

Short Communication

Mating system parameters in *Stylosanthes guianensis* (Aubl.) Sw. based on RAPD markers

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This study investigated the mating systems in *Stylosanthes guianensis*, an important tropical forage legume. Twelve progenies were analyzed with 21 polymorphic random amplified polymorphic DNA (RAPD) loci. The mating parameters estimated using the mixed mating model showed that this population practices a mixture of selfing (61.4%) and out-crossing (38.6%) for reproduction. The results also indicated that the fraction of multilocus out-crossing of about 8.2% occurred among related individuals. Low values were obtained for correlation of paternity (0.212 ± 0.046) and correlation of selfing among families (0.102 ± 0.001). These results have important implications to establish strategies of maintenance of germplasm and genetic breeding.

Key words: Mixed mating model, out-crossing rate, tropical forage legume.

INTRODUCTION

The mating system plays a crucial role in the genetic composition of populations, as it determines the frequency of the individual genotypes in subsequent generations, with great influence on the distribution and content of the genetic variation within and among populations (Brown, 1990). Moreover, the knowledge of the mating system of species or population is essential for an adequate plant breeding program, since breeding methods applicable to self-pollinated crops are distinct from those that apply to cross-pollinated ones and those that are adequate for crops with a mixed mating system (Ferreira et al., 2000).

Studies about mating systems can be conducted to know the out-crossing rates, self-fertilization rates, in-breeding crossings, biparental crossings and other parameters. These studies can be efficiently carried out using genetic markers, especially, molecular markers,

such as isozymes (Ribeiro and Lovato, 2004), random amplified polymorphic DNA (RAPD) (Wadt and Kageyama, 2004), amplified fragment length polymorphism (AFLP) (Muluvi et al., 2004) and microsatellites (Rao et al., 2008).

In this paper, mating system parameters were estimated in the tropical forage legume *Stylosanthes guianensis*. This species is native to South and Central America, where it is widely distributed. It is adapted to a wide range of soils and climates but is one of the few herbaceous legumes which will grow well on infertile and acidic soils (Quecini et al., 2002). Commercial cultivars of this species are employed as protein source in cattle feeding, organic fertilizer and mainly as a forage crop in consortium with a wide range of grasses in tropical regions (Kolver, 2003).

Stylosanthes species were considered to be mainly self-pollinated (Bray and Hutton, 1976), but reports based on isozyme loci or morphological traits have evidenced that out-crossing can occur in some of these species (Stace, 1982; Miles, 1983, 1985). The main aim of this study was to obtain mating system parameters in *S. guianensis* under experimental conditions based on progeny array using RAPD markers.

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Abbreviations: RAPD, random amplified polymorphic DNA; AFLP, amplified fragment length polymorphism.

Table 1. Estimates of mating system parameters in a breeding population of *S. guianensis* obtained with RAPD markers using maximum likelihood method. The standard errors (values in parentheses) were calculated from 1,000 bootstrap samples.

Parameter	Estimates	Percentage (%)
Multilocus out-crossing rate (t_m)	0.386 (0.070)	38.6
Single-locus out-crossing rate (t_s)	0.304 (0.041)	30.4
Self-fertilization rate ($s = 1 - t_m$)	0.614	61.4
Biparental inbreeding ($t_m - t_s$)	0.082 (0.038)	8.2
Maternal inbreeding coefficients (F_m)	0.622 (0.058)	62.2
Correlation of paternity (r_p)	0.212 (0.046)	21.2
Correlation of selfing (r_s)	0.102 (0.001)	10.2

MATERIALS AND METHODS

Twenty accessions from the germplasm bank of Embrapa Beef Cattle were grown in the field in two randomized block design experiments. Seeds were collected from those accessions for the progeny array. About 30 pollinated seeds from each of the maternal plants were grown and 10 plants per progeny were evaluated in this study, making a total of 200 plants.

The DNAs were extracted from young leaves using the Bonato et al. (2002) method. The RAPD reactions and amplification was performed according to the Chiari et al. (2006) procedure. The 200 individual plants were analyzed with nine random decamer primers (OPAN10, OPBA01, OPBA03, OPD03, OPG02, OPG03, OPG07, OPG17 and OPJ16) which were previously selected in *S. guianensis* (Chiari et al., 2006).

Scoring of the RAPD fragments (bands) was carried out considering only two possible alleles: band presence or band absence. The mating system was analyzed by multilocus mixed program (MLDT) of Ritland (2002). From progeny array data, the program simultaneously estimated: multilocus out-crossing rate (t_m); single-locus out-crossing rate (t_s); selfing ($s = 1 - t_m$); biparental inbreeding ($t_m - t_s$); inbreeding coefficient of the maternal parents (F); correlation of paternity (r_p) and correlation of selfing among family (r_s). These estimates were calculated using the maximum likelihood method, numerical algorithm expectation-maximization (EM) and the variances of the above quantities using the bootstrap method where the progeny array (within families) is the unit of resampling (1,000 bootstraps).

RESULTS AND DISCUSSION

The nine cited primers amplified a total of 62 bands that were reproducible and easily analyzed. From these bands, 21 (33.87%) that were polymorphic were scored for data analysis.

This present paper provides the first estimate of out-crossing rate in *S. guianensis* done under experimental conditions in Brazil, where this species is native and broadly distributed, and is also their pollinator insects. The mating system parameters estimated are presented in Table 1.

Multilocus out-crossing rate estimate was significantly different from zero and higher than 5% (38.6%), pointing a mating mixed system for this population, with predo-

minance of selfing (61.4%). The detection of out-crossing in *S. guianensis* agrees with previous reports (Miles, 1985). In experiments done in Colombia, Miles (1985) estimated the rate of out-crossing to be 13.8% in *S. guianensis* when studying the color of the flower as genetic marker. Differences in out-crossing rates on the same species seem to be common in animal-pollinated species, in accordance with Vogler and Kalisz (2001) report in which 49% of these species presents out-crossing rates between 20 and 80%.

The difference between the multilocus out-crossing rate estimates and the mean of the single-locus estimate (Table 1) indicated that the fraction of multilocus out-crossing of around 8.2% had occurred between related individuals (biparental inbreeding). The correlation of paternity suggested that 8% of the out-crossing progeny are full sibs ($t_m r_p$) and 30% are half sibs ($t_m (1-r_p)$). Even though the progenies were derived from 20 parental plants, only five plants ($1/r_p$) contributed as pollen donors in this experiment.

The detection of a substantial level of out-crossing in *S. guianensis* has important practical implications in the maintenance of germplasm collections and to delineate strategies of genetic breeding. Seed multiplication procedures should be reviewed in order to avoid mixture of accessions on germplasm collection. Moreover, the presence of selfing and cross-fertilization might provide an alternative breeding program for this species as compared with traditional directional selection. In this case, it is possible to use breeding schemes involving inbred lines and hybridization or a recurrent selection, which should provide ample opportunity for genetic recombination through random out-crossing and should result in continuing genetic progress as long as a sufficiently large effective population size is maintained.

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