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INCREASE OF GENETIC VARIABILITY OF FORAGE *Arachis* BY INTRA AND INTERSPECIFIC HYBRIDIZATION

J. F. M. VALLS[1], C. M. CASTRO[2], M. A. P. OLIVEIRA[2] and C. C. TEIXEIRA[2]

[1]EMBRAPA Recursos Genéticos e Biotecnologia, Parque Estação Biológica- PqEB- Final Av. W5 Norte, Brasília – DF, Brasil, 70770-900

[2]Pós-graduação em Genética/ UNESP/ Rubião Jr., Botucatu – SP, Brasil,18618-000

Abstract

This study was conducted to examine the potencial to increase the genetic variability of forage *Arachis* species, involving crosses between accessions of same species and crosses between different species, mostly of distinct taxonomic sections. Hybrids were produced between creeping, stoloniferous accessions belonging to section *Caulorrhizae*, *Arachis pintoi* x *A. pintoi* and *A. pintoi* x *A. repens*. Additional crosses involved section *Caulorrhizae versus* representatives of *Erectoides* (*A. hermannii* and *A. paraguariensis*) and *Procumbentes* (*A. appressipila*, *A. kretschmeri*, *A. lignosa* and *A. vallsii*). In intersectional crosses, the stoloniferous accessions were used as male parents. All hybrids were perennial and inherited the stoloniferous habit, being suitable for field propagation. Most intersectional hybrids failed to produce flowers, and in those that flowered, pollen stainability was low, from zero to 7 %, exceptionally 68 % in one plant. *Arachis pintoi* x *A. repens* hybrids were sterile, although showing pollen counts around 90 %. Yellow flowers were dominant over orange, cream and white, and appeared in all F_1 crosses between parents with distinct flower colors. F_2 plants were only obtained from intraspecific F_{1s}. They segregate for flower color, always including the original parental colors and yellow. This work shows that new forage materials, with variable plant types, can be produced to enrich the forage *Arachis* germplasm, either by intraspecific hybridization of *Arachis pintoi*, or by interspecific hybridization, using stoloniferous male parents. Intraspecific hybrids produce segregating lines for breeding programs, while interspecific and intersectional F_{1s} can be clonally propagated, incorporating genetic features of two additional sections.

Keywords: Forage peanut, hybrids

Introduction

The forage potential of *Arachis* species of section *Caulorrhizae* is recognized. Ten commercial cultivars of *A. pintoi* have been released in the last 12 years (Valls, 1999). Until 1981, research on stoloniferous *Arachis* species had been concentrated in a single accession of *A. pintoi*, collected in nature in 1954 by Geraldo Pinto, and in one accession of *A. repens*, collected in 1941 by Jorge Otero (Valls, 1992). Natural populations of these species occur exclusively in Brazil, in the valleys of the rivers Jequitinhonha, São Francisco and Paranã (Krapovickas and Gregory, 1994). Since the discovery of the third stoloniferous population in nature, in 1981, over 150 accessions have been obtained (Valls, 1999). Besides the incorporation of novel germplasm from nature, and eventual selection of off-types in genebanks, the possibility of increasing the available variability by crosses was anticipated. The objective of this study was to determine the potencial of intra and interspecific hybridization, as well as intersectional hybridization, to produce useful variation and new plant types with the stoloniferous habit.

Material and Methods

Intraspecific crosses involved five accessions of *A. pintoi*. Other intrasectional crosses involved two accessions of *A. pintoi* and two of *A. repens* (Table 1). Intersectional crosses used the *Caulorrhizae* accessions as male parents. This involved eight accessions of *A. pintoi* and one of *A. repens*, which were used to pollinate two accessions of section *Erectoides* (*A. hermannii* and *A. paraguariensis*), and five of the section *Procumbentes* (*A. kretschmeri*, *A. lignosa*, *A. vallsii* and two of *A. appressipila*) (Table 1). The crosses were done according to Nigam et al., 1990. F_1 hybrids were grown in a screenhouse, and were analysed for the presence or absence of stolon, life cicle, flower color and pollen viability. Hybrids that produced seed were planted in pots and analysed for segregation of flower color in the F_2 generation. Pollen viability was estimated by removing the pollen from flowers collected between 8:00 and 10:00 am, and staining the grains on a slide with aceto carmine: glicerin (1:1). Counts were made after 30 minutes in samples of 100 pollen grains, with five replications.

Results and Discussion

Intrasectional crosses - Twenty hybrids were obtained out of 1420 pollinations representing seven parental combinations. Intraspecific F_{1s} produced progenies, while interspecific hybrids were sterile. The stainability of pollen grains was high in all crosses, ranging from 71. 2 % to 99 %, so the sterility in interspecific hybrids is not because of unviable pollen. Gregory and Gregory (1979) also found a high pollen count in crosses between *A. pintoi* x *A. repens*. Intersectional crosses - Twenty four hybrids were obtained out of 2968 pollinations. The success of hybridization ranged from zero to 14.71 %. Four combinations could not be accomplished because of different flowering time between progenitors. Crosses between *A. hermannii* (*Erectoides*) and all accessions of *Caulorrhizae* failed, but those involving *A. paraguariensis* (V7677) and two accessions of *A. pintoi*, V6784 and V13312, were successful, with pollen grain stainability ranging from zero to 7 % in the first hybrid, and null in the second. V7677 also crossed with *A. repens* (Nc1579), with no stainable pollen. From section *Procumbentes,* accessions of *A. kretschmeri* and *A. lignosa* did not produced hybrids with any *Caulorrhizae.* The same occurred with one of the accesions of *A. appressipila*, V9077, but the other accession of this species, GKP10002, produced hybrids when crossed to five of the eight accessions of *A. pintoi*, and with the accession of *A. repens*. Of six hybrids produced by GKP10002, only those with the male parents V6784 and V13312 flowered. The hybrid GKP10002 x V6784 showed pollen stainability ranging from two to 28 %, while that with V13312 showed a high stainability, ranging from 56 to 68 %. *Arachis vallsii*, V7635, produced several hybrids when crossed with three of the eight accessions of *A. pintoi*, W647, V6791-wf and W34, but did not cross with the other five and *A. repens*. Five out of six F_{1s} hybrids V7635 x W647 flowered, and their pollen stainability ranged from zero to 7 %. The only accession of *Caulorrhizae* that did not produce intersectional hybrids, when used as a male parent, was V13167, although it is similar to the other accessions of *A. pintoi* in morphological and molecular grounds (Monçato, 1995; Bertozo, 1997). The results of color flower inheritance are in Table 2. In all intra and interspecific crosses the yellow flower was dominant over orange, cream and white. It also appeared in F_1 in any crosses involving parents with distinct flower color. F_2 plants were only obtained from intraspecific F_{1s}. They showed segregation for flower color, always including the original parental colors and yellow. The number of plants in F_2 progenies is still too small to define the model of inheritance of flower color, but preliminary results suggest the possibility of control by two independent genes. The flower color showed to be an excellent morphological marker to confirm hibridization, and the understanding of the inheritance of this trait can enhance its use in commercial cultivars. All hybrids were perennial, even those obtained from crosses between *A. vallsii* (annual) x *A. pintoi* (perennial), and inherited the stoloniferous habit, being suitable for field propagation. The results of this work show that new forage material, with variable plant types, can be produced to enrich the already expanded forage *Arachis* germplasm, either by intraspecific hybridization of *Arachis pintoi* accessions, or by interspecific hybridization, using stoloniferous male parents. Intraspecific hybrids give rise to segregating lines for breeding programs, while interspecific and intersectional F_{1s} can be clonally propagated, incorporating genetic features of species from two additional taxonomic sections.

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Table 1 - *Arachis* accessions involved in experimental crosses, collector numbers and origin

Section	Species	BRA-	Accession	Lat.	Long.	Alt.
Intrasectional crosses						
Caulorrhizae	*A. pintoi*	013251	GK 12787	15 52 S	39 08 W	50
	A. pintoi	030490	V 13167	16 21 S	46 54 W	600
	A. pintoi	034100	V 13338	15 26 S	47 21 W	600
	A. pintoi	031984	V 13468	13 18 S	46 42 W	600
	A. pintoi	031097	V 6791-wf	15 26 S	47 21 W	500
	A. repens	029211	Nc 1578	20 06 S	44 52 W	600
	A. repens	029220	Nc 1579	15 10 S	44 22 W	420
Intersectional crosses						
Erectoides	*A. hermannii*	017167	V 7560	18 32 S	54 45 W	250
	A. paraguariensis	017621	V 7677	22 08 S	56 34 W	220
Procumbentes	*A. appressipila*	013099	GKP 10002	19 01 S	57 39 W	170
	A. appressipila	022764	V 9077	19 25 S	57 41 W	120
	A. kretschmeri	020613	KrRy s/n	20 07 S	56 43 W	-
	A. lignosa	032808	V 13570	21 32 S	57 49 W	120
	A. vallsii	017493	V 7635	20 07 S	56 42 W	150
Caulorrhizae	*A. pintoi*	013251	GK 12787	15 52 S	39 08 W	50
	A. pintoi	030490	V 13167	16 21 S	46 54 W	600
	A. pintoi	015083	V 6784	13 23 S	44 05 W	450
	A. pintoi	015253	W 34	17 03 S	42 21 W	360
	A. pintoi	031097	V 6791-wf	15 26 S	47 21 W	500
	A. pintoi	030996	V 13312	14 28 S	46 29 W	500
	A. pintoi	032450	W 225	16 09 S	46 10 W	560
	A. pintoi	034142	W 647	14 25 S	46 23 W	750
	A. repens	029220	Nc 1579	15 10 S	44 22 W	420

Colectors: GKP- W. C. Gregory, A. Krapovickas & J. Pietrarelli; KrRy- A. Kretschmer & P. Rayman; Nc- N. M. S. Costa; V- J. F. M. Valls; W- W. Werneck;

Table 2 - Intra and intersectional crosses of species of *Arachis*, considering flower color in the progenitors, F_1 hybrids and in the F_2 progeny

Crossing	Flower color			
	Parents	F_1 hybrid	F_2 progeny	
			N° plants	Flower color[1]
A. pintoi x *A. pintoi*				
V 13468 x GK 12787	cream x yellow	yelow	4	4 y
V 13167 x GK 12787	orange x yellow	yellow	28	20 y : 7 or
V 13338 x GK 12787	cream x yellow	yellow	119	40 y : 14 cr
V 13167 x V 6791-wf	orange x white	yellow	21	12 y : 4 wh : 4 or
V 13468 x V 13167	cream x orange	yellow	1	1 y
A. pintoi x *A. repens*				
GK 12787 x Nc 1579	yellow x yellow	yellow	zero	
V 13167 x Nc 1578	orange x orange	orange	zero	
A. appressipila x *A. pintoi*				
GKP 10002 x V 13312	orange x yellow	yellow	zero	
A. paraguariensis x *A. pintoi*				
V 7677 x V 6784	orange x yellow	yellow	zero	
A. paraguariensis x *A. repens*				
V 7677 x Nc 1579	orange x yellow	yellow	zero	
A. vallsii x *A pintoi*				
V 7635 x W 647	orange x cream	yellow	zero	

[1]Based on plants that reached flowering; y = yellow; cr = cream; or = orange; wh = white