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Short Communication

Mode of reproduction of Brazilian species of *Adesmia* (Leguminosae)

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Abstract

Mode of reproduction was studied in 15 species of *Adesmia* DC. (Leguminosae). In six species, three treatments were used: mutual pollination, mechanical stimulation and control. Fifty-four plants of these six species were grown in a greenhouse, individually isolated in nylon screen boxes. Flowers were labelled and submitted to the different treatments. In addition, the frequency of spontaneous self-pollination in the absence of pollinators was studied in 200 plants of nine other species. These 200 plants were kept in a greenhouse, which avoided contact with any possible pollinator. *Adesmia bicolor, A. muricata, A. punctata* and *A. riograndensis* produced seed both by cross- and self-pollination. *Adesmia punctata* and *A. riograndensis* need mechanical stimulation for self-pollination. *Adesmia incana* reproduced by self-pollination; however, the possibility of cross-pollination cannot be totally ruled out. *Adesmia tristis* reproduced mainly by cross-pollination and a mechanism of self-incompatibility is suggested. Among the nine species that were not exposed to pollinators, *A. securigerifolia* produced a large amount of seed, indicating that it is a self-pollinating species. *Adesmia arillata, A. araujoi, A. ciliata, A. psoraleoides, A. rocinhensis, A. reitziana, A. sulina* and *A. vallsii* did not produce any seed under the experimental conditions, suggesting that they are cross-pollinated or that they need mechanical stimulation to reproduce.

INTRODUCTION

The South American genus *Adesmia* DC. belongs to tribe Adesmieae (Benth.) Hutch. and comprises about 200 species. Seventeen species and one variety, annual or perennial, herbaceous or shrubby, are found in Brazil. All of them are endemic to the southern region, which includes the States of Rio Grande do Sul, Santa Catarina and Paraná (Miotto and Leitão-Filho, 1993).

Many of the Brazilian species are promising as forage crops and have attracted the interest of researchers. Several species of *Adesmia* from southern Brazil present good winter growth, when there is normally lack of cattle forage. They are well adapted to the regional environment, are widespread and have a high nutritional value. Crude protein percentages range from 6.9 to 17.5 in *A. tristis*, 18.6 in *A. latifolia*, 17.9 to 21.5 in *A. ciliata*, 19.7 in *A. psoraleoides* and 23.4 in *A. punctata* (Dall´Agnol and Gomes, 1994). High crude protein values, as well as good *in vitro* organic matter digestibility (IVOMD), were also found for *A. latifolia, A. tristis* and *A. punctata*, by Scheffer-Basso (1999).

Germplasm accessions of the Brazilian species of *Adesmia* have been stored at Embrapa Recursos Genéticos e Biotecnologia/CENARGEN, Brasília, DF, since 1984, when intensive efforts to collect and conserve this germplasm were initiated. Germplasm characterization, including knowledge about the mode of reproduction, is essential for any further research. The reproductive success of a given species depends on the reproductive strategy used to ensure perpetuation.

The determination of the mode of reproduction should be one of the first steps in germplasm characterization, since the process of collecting, multiplication, and further plant breeding procedure depends on how each species reproduces (Valls, 1988). Depending on the mode of reproduction, different plant breeding methods should be used (Poehlman, 1965; Borém, 1997). Native legumes are important components of many natural pastures in several countries and regions, such as in southern Brazil; nevertheless the reproductive processes are unknown for many species (Izaguirre *et al.*, 1994). Very little is known about the mode of reproduction of *Adesmia* (Valls, 1984). *Adesmia latifolia* is considered a versatile species, allowing for self and cross fertilization (Tedesco *et al*., 1998), but there is no published information about the other Brazilian species. Visual observation has shown that the flowers are visited and pollinated by insects.

We determined the mode of reproduction of 15 Brazilian species of *Adesmia*. This is expected to be useful for plant breeding.

MATERIAL AND METHODS

Fifty-four plants of six species (*Adesmia bicolor, A. incana, A. muricata, A. punctata, A. riograndensis* and *A. tristis* (Table I)) were grown in pots in a greenhouse, indi-

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Table I - List of *Adesmia* accessions utilized for the determination of the mode of reproduction (Experiment 1).

Species	Accessions code[1]	Geographic origin[2]	Source of germplasm[3]
A. riograndensis Hert.	BRA-000761	Santana da Boa Vista/RS	CENARGEN
	BRA-001147	Bagé/RS	CENARGEN
A. muricata (Jacq.) DC	BRA-001940	Caçapava/RS	CENARGEN
	BRA-001058	Caçapava/RS	CENARGEN
A. tristis Vog.	BRA-001325	Vacaria/RS	CENARGEN
	BRA-001546	Lages/SC	EEL
	BRA-001678	Vacaria/RS	UFRGS
A. incana Vog.	BRA-000311	Santana do Livramento/RS	CENARGEN
	BRA-001015	Bagé/RS	CENARGEN
A. punctata (Poir.) DC.	BRA-001104	Vacaria/RS	CENARGEN
	BRA-001112	Vacaria/RS	CENARGEN
A. bicolor (Poir.) DC.	BRA-000175	Bagé/RS	CENARGEN
	BRA-001520	Bagé/RS	UFRGS

1.Brazilian accession code, provided by CENARGEN. 2.Brazilian States: PR = Paraná; RS = Rio Grande do Sul; SC = Santa Catarina. 3.CENARGEN = Embrapa Recursos Genéticos e Biotecnologia, Brasília/DF; EEL = EPAGRI, Estação Experimental de Lages; UFRGS = Universidade Federal do Rio Grande do Sul, Porto Alegre/RS.

Table II - List of *Adesmia* accessions utilized for the observation of the mode of reproduction (Experiment 2).

Species	Accession code[1]	Geographic origin[2]	Source of germplasm[3]
A. securigerifolia Hert.	BRA-000621	Bagé/RS	CENARGEN
	BRA-000060	Bagé/RS	CENARGEN
	BRA-000043	Bagé/RS	CENARGEN
	BRA-001180	Bagé/RS	CENARGEN
	BRA-001481	Bagé/RS	UFRGS
	BRA-001490	Bagé/RS	UFRGS
A. araujoi Burk.	BRA-001473	Passo Fundo/RS	UPF
	BRA-000817	Soledade/RS	CENARGEN
A. sulina Miotto	BRA-001261	Água Doce/SC	CENARGEN
	BRA-001210	Água Doce/SC	CENARGEM
	BRA-001279	Palmas/PR	CENARGEM
	BRA-001201	Guarapuava/PR	CENARGEM
A. vallsii Miotto	BRA-001392	Palmas/PR	CENARGEN
	BRA-001376	Palmas/PR	CENARGEN
	BRA-001368	Palmas/PR	CENARGEN
A. arillata Miotto	BRA-000825	Guarapuava/PR	CENARGEN
	BRA-000841	Guarapuava/PR	CENARGEN
	BRA-000850	Guarapuava/PR	CENARGEN
A. psoraleoides Vog.	BRA-001091	Abelardo Luz/SC	CENARGEN
	BRA-001066	Lagoa Vermelha/RS	CENARGEN
	BRA-001082	Guarapuava/PR	CENARGEN
	BRA-001074	Guarapuava/PR	CENARGEN
	BRA-001686	Bom Jardim Serra/SC	UFSM
	BRA-001554	Lages/SC	EEL
	BRA-001562	Lages/SC	EEL
	BRA-001694	Lages/SC	EEL
A. reitziana Burk.	BRA-001708	Urubici/SC	UFRGS
A. ciliata Vog.	BRA-000272	Lages/SC	CENARGEN
	BRA-000914	Balsa Nova/PR	CENARGEN
	BRA-000922	Balsa Nova/PR	CENARGEN
	BRA-000949	Palmeira/PR	CENARGEN
	BRA-000965	Guarapuava/PR	CENARGEN
	BRA-000973	Guarapuava/PR	CENARGEN
	BRA-000990	Palmas/PR	CENARGEN
	BRA-001007	Palmas/PR	CENARGEN
A. rocinhensis Burk.	BRA-001163	Palmas/PR	CENARGEN
	BRA-001171	Palmas/PR	CENARGEN

UFSM = Universidade Federal de Santa Maria, Santa Maria/RS; UPF = Universidade de Passo Fundo, Passo Fundo/RS. For other abbreviations see legend to Table I.

vidually isolated in nylon screen boxes of 0.5 x 0.5 or 0.5 x 1.0 m, depending on plant height. The effects of three treatments were tested: T1: pairs of plants, which were mutually pollinated by reciprocally transferring pollen from one to the other. Flowers were labelled and manually pollinated with a small cuneiform piece of cardboard, suitable to pollinate without damaging the flowers; T2: flowers were labelled and mechanically stimulated by manually pressing the keel with a piece of cardboard as in T1, one per flower, thus liberating the anthers and releasing pollen. This artificial tripping simulates the action of the pollinating insect; T3: flowers were labelled and left undisturbed. The number of flowers in each treatment varied among species and among treatments, depending on the availability of exposed flowers. T1 and T2 were carried out from 10:00 a.m. to 1:00 p.m. for several days. The number of fruits produced was counted for each plant and treatment, and compared by a χ^2 test.

For nine other species, *Adesmia araujoi*, *A. arillata*, *A. ciliata*, *A. psoraleoides*, *A. securigerifolia*, *A. sulina*, *A. reitziana*, *A. rocinhensis* and *A. vallsii*, plants of 37 accessions (Table II) were kept in a greenhouse, totally isolated from pollinators. After flowering, fruiting and seed formation were observed. *Adesmia securigerifolia*, *A. riograndensis* and *A. muricata* are annual, while the other species are perennial (Miotto and Leitão-Filho, 1993).

RESULTS AND DISCUSSION

Manual stimulation was the most efficient treatment for *A. riograndensis* (Table III), suggesting that this species is autogamous, but needs mechanical stimulation. Percentages of cross-pollination are only estimates, since there was no emasculation, but just mutual pollen transfer, so that some of the fruits may have been formed by self-pollination. As *A. riograndensis* is annual, a higher percentage of autogamy would be expected. *A. bicolor* needed mechanical stimulation to set fruit, suggesting that it is a preferentially allogamous species (Table III), even considering that some of these fruits could have been formed by self-pollination. Similar results were found for *A. punctata* and *A. tristis*.

Versatility in mode of reproduction has been described for other native and exotic legumes, as for example, *Trifolium riograndense* (Becker *et al.*, 1987), *Desmodium intortum* and *D. uncinatum* (Hutton, 1977), and *Adesmia latifolia* (Tedesco *et al.*, 1998).

A. muricata is probably mainly cross-pollinating (Table III). In *A. incana*, no fruits were formed by T1 and T3 but 6.77% of fruits were formed by T2 (mechanical stimulation). The number of flowers treated was small (Table III), but the hypothesis that this species is an inbreeder that needs mechanical stimulation cannot be ruled out.

Apparently *A. tristis*, *A. bicolor*, *A. incana* and *A. punctata* need mechanical stimulation for self-pollination, which in nature is done by pollinating insects. There may be a self-incompatibility mechanism in *A. tristis*, as the percentage of fruits formed was small (Table III).

Self-incompatibility may hinder genetic breeding, avoiding selfing, but at the same time assures maintenance of high heterozygosis. It is genetically controlled (Nettancourt, 1997) and common in several economically important legumes of the genera *Trifolium*, *Lotus*, *Melilotus* and

Table III - Percentage of fruits formed under treatments T1, T2 and T3 to determine the mode of reproduction of Brazilian species of *Adesmia*.

Species	Treatment	Number of flowers labelled	Number of fruits formed	Percentage of fruits formed[1]
A. riograndensis Miotto	T1	120	15	23 b
	T2	33	26	45 a
	T3	344	01	0.3 c
A. muricata (Jacq.) DC.	T1	99	68	69 a
	T2	24	34	8 b
	T3	257	02	13 b
A. tristis Vog.	T1	177	12	7 a
	T2	57	01	2 b
	T3	165	00	0 b
A. bicolor (Poir.) DC.	T1	143	84	59 a
	T2	76	17	22 b
	T3	250	00	0 c
A. incana Vog.	T1	28	00	0 a
	T2	59	04	7 a
	T3	350	00	0 a
A. punctata (Poir.) DC.	T1	71	29	41 a
	T2	91	14	15 b
	T3	252	00	0 c

Values followed by the same letters do not differ by the χ^2 test ($P > 0.05$). T1- Mutual pollination; T2- mechanical stimulation; T3- control.

Table IV - Number of fruits formed in plants of *Adesmia securigerifolia* by non-induced self-pollination.

Germplasm accessions	Number of plants	Mean number of flowers per plant	Percentage of fruits formed
BRA-001180	6	463	90.5
BRA-001481	5	403	92.1
BRA-000043	8	171	85.4
BRA-000621	8	392	93.4
BRA-001490	12	481	94.6

Phaseolus (Brewbaker, 1982). Several perennial, winter-producing forage legumes are self-incompatible (Paim, 1980).

Observations with the other nine species suggest that *A. securigerifolia* is an inbreeder, since it produced a large amount of seed by self-pollination (Table IV). The other species did not produce any seed at all in the absence of pollinators, suggesting that they are obligate outbreeders or need mechanical stimulation for selfing.

Data on mode of reproduction should be taken into account when sampling new populations, to assure a representative portion of the existing genetic variability, as well as when plant breeding experiments are designed. Studies with molecular markers may help to elucidate the mode of reproduction of these *Adesmia* species; they are being planned by our research group.

RESUMO

Foram estudadas 15 espécies do gênero *Adesmia* DC. (Leguminosae), quanto ao modo de reprodução. Em seis espécies do gênero *Adesmia*, o modo de reprodução foi determinado através de três tratamentos: polinização mútua, estímulo mecânico e controle. As 54 plantas submetidas aos tratamentos foram cultivadas em casa de vegetação e mantidas isoladas individualmente, através de armações de tela de náilon. As flores foram marcadas e submetidas aos distintos tratamentos. Adicionalmente, foram observadas 200 plantas de outras 9 espécies do mesmo gênero quanto à ocorrência de autofecundação na ausência de polinizadores. As 200 plantas apenas isoladas e observadas foram cultivadas em vasos e mantidas em casa de vegetação telada, sem acesso de polinizadores. Os resultados mostraram que, das espécies investigadas pelos três tratamentos, *A. bicolor*, *A. muricata*, *A. punctata* e *A. riograndensis* são versáteis, pois permitiram a reprodução por fecundação cruzada e autofecundação. As duas últimas, para se autofecundarem, necessitaram de estímulo mecânico. *Adesmia incana* se reproduziu por autofecundação, mas não se descarta a possibilidade de ocorrer fecundação cruzada. *Adesmia tristis* se reproduziu quase que totalmente por fecundação cruzada e é possível a ocorrência de mecanismos de autoincompatibilidade. Das nove espécies apenas observadas, *Adesmia securigerifolia* se reproduziu por autofecundação, formando elevado número de sementes por planta. *A. arillata*, *A. araujoi*, *A. ciliata*, *A. psoraleoides*, *A. rocinhensis*, *A. reitziana*, *A. sulina* e *A. vallsii* não produziram sementes por autofecundação espontânea. Estas espécies são de fecundação cruzada ou necessitam do estímulo do polinizador.

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