Genetic resources of *Centrosema* spp.: genetic changes associated to the handling of an active collection

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*Summary*

The potentials of the species of *Centrosema* as pasture and fodder crops in Tropical and Subtropical areas have promoted their germplasm collection and evaluation in Brazil and other countries of Central and South America. The species *C. acutifolium*, *C. pubescens* and *C. brasilianum* are of particular interest. Samples of the same accessions from wild materials collected in Brazil have been agronomically evaluated in Brazil and Colombia, and handled as self-pollinating species. Pairs of samples (one from Brazil and the other from Colombia) of four accessions have been genetically analyzed using isozyme markers at 16 loci. A noticeable genetic differentiation has occurred between members of each pair in few generations. Experimental evidences indicate that frequency of outcrossing are relatively high in these species. It is proposed that outcrossing between non-isolated neighboring accessions and genetic drift in small size plantings are the causes of the genetic differentiation between Brazilian and Colombian samples. Guides to evaluate and multiply *Centrosema* germplasm are suggested.

*Introduction*

The family Leguminosae includes a large number of species, many of which have a relevant economic and/or scientific interest. The genus *Centrosema* (DC.) Benth. belongs to the tribe Phaseolae which, with approximately 84 genera and 1500 species, represents the largest and economically most important tribe of the subfamily Papilionoideae.

The species of *Centrosema* are native to Central and South America. Brazil is one of the most important centers of genetic diversity. Among the nearly 35 described species of the genus, 26 are indigenous to Brazil (Barbosa-Fevereiro, 1977; Schultzze-Kraft & Clements, 1990). These species show a large morphologic diversity and exhibit a great potential for being utilized as fodder plants (For revisions on taxonomy and utilization see Clements et al., 1983; Schultzze-Kraft & Clements, 1990; Thomas & Schultzze-Kraft, 1992).

Beef cattle in Brazil are mainly raised in the Central West Region, in extensive systems, where animal feeding is based on grazing. The weather conditions in this region show a very drastic dry season, and in general soils are acid and poor in phosphorus. Grasses, the most important fodders in that region, are characterized by a low protein content and show a large decrease in production during the dry season. Therefore, under these conditions, the possibility of using legumes as forage represents an important alternative. Legumes bring to the system a major source of protein, improve the physico-chemical conditions of soils and also increase the productivity and nutritive value of grasses.

The genus *Centrosema* has been agronomically evaluated for many years and its potential as forage has been studied extensively (Clements et al., 1983; Schultzze-Kraft & Clements, 1990). Three species are particularly interesting: *C. acutifolium* Benth., *C. pubescens* Benth. and *C. brasilianum* (L.) Benth.. These species are short-lived perennials and, as oth-
er species from the same taxonomic group, have been considered as self-pollinating (Hutton, 1960). Although some natural outcrossing was suspected to occur by the observation of a high phenotypic variability within accessions in field-trial evaluations (Penteado, 1986; Penteado et al., 1990) and although evidences of outcrossing in some species of this genus were reported (Battistin, 1983; Schultze-Kraft & Belalcazar, 1988), Centrosema germplasm has been and still is handled as if self-pollinated. Recently, outcrossing estimates of 31.2% and 53.3% were reported in two accessions of C. brasilianum (Maass & Tortes, 1992).

Although Centrosema has probably received more attention from plant breeders than other tropical legume forages, basic genetic knowledge on its species are particularly scarce. Aspects such as floral biology, mating system and genetic variability are not well studied and very few papers are available on these subjects. Even chromosome numbers are controversial, thus 2n numbers of 18, 20 and 22 have been reported in these and other Centrosema species (Clements et al., 1983; Battistin & Vargas, 1989; Miles et al., 1990). The number of all species and accessions used in this work was observed to be 2n = 22 (Novaes & Penteado, 1993).

The object of this work is to evaluate the genetic structure of different accessions of three species of Centrosema, selected for its agronomic importance, and to study genetic implications for germplasm conservation.

Materials and methods

The materials analyzed in this experiment consist of four Centrosema accessions, belonging to the species C. acutifolium (2n = 22), C. brasilianum (2n = 22) and C. pubescens (2n = 22). The registered numbers of accessions at the Brazilian Germplasm Bank are: C. acutifolium (BRA-013501 and BRA-004990), C. pubescens (BRA-017764) and C. brasilianum (BRA-007382), but they will be designated throughout this work, respectively as GC 350, GC 351, GC 495 and GC 489, which are their numbers in the active collection under agronomical evaluation.

All of these accessions were collected in Brazil at the end of the 70's and multiplied in two different sites. Some of the original seeds were grown by the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) at the Centro Nacional de Pesquisa de Gado de Corte (CNPGC) in Campo Grande, Mato Grosso do Sul (Brazil), and part by 'Centro Internacional de Agricultura Tropical' (CIAT) at the Experimental Station of Quilichao in Cali (Colombia). Two samples of each accession were studied, one grown in Brazil and the other in Colombia and designated with the letters B and C, respectively. Accessions under field evaluation were planted in small plots with between 20 and 40 adult plants.

 Seeds of each accession collected in 1989 were germinated under controlled conditions. Crude extracts from leaf tissue of two-week-old seedlings were analyzed by standard starch gel electrophoresis, in two discontinuous buffer systems, Tris-Citric Acid pH 7.0 and Tris-Boric Acid pH 8.5 (Penteado, 1994).

The isozymatic systems analyzed were glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1), glucose phosphate isomerase (GPI, EC 5.3.1.9), phosphoglucone mutase (PGM, EC 2.7.5.1), peroxidase (PRX, EC 1.11.1.7), malate dehydrogenase (MDH, EC 1.1.1.37), 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44) and isocitrate dehydrogenase (IDH, EC 1.1.1.42). The staining procedures were, with minor modifications, those described by Vallejos (1983). The genetic control of these isozymatic systems was studied by means of segregating progenies (Penteado, 1994). The estimation of mating system parameters was performed by a multilocus method (Shaw et al., 1981) from family array data of seeds sampled in larger Brazilian populations. Several polymorphism parameters were estimated from genotypic and allelic data.

Results

Allelic frequencies estimated from genotypic frequencies (not shown) of the analyzed samples are shown in Table 1. Three additional monomorphic loci (Got2, Mdh3 and Mdh4) fixed for a single allele at the three species have not been included in Table 1, but parameters of genetic variability have been estimated from the total sixteen loci. It is clear from the data in Table 1 that, in general, samples grown in Colombia have a greater genetic variability than the Brazilian samples: the former show a higher number of alleles with less different frequencies. These results can be observed in Table 2 which summarizes the values observed for some polymorphism parameters. With the exception of accessions GC 350 of C. acutifolium the average number of alleles per locus (n), number of polymorphic loci (P), mean heterozygosity (H) and polymorphic index (P*) are equal or higher in the samples grown in Colombia than in Brazil. Figure 1 shows the number of alleles.