomic traits (Table 2) indicated that they were influenced by the general environment variance (Falconer and Mackay, 1996); thus, the general environment variance can be confounded with the genotypic variance when only the repeatability is estimated. This information is important because it indicates that a low proximity of the genotypic value of the individual candidate to clonal selection will be obtained based solely on the use of repeatability as the regressor of the phenotypic value.

The *Arachis* ecotypes produced 2·7–13·1 t DM ha^{-1} annually in Campo Grande, MS. The DM yield, in g m^{-2}, was lower than that obtained by Assis *et al.* (2008) in the more rainy Brazilian North region, for the same ecotypes. The *Arachis* herbage production in Cerrado is comparable to that of other forage legumes, such as *Trifolium repens* (7·0–15·5 t DM ha^{-1}) in Britain (Frame and Newbould, 1986) and *Lotus corniculatus* (4·3–14·2 t DM ha^{-1}) in Europe (Halling *et al.*, 2004). *Arachis* herbage production was lower than *Medicago sativa* production in a variety of experiments performed worldwide according to Phelan *et al.* (2015). However, we must also consider the 12 months prior to establishment of the sward plots of *Arachis* ecotypes before the start of clipping evaluations. Similar to temperate forage legumes (Moot, 2013; Phelan *et al.*, 2015), the establishment of tropical forage legumes is also slow, and this characteristic is one of the reasons for their poor adoption by farmers (Shelton, 2005; Phelan *et al.*, 2015). Focusing exclusively on ecotypes selection, we have provided evidence that the first clipping DM yield resulted in a ranking of ecotypes similar to that based on the all five subsequent clippings (Figure 2). Therefore, despite their slow establishment, A*rachis* first clippings are informative for selection and may be useful for establishment-ability experiments in monoculture situations. Additional studies on persistence under grazing and grass competition ability are necessary after this initial selection in monoculture sward plots.

A better distribution of wet- and dry-season yields in tropical forages is a well-recognized demand of livestock producers, particularly in central Brazil, where animals are bred and finished under grazing (Euclides *et al.*, 2007). However, the *Arachis* ecotypes showed poor ability to produce herbage during the dry season, similar to the more important grasses cultivated in the Cerrado region (Valle *et al.*, 2013).

Forage legumes have a strong potential to overcome the low protein content in tropical grasses, in which the CP content is, on average, <150 g kg^{-1} DM and less absorbable in the digestive tracts of ruminants (Euclides and Medeiros, 2003). Ecotypes of *Arachis* spp. presented a CP concentration of 224 g kg^{-1} DM in the leaves and 138 g kg^{-1} DM in stems, which was somewhat lower than the 191–206 g kg^{-1} DM obtained in three *A. pintoi* ecotypes evaluated by Villarreal *et al.* (2005) for an entire plant. According to Phelan *et al.* (2015), the CP content of temperate forage legumes was found to be approximately 170 g kg^{-1} DM. Therefore, the selected *Arachis* ecotypes can, as a protein source for growth livestock, contribute to the ruminant production system in the Cerrado, as has been demonstrated in the northern regions of Brazil (Andrade *et al.*, 2015). The absence of genetic variability in this trait in leaves demands a search for another germplasm source, aimed at its improvement.

Feed quality evaluations were performed after 79 d of growth (the second clipping) and after 143 d of growth (fifth clipping). Among these first and second evaluations, the mean of the traits associated with low nutritional quality in leaves declined, in contrast to the evidence from stems, mostly in lignin (Table 3). Nevertheless, the *Arachis* data reinforce the recognized advantage shown by several species of forage legumes, and there is a lower decline in quality with increasing regrowth duration (Peyraud *et al.*, 2009), in contrast to grasses (Phelan *et al.*, 2015).

The improvement of forage legumes has a final economic objective of maximizing the weight gain per animal and per area. In this regard, the following three characteristics are fundamental as criteria for forage legume selection to achieve their economically viable use in pastures (Resende *et al.*, 2008; Annicchiarico *et al.*, 2014; Phelan *et al.*, 2015): (i) greater herbage mass accumulation; (ii) high persistence associated with survival, earliness, rapid establishment, competition capacity and complementarity when grown together with grass and (iii) better nutritional quality associated with protein content, greater digestibility and reduced levels of components of low nutritional quality. All of these traits are strongly related to each other and to the objectives of overall improvement.

The genetic improvement of forage legumes is a challenge when considering the number of target species, different crossing systems, available germplasm, target environments for a new potential cultivar and the field-evaluation requirements for the species of interest (Phelan *et al.*, 2015), as well as the ability to exert meaningful selection pressure, and the type of cultivar to be developed (Resende *et al.*, 2013). The development of tropical forage legume cultivars introduces new challenges because of their domestication is low or absent and there is a lack of information about inheritance and correlations among important traits. This study therefore contributes new information on *Arachis* spp. genetic parameters.

The breeding of *Arachis* species continues to require information in research areas such as crop establishment (Walker and King, 2010; Lüscher *et al.*, 2014), grass competition ability (Wachendorf *et al.*, 2001; Annicchiarico and Proietti, 2010; Castillo *et al.*, 2013) and survival (tolerance) under animal grazing (Bouton and Smith, 1998; Solomon *et al.*, 2012). All of the characteristics mentioned here are typically evaluated only at the end of an artificial selection procedure and after constraining the available genetic variability, if they are evaluated at all.

Forage legume breeders are consistently concerned with selection efficiency, primarily because most legume cultivars are used in combined grass–legume pasture swards,, which introduce interspecies competition and other factors that affect legume persistence, as described by Lüscher *et al.* (2014). Over the course of improvement, as in our work, forage legumes are generally evaluated and selected without grass competition and under a clipping regime that differs from the pasture management used for combinations, in which the plants will likely suffer more frequent defoliation. To address this question, breeders may start selection of genotypes under competition (Annicchiarico, 2003), but such procedures are restricted to selection methods that do not consider or fall within progeny investigations of genetic variability, which may result in considerable experimental sizes and labour requirements when more than one species combination is tested. Annicchiarico and Proietti (2010) evaluated forage legume cultivars that were combined with forage grass cultivars, and this approach is recommended. In this case, however, the forage legume cultivar was validated on the basis of its competitive ability, which was not effectively improved.

The ecotypes that we selected must be validated in the long-term in grass–*Arachis* pastures under competition and with adjusted grazing. Only then we will know if the evaluation and selection procedure that we adopted can contribute effectively to the selection of superior ecotypes and their adequate performance when used under grazing in the Cerrado region.

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