

Note

CHARACTERIZATION OF RUST, EARLY AND LATE LEAF SPOT RESISTANCE IN WILD AND CULTIVATED PEANUT GERMPLASM

Alessandra Pereira Fávero^{1*}; Sérgio Almeida de Moraes²; Antonio Augusto Franco Garcia³;
José Francisco Montenegro Valls¹; Natal Antonio Vello³

¹Embrapa Recursos Genéticos e Biotecnologia - SAIN Parque Estação Biológica - C.P. 02372 - 70770-900 - Brasília, DF - Brasil.

²IAC - Centro de Pesquisa e Desenvolvimento de Fitossanidade, C.P. 28 - 13001-970 - Campinas, SP - Brasil.

³USP/ESALQ - Depto. De Genética, C.P. 09 - 13418-900 - Piracicaba, SP - Brasil.

*Corresponding author <favero@cenargen.embrapa.br>

ABSTRACT: Groundnut (*Arachis hypogaea*) has an AB genome and is one of the most important oil crops in the world. The main constraints of crop management in Brazil are fungal diseases. Several species of the genus *Arachis* are resistant to pests and diseases. The objective of our experiments was to identify wild species belonging to the taxonomic section *Arachis* with either A or B (or “non-A”) genomes that are resistant to early leaf spot (*Cercospora arachidicola*), late leaf spot (*Cercosporidium personatum*) and rust (*Puccinia arachidis*). For the identification of genotypes resistant to fungal diseases, bioassays with detached leaves were done in laboratory conditions, with artificial inoculation, a controlled temperature of 25°C and a photoperiod of 10 h light/14 h dark, for 20–42 days, depending on the fungi species. Most of the accessions of wild species were more resistant than accessions of *A. hypogaea* for one, two or all three fungi species studied. *Arachis monticola*, considered to be a possible tetraploid ancestor or a derivative of *A. hypogaea*, was also more susceptible to *Cercosporidium personatum* and *Puccinia arachidis*, as compared to most of the wild species. Therefore, wild germplasm accessions of both genome types are available to be used for the introgression of resistance genes against three fungal diseases of peanut.

Key words: *Puccinia arachidis*, *Cercospora arachidicola*, *Cercosporidium personatum*, groundnut, *Arachis* spp.

CARACTERIZAÇÃO DA RESISTÊNCIA À FERRUGEM, MANCHA PRETA E MANCHA CASTANHA EM GERMOPLASMA SILVESTRE E CULTIVADO DE AMENDOIM

RESUMO: O amendoim (*Arachis hypogaea*) possui genoma AB e é uma das mais importantes culturas oleaginosas em todo o mundo. Os principais problemas da cultura no Brasil são as doenças fúngicas. Várias espécies do gênero *Arachis* são resistentes a pragas e doenças. Este trabalho visou a identificar espécies silvestres pertencentes à seção *Arachis* associadas aos genomas A ou B (ou “não-A”) do amendoim que são resistentes à mancha castanha (*Cercospora arachidicola*), mancha preta (*Cercosporidium personatum*) e ferrugem (*Puccinia arachidis*). Para a identificação de genótipos resistentes a doenças fúngicas, bioensaios utilizando folhas destacadas foram realizados em condições de laboratório, com inoculação artificial, temperatura controlada de 25°C e fotoperíodo de 10h luz/14h escuro, por 20–42 dias, de acordo com a espécie fúngica. A maioria dos acessos das espécies silvestres foram mais resistentes que os acessos de *A. hypogaea* para uma, duas ou todas as espécies fúngicas estudadas. *Arachis monticola*, considerada como o possível ancestral tetraplóide ou como um derivativo de *A. hypogaea*, também mostrou-se mais suscetível a *Cercosporidium personatum* e *Puccinia arachidis*, quando comparado à maioria das espécies silvestres. Portanto, acessos de germoplasma silvestre com genoma A ou B estão disponíveis para serem utilizados na introgressão de genes de resistência a doenças fúngicas no amendoim.

Palavras-chave: *Puccinia arachidis*, *Cercospora arachidicola*, *Cercosporidium personatum*, *Arachis* spp.

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is the fourth most important oleaginous plant in the world. It is used mainly for oil and candy production, or for consumption *in natura*. World production is thought to be more than 30 million tons per year (CONAB, 2003). Brazil has about 90,000 ha planted with peanut, with a production of about 220,000 tons in 2006. The main constraints of the peanut production in Brazil, and indeed in the world, are fungal diseases, such as web blotch (*Phoma arachidicola* Marasas, Pauer & Boerema), early leaf spot (*Cercospora arachidicola* Hori), late leaf spot (*Cercosporidium personatum* (Berk & Curt.) Deighton), rust (*Puccinia arachidis* Speg.) and scab (*Sphaceloma arachidis* Bitancourt & Jenkins) (Godoy et al., 1999).

Species in the genus *Arachis* have potential for peanut improvement (Fávero et al., 2006). Several species have higher resistance levels to diseases when compared to *A. hypogaea* germplasm accessions (Pande & Rao, 2001; Stalker & Moss, 1987). The genus has nine taxonomic sections, and *A. hypogaea* is in the section *Arachis*, along with 30 wild species (Krapovickas & Gregory, 1994; Valls & Simpson, 2005).

The objective of the present study was to test diploid and tetraploid wild species within the section *Arachis*, with A and/or B or "non A" genomes, as well as several *A. hypogaea* varieties, for resistance against three fungal diseases - early and late leaf spot and rust, with the aim of future introgression of disease resistance genes into a breeding program of the cultivated peanut. Different accessions of a particular species may have different rates of resistance to fungal diseases. Although several previous publications have demonstrated the resistance of assorted wild *Arachis* germplasm against fungal diseases, old and newly collected accessions of many species were put together in the present work, and were tested with fungi isolates from São Paulo State, where 80% of the total Brazilian peanut acreage is concentrated. Isolates from Brazil may be different from those from other countries, so, this work was necessary as a basic step to implement a Peanut Pre-breeding project in Brazil.

MATERIAL AND METHODS

Accessions used in this study are shown in Table 2 and were obtained from the *Arachis* germplasm bank of Embrapa Recursos Genéticos e Biotecnologia, Brasília, Brazil. Only accessions of the section *Arachis* were selected because crossability with the cultivated peanut has not been accomplished so far with species of other taxonomic sections. The three diploid species of section *Arachis* with $2n = 18$ chromosomes *Arachis praecox*, *A. decora* and *A. palustris* (Peñaloza & Valls, 1997; Lavia, 1998) were not included. Diploid species with $2n = 20$ were assigned the A or B (non-A) genome according to the available documentation of the presence or absence of the small "A" chromosome pair in at least one accession of each in the literature (Fernández & Krapovickas, 1994; Lavia, 1998; Lavia, 1999; Peñaloza & Valls, 2005). The tetraploid wild species *Arachis monticola* Krapov. & Rigoni ($2n = 40$) is considered to share the same AB genome of *A. hypogaea*. Seeds from 102 accessions were treated with Carboxim and Thiram (0.5 g L^{-1}) fungicide and germinated at 25°C in germitest paper immersed in Ethrel solution (6 mL L^{-1}) to break dormancy. Plants were maintained under greenhouse conditions, with four replications per each accession.

Cercospora arachidicola and *Cercosporidium personatum* spores were collected from infected plants at the Experimental Station of Ribeirão Preto ($21^{\circ}10' \text{ S}$, $47^{\circ}48' \text{ W}$), from the Agronomic Institute of São Paulo State, Brazil (IAC), and *Puccinia arachidis* spores were collected from infected plants at the Experimental Station of Pindorama, ($21^{\circ}11' \text{ S}$, $48^{\circ}54' \text{ W}$), from the Agronomic Institute of São Paulo State, Brazil (IAC).

For *Cercosporidium personatum* and *Cercospora arachidicola*, fungi cultures were grown in oat-agar medium (Moraes & Salgado, 1979). In addition, some fungi from the IAC bank of fungal isolates (1436-1 and 1595-0) were used (Table 1). New isolates were given the numbers 11576-0 and 11576-1, respectively. The *P. arachidis* spores were collected and stored in jelly capsules in refrigerator (about 5°C), for about one week. It was periodically necessary to inoculate susceptible peanut leaves and re-isolate the spores.

Table 1 - Accession code at Agronomic Institute of São Paulo State, Brazil (IAC) fungi collection, fungi species names and municipality of São Paulo State where they were collected.

Code	Name	Region	Coordinates
1436-1	<i>Cercospora arachidicola</i>	Pompéia	$22^{\circ}06' \text{ S}$; $50^{\circ}10' \text{ W}$
11576-1	<i>C. arachidicola</i>	Ribeirão Preto	$21^{\circ}10' \text{ S}$; $47^{\circ}48' \text{ W}$
1595-0	<i>Cercosporidium personatum</i>	Jaú	$22^{\circ}17' \text{ S}$; $48^{\circ}33' \text{ W}$
11576-0	<i>C. personatum</i>	Ribeirão Preto	$21^{\circ}10' \text{ S}$; $47^{\circ}48' \text{ W}$

Table 2 - Species names, collector's number, the screening for resistance to *Cercospora arachidicola*, *Cercosporidium personatum* and *Puccinia arachidis* and sum of points related to resistance to all three diseases.

Species	Accession	<i>Cercospora arachidicola</i>	<i>Cercosporidium personatum</i>	<i>Puccinia arachidis</i>		Σ
		0-f (0-6)	0-d	Group 1 (0-d)	Group 2 (0-b)	
<i>A. batizocoi</i>	K 9484	sl *(0)	sl (0)	-	-	0*
<i>A. cardenasii</i>	GKP 10017	sl (0)	sl (0)	0.00228a (1)	-	1
<i>A. aff. diogoi</i>	VSPmSv 13774	0.00035a (1)	sl (0)	sl (0)	-	1
<i>A. helodes</i>	CoSzSv 6862	sl (0)	sl (0)	0.00337a (1)	-	1
<i>A. helodes</i>	Pa s/n	0.00055a (1)	sl (0)	sl (0)	-	1
<i>A. helodes</i>	VPoJSv 10470	sl (0)	sl (0)	0.000813a (1)	-	1
<i>A. helodes</i>	VK 12083	0.00142a (1)	sl (0)	sl (0)	-	1
<i>A. hoehnei</i>	VMPzW 13985	-	sl (0)	0.00276a (1)	-	1
<i>A. linearifolia</i>	VPoBi 9401	sl (0)	sl (0)	0.00631a (1)	-	1
<i>A. simpsonii</i>	VSPmSv 13710	0.00049a (1)	sl (0)	sl (0)	-	1
<i>A. stenosperma</i>	VSMGeSv 7379	0.00134a (1)	sl (0)	-	-	1*
<i>A. stenosperma</i>	VGaRoSv 12488	sl (0)	sl (0)	0.00474a (1)	-	1
<i>A. diogoi</i>	GK 10602	0.00054a (1)	sl (0)	0.00397a (1)	-	2
<i>A. duranensis</i>	VNvEv 14167	-	0.00064a (1)	0.00282a (1)	-	2
<i>A. hoehnei</i>	KG 30006	sl (0)	sl (0)	0.00974b (2)	-	2
<i>A. hoehnei</i>	VPoBi 9094	sl (0)	-	0.02177b (2)	-	2*
<i>A. kempff-mercadoi</i>	V 13250	sl (0)	0.00046a (1)	0.00491a (1)	-	2
<i>A. kuhlmannii</i>	VRGeSv 7639	sl (0)	-	0.00875b (2)	-	2*
<i>A. kuhlmannii</i>	VPoBi 9235	0.00035a (1)	-	0.00709a (1)	-	2*
<i>A. kuhlmannii</i>	VPoJSv 10506	0.00020a (1)	0.00029a (1)	sl (0)	-	2
<i>A. microsperma</i>	VRGeSv 13545	-	sl (0)	0.01195b (2)	-	2*
<i>A. microsperma</i>	VMPzW 14042	0.00011a (1)	sl (0)	0.00322a (1)	-	2
<i>A. stenosperma</i>	HLK 408	-	sl (0)	0.02340b (2)	-	2*
<i>A. stenosperma</i>	Lm 5	sl (0)	sl (0)	0.01380b (2)	-	2
<i>A. stenosperma</i>	VSSStGdW 7762	sl (0)	sl (0)	0.02588b (2)	-	2
<i>A. gregoryi</i>	VSGr 6389	0.00275b (2)	0.00289a (1)	sl (0)	-	3
<i>A. helodes</i>	VSGr 6325	0.00020a (1)	0.00009a (1)	0.00067a (1)	-	3
<i>A. kuhlmannii</i>	VSGr 6344	0.00091a (1)	0.00745a (1)	0.00040a (1)	-	3
<i>A. kuhlmannii</i>	VSGr 6352	0.00122a (1)	sl (0)	0.02217b (2)	-	3
<i>A. kuhlmannii</i>	VSGr 6380	0.00112a (1)	sl (0)	0.01785b (2)	-	3
<i>A. kuhlmannii</i>	VKSSv 8916a	0.00012a (1)	sl (0)	0.01876b (2)	-	3
<i>A. kuhlmannii</i>	VPoBi 9470	0.00006a (1)	sl (0)	0.01698b (2)	-	3
<i>A. kuhlmannii</i>	VPoBi 9479	0.00026a (1)	sl (0)	0.01977b (2)	-	3
<i>A. kuhlmannii</i>	VSW 9912	0.00004a (1)	sl (0)	0.02308b (2)	-	3
<i>A. kuhlmannii</i>	VSPmSv 13721	0.00014a (1)	0.0000818a (1)	0.00039a (1)	-	3
<i>A. magna</i>	KGSSc 30097	0.00377b (2)	sl (0)	0.00125a (1)	-	3
<i>A. stenosperma</i>	Jt 2	sl (0)	sl (0)	0.03807c (3)	-	3
<i>A. stenosperma</i>	Lm 3	0.00080a (1)	sl (0)	0.01186b (2)	-	3
<i>A. stenosperma</i>	SvW 3755	sl (0)	sl (0)	0.06715c (3)	-	3
<i>A. stenosperma</i>	VKSSv 9010	0.00028a (1)	sl (0)	0.01302b (2)	-	3
<i>A. stenosperma</i>	VMiSv 10229	sl (0)	sl (0)	0.04220c (3)	-	3
<i>A. stenosperma</i>	VSPmSv 13832	sl (0)	0.00372a (1)	0.02715b (2)	-	3

Continue...

Table 2 - Continuation.

<i>A. stenosperma</i>	WPz 422	0.00033a (1)	sl (0)	0.02057b (2)	-	3
<i>A. villosa</i>	VGoMrOv 12812	0.00011a (1)	0.00215a (1)	0.00072a (1)	-	3
<i>A. batizocoi</i>	K 9484 mut	0.00862c (3)	-	0.00134a (1)	-	4*
<i>A. cruziana</i>	WiSVg 1302	0.00940c (3)	sl (0)	0.00471a (1)	-	4
<i>A. duranensis</i>	K 7988	0.00091a (1)	-	0.05935c (3)	-	4*
<i>A. hoehnei</i>	VPoBi 9146	0.00071a (1)	sl (0)	0.03226c (3)	-	4
<i>A. kuhlmannii</i>	VSGr 6351	0.00016a (1)	0.00048a (1)	0.02434b (2)	-	4
<i>A. kuhlmannii</i>	VKSSv 8979	sl (0)	0.00415a (1)	0.04841c (3)	-	4
<i>A. kuhlmannii</i>	VPoBi 9230	0.00767c (3)	0.00091a (1)	sl (0)	-	4
<i>A. kuhlmannii</i>	VPoBi 9394	0.00203b (2)	0.00017a (1)	0.00314a (1)	-	4
<i>A. magna</i>	VSPmSv 13751	0.00071a (1)	0.00016a (1)	0.01040b (2)	-	4
<i>A. magna</i>	VSPmSv 13765	sl (0)	sl (0)	0.10415d (4)	-	4
<i>A. simpsonii</i>	VSPmSv 13716	0.00016a (1)	sl (0)	0.05585c (3)	-	4
<i>A. simpsonii</i>	VSPmSv 13728	0.00301b (2)	sl (0)	0.01221b (2)	-	4
<i>A. simpsonii</i>	VSPmSv 13745	0.00188b (2)	0.00012a (1)	0.00054a (1)	-	4
<i>A. stenosperma</i>	Lm 1	0.00065a (1)	sl (0)	0.04328c (3)	-	4
<i>A. stenosperma</i>	VSSv 7382	0.00009a (1)	sl (0)	0.04287c (3)	-	4
<i>A. stenosperma</i>	VSv 10309	0.00018a (1)	sl (0)	0.04461c (3)	-	4
<i>A. stenosperma</i>	VSPmSv 13670	sl (0)	sl (0)	0.16580d (4)	-	4
<i>A. stenosperma</i>	VKSSv 9017	0.01119c (3)	0.00024a (1)	0.00119a (1)	-	5
<i>A. stenosperma</i>	WPz 421	0.00133a (1)	0.00179a (1)	0.05112c (3)	-	5
<i>A. kuhlmannii</i>	VSGr 6413	0.00359b (2)	0.00397a (1)	0.01262b (2)	-	5
<i>A. kuhlmannii</i>	VPoBi 9375	0.00252b (2)	0.00168a (1)	0.00837b (2)	-	5
<i>A. magna</i>	VSPmSv 13748	0.00275b (2)	sl (0)	0.10855c (3)	-	5
<i>A. schinirii</i>	VSW 9923	0.00686c (3)	0.00067a (1)	0.00034a (1)	-	5
<i>A. stenosperma</i>	SvPzSz 3042	0.00005a (1)	sl (0)	0.11090d (4)	-	5
<i>A. stenosperma</i>	VGaSv 12646	0.00032a (1)	sl (0)	0.16580d (4)	-	5
<i>A. stenosperma</i>	VSPmWiSv 13262	0.00048a (1)	0.00103a (1)	0.06378c (3)	-	5
<i>A. stenosperma</i>	VSPmSv 13672	sl (0)	sl (0)	-	0.03711a (5)	5
<i>A. stenosperma</i>	VSPmSv 13693	0.00085a (1)	sl (0)	0.19504d (4)	-	5
<i>A. stenosperma</i>	VSPmW 13828	sl (0)	sl (0)	-	0.03166a (5)	5
<i>A. stenosperma</i>	VSPmW 13844	0.00134b (2)	sl (0)	0.05125c (3)	-	5
<i>A. valida</i>	VPoBi 9153	-	sl (0)	-	0.01718a (5)	5*
<i>A. valida</i>	VPzRcSgSv 13514	0.00532b (2)	sl (0)	0.06793c (3)	-	5
<i>A. hypogaea</i>	Mf 1538	0.00073a (1)	-	-	0.01112a (5)	6*
<i>A. kuhlmannii</i>	VPoBi 9214	0.01858d (4)	0.00041a (1)	0.00221a (1)	-	6
<i>A. magna</i>	VSPmSv 13761	sl (0)	0.00657a (1)	-	0.03247a (5)	6
<i>A. stenosperma</i>	VGaRoSv 12575	0.00022a (1)	sl (0)	-	0.04907a (5)	6
<i>A. stenosperma</i>	VSSv 13258	0.00034a (1)	0.01924b (2)	0.05798c (3)	-	6
<i>A. stenosperma</i>	VSPmW 13824	0.00007a (1)	-	-	0.03898a (5)	6*
<i>A. williamsii</i>	WiDc 1118	0.00112a (1)	sl (0)	-	0.07156a (5)	6
<i>A. ipaënsis</i>	KGBPScS 30076	0.00287b (2)	-	-	0.06128a (5)	7*
<i>A. kuhlmannii</i>	VPoBi 9243	0.14491d (4)	0.00004a (1)	0.01002b (2)	-	7
<i>A. stenosperma</i>	SvSz 2411	0.00035a (1)	sl (0)	-	0.20452b (6)	7
<i>A. stenosperma</i>	SvW 3712	0.00030a (1)	0.00029a (1)	-	0.06331a (5)	7

Continue...

Table 2 - Continuation.

<i>A. stenosperma</i>	VSPmW 13796	0.00338b (2)	sl (0)	-	0.05784a (5)	7
<i>A. valida</i>	VPoBi 9157	0.00580b (2)	-	-	0.07219a (5)	7*
<i>A. valida</i>	VPzRcSgSv 13516	0.00042a (1)	-	-	0.20794b (6)	7*
<i>A. hypogaea</i>	US 224	0.00251b (2)	0.00073a (1)	-	0.02885a (5)	8
<i>A. monticola</i>	VOa 14165	0.00776c (3)	-	-	0.03144a (5)	8*
<i>A. hypogaea</i>	VGaRoSv 12549	0.00802c (3)	0.01083b (2)	-	0.04417a (5)	10
<i>A. hypogaea</i>	cv. Tatu	0.01446d (4)	0.00946b (2)	-	0.03613a (5)	11
<i>A. hypogaea</i>	Mf 1560	0.01706d (4)	0.02179b (2)	-	0.00968a (5)	11
<i>A. hypogaea</i>	VGaRoSv 12548	0.01423d (4)	0.01859b (2)	-	0.02358a (5)	11
<i>A. hypogaea</i>	cv. Tatuí	0.02693e (5)	0.01587b (2)	-	0.03717a (5)	12
<i>A. hypogaea</i>	cv. Caiapó	0.02663e (5)	0.03075c (3)	-	0.03125a (5)	13
<i>A. hypogaea</i>	Mf 1678	0.05183f (6)	0.01747b (2)	-	0.03713a (5)	13
<i>A. hypogaea</i>	Runner 886	0.02812e (5)	0.03901c (3)	-	0.03840a (5)	13
<i>A. hypogaea</i>	F 1334	0.02391e (5)	0.05477d (4)	-	0.07898a (5)	14
<i>A. hypogaea</i>	cv. BR1	0.05034f (6)	0.05972d (4)	-	0.07430a (5)	15

sl means no lesion. Number inside parenthesis means the punctuation of the genotypes to do the ranking. They are related to letters obtained in the average test (Scott & Knott test). The signal * means that there is data missing so with this data the ranking may change.

Five isolates were prepared: two of *Cercospora arachidicola*, two of *Cercosporidium personatum* and one of *P. arachidis*. For *Cercospora arachidicola* and *Cercosporidium personatum* isolates were replicated by the use of oat-agar medium into Erlenmeyers that were shaken for about 20 minutes. The cultures were replicated at each 14 days, during approximately three months.

A detached leaf technique was used for the establishment of bioassays (Moraes & Salgado, 1982) mostly using leaves of the main stem of the plants. Cotton and germitest paper layers and a slide were used to keep the leaves well conditioned in Petri dishes. Destilated water was used to maintain leaves alive and turgid for several weeks. For the inoculation of *Cercospora arachidicola* and *Cercosporidium personatum*, the first expanded leaves were placed in Petri dishes, with the adaxial side upward. For *Puccinia arachidis*, the abaxial side was set upward. Inoculation was done with a mixture of the isolates of each leaf spot by spraying Tween 20 at 0.5% in a concentration of 50.000 spores mL⁻¹. Plates were maintained at 23–25°C, photoperiod of 10 h light and 14 h darkness, in four random blocks. During the first 48 h, the Petri dishes were sealed in a plastic bag.

Puccinia arachidis bioassays were analysed at 20 and 27 days, *Cercospora arachidicola* at 27 days and *Cercosporidium personatum* at 42 days. For *P. arachidis* evaluation, the number of lesions per leaflet area (mm²) was recorded and the lesions scored for presence or absence of spores. For *Cercospora arachidicola* and *Cercosporidium personatum*, the ratio of lesion area to leaflet area was evaluated (Foster et al., 1981).

The SAS program was used for the analysis of mathematic model according to the random blocks experiments. Different transformations were used for each group of data, according to ANOVA conditions. For *Cercospora arachidicola*, the transformation was arcsin (x + 0.5). For *Cercosporidium personatum* and *P. arachidis* a $\sqrt{\log(x+1)}$ transformation was used. The ANOVA presuppositions were verified: independence, homogeneity, and normal distribution of residues. Only data from accessions with lesions were used in the analyses. The Scott & Knott method (Scott & Knott, 1974) was used at 5% probability of Type I Error for the multiple comparisons among averages of different accessions. A sum of points, adapted to an index of selection (rank sum) of Mulamba & Mock (1978), was done to determine the most resistant and most susceptible genotypes. The t test was used to observe significant differences between the three groups, the species that have the A genome, species with non-A genome, and species that possess the AB genome. For *Cercospora arachidicola* and *Cercosporidium personatum*, this test was done using the average data of the species. For *P. arachidis*, the t test was used in the punctuation data to have the same screening for accessions that had lesions without pustule and lesions with pustule.

RESULTS AND DISCUSSION

Results for all diseases studied are summarized in Table 2.

Cercospora arachidicola

From 97 accessions investigated, 22 were shown to be highly resistant (Table 2 - third column)

without fungal lesions. From these accessions, seven are perennial and with A genome (*A. kuhlmannii* Krapov. & W.C. Greg. (2 accessions), *A. cardenasii* Krapov. & W.C. Greg. (1), *A. helodes* Mart. ex Krapov. & Rigoni (2), *A. kempff-mercadoi* Krapov., W.C. Greg. & C.E. Simpson (1), *A. linearifolia* Valls, Krapov. & Simpson (1)), ten are annual and with A genome (*A. stenosperma* Krapov. & W.C. Greg.) and five are annual and with “non-A” genome (*A. magna* Krapov., W.C. Greg. & C.E. Simpson (2), *A. hoehnei* Krapov. & W.C. Greg. (2) and *A. batizocoi* Krapov. & W.C. Greg. (1)). Therefore, there are highly resistant wild species associated to both genomes of *A. hypogaea*.

From 75 accessions considered in the statistical analysis, 43 were in the “a” group, with a smaller number of lesions. In this group, there were A genome species (*A. stenosperma* (17 accessions), *A. kuhlmannii* (11), *A. helodes* (3), *A. diogoi* Hoehne (1), *A. aff. diogoi* (1), *A. simpsonii* Krapov. & W.C. Greg. (2), *A. duranensis* Krapov. & W.C. Greg. (1), *A. microsperma* Krapov., W.C. Greg. & Valls (1), *A. villosa* Benth. (1)), and “non-A” genome species (*A. magna* (1), *A. hoehnei* (1), *A. valida* Krapov. & W.C. Greg. (1) and *A. williamsii* Krapov. & W.C. Greg. (1) and one accession of *A. hypogaea* var. *hirsuta* Köhler (Mf 1538). The “b” group included 14 accessions distributed among the A genome species *A. kuhlmannii* (3), *A. simpsonii* (2), *A. stenosperma* (2), and the “non-A” genome species *A. magna* (2), *A. valida* (2), *A. gregoryi* C.E. Simpson, Krapov. & Valls (1) and *A. ipaënsis* Krapov. & W.C. Greg. (1), and one accession of *A. hypogaea* var. *hypogaea* (US 224). In the “c” group, seven accessions were observed, three with A genome (*A. kuhlmannii* (1), *A. stenosperma* (1)) and *A. schininii* Krapov., Valls & C.E. Simpson (1), two with “non-A” genome, (*A. batizocoi* and *A. cruziana* Krapov., W.C. Greg. & C.E. Simpson) and two were allotetraploids (*A. monticola* (1) and *A. hypogaea* (1)). The “d” group presented accessions of *A. kuhlmannii* (2) and *A. hypogaea* (3). The “e” and “f” groups showed accessions with more lesions than the other groups, so these were the most susceptible. In these two final groups, only accessions of *A. hypogaea* (6) were observed. These results were already expected, confirming the greater resistance of many wild species when compared to *A. hypogaea* and their potential use in the improvement of the cultivated peanut, like A and “non-A” peanut genome substitutes. Resistance to *C. arachidicola* was also found in accessions of *A. stenosperma*, *A. diogoi*, *A. correntina* (Burkart) Krapov. & W.C. Greg. and *A. duranensis* by Foster et al. (1981).

The resistances are very heterogeneous among accessions of the same species, as in *A. kuhlmannii* and *A. stenosperma*. So, a precise analysis of each accession is necessary, as the data do not support the species as always resistant or susceptible. A high coefficient of variation of 49.68%, was obtained. However, it is common to find values as great as this in disease evaluation data.

Cercosporidium personatum

From 91 accessions submitted to the *C. personatum* resistance test, 54 appeared highly resistant. No lesion was observed (Table 2 -fourth column) in these 54 accessions of different species of *Arachis*, from A genome species (*A. stenosperma* (24 accessions), *A. kuhlmannii* (6), *A. helodes* (4), *A. simpsonii* (3), *A. diogoi* (1), *A. aff. diogoi* (1), *A. microsperma* (2), *A. linearifolia* (1), *A. cardenasii* (1)) and “non-A” genome species (*A. cruziana* (1), *A. hoehnei* (3), *A. magna* (3), *A. valida* (2), *A. batizocoi* (1), *A. williamsii* (1)). As for resistance to *Cercospora arachidicola*, it was observed that wild germplasm accessions associated to both *A. hypogaea* genomes are available to be used in the introgression of *Cercosporidium personatum* resistance genes into the cultivated peanut. The results may suggest that the above accessions are immune to the pathogen. However, it seems too hasty to come to this conclusion based just on a laboratory test. It would be necessary to repeat the bioassay or to test the material in the field.

In the “a” group, which includes the most resistant of those accessions statistically analyzed, 22 accessions associated to the A genome species were observed, *A. kuhlmannii* (11 accessions), *A. stenosperma* (5), *A. duranensis* (1), *A. helodes* (1), *A. kempff-mercadoi* (1), *A. schininii* (1), *A. simpsonii* (1), *A. villosa* (1) and three of “non-A” genome, *A. magna* (2) and *A. gregoryi* (1). An accession of *A. hypogaea* (US 224) showed higher resistance than the others, also being located in the “a” group. It is interesting to mention that this peanut accession, from Rondonia State, in Brazil, which also presented some resistance to *C. arachidicola*, is the source of resistance to Tomato Spotted Wilt Virus/TSWV incorporated in the Tamrun 96 cultivar (Smith et al., 1998). The “b” group only includes one accession of *A. stenosperma* and six of *A. hypogaea*. The “c” and “d” groups only include accessions of *A. hypogaea* (4), confirming that the cultivated species is significantly more susceptible than the wild species used in the experiment.

The coefficient of variation was 59.06%. As in the tests for *Cercospora arachidicola* resistance, it is normal to find high values like this in disease evaluation data.

Puccinia arachidis

In the rust resistance tests, seven out of 100 accessions appeared highly resistant, without evidence of any lesion (Table 2 - fifth column), 67 accessions showed different lesion levels, however, without pustules but with hypersensitivity reactions (Table 2 - fifth column), and 26 accessions were shown to be more susceptible, presenting pustules (Table 2 - sixth column).

Six of the highly resistant accessions belong to A genome species, *A. helodes* (2 accessions), *A. kuhlmannii* (2), *A. aff. diogoi* (1), *A. simpsonii* (1), and a single one to the “non-A” genome species, *A. gregoryi* (1).

In Table 2, from 67 accessions that had just hypersensitivity reactions, 23 were in the “a” group, being the most resistant among those encompassed by the statistical analysis. The accessions found in the “a” group were in 19 species with “A” genome - *A. kuhlmannii* (5 accessions), *A. helodes* (3), *A. stenosperma* (2), *A. duranensis* (1), *A. cardenasii* (1), *A. diogoi* (1), *A. kempff-mercadoi* (1), *A. linearifolia* (1), *A. microsperma* (1), *A. schininii* (1), *A. simpsonii* (1), *A. villosa* (1) - and four with “non-A” genome species - *A. batizocoi* (1), *A. cruziana* (1), *A. hoehnei* (1), *A. magna* (1). In the “b” group, other 23 accessions were observed: 20 with A genome - *A. kuhlmannii* (11), *A. stenosperma* (7), *A. microsperma* (1), *A. simpsonii* (1) - and three with “non-A” genome - *A. magna* (1), *A. hoehnei* (2). The “c” group involved 16 accessions, 13 with A genome - *A. stenosperma* (10), *A. duranensis* (1), *A. kuhlmannii* (1), *A. simpsonii* (1) and three with “non-A” genome - *A. hoehnei* (1), *A. magna* (1) and *A. valida* (1). The “d” group presented four accessions of *A. stenosperma* (A genome) and one of *A. magna* (“non-A” genome). Such results are reasonably in line with those of Subrahmanyam (1983), who found immunity against *P. arachidis* in *A. batizocoi* (accession K 9484), *A. cardenasii* (GKP 10017), *A. diogoi* (GK 10602) and high resistance in *A. stenosperma* (HLK 408). He also found susceptibility in *A. monticola*.

In the same way as for resistance to *Cercospora arachidicola* and *Cercosporidium personatum*, wild germplasm accessions relating to both genomes of *A. hypogaea* were observed, and are available to be used in the introgression of *P. arachidis* resistance genes in the cultivated peanut.

All the accessions in the sixth column showed sporulation of *P. arachidis*. In the “a” group, there were 12 accessions of *A. hypogaea*, 6 of *A. stenosperma*, 2 of *A. valida*, 1 of *A. monticola*, 1 of *A. magna*, 1 of *A. ipaënsis* and 1 of *A. williamsii* (Table 2). In the “b” group, only 2 accessions were

observed, 1 of *A. stenosperma* and 1 of *A. valida*. All accessions of *A. hypogaea* showed pustules, so the cultivated peanut is more susceptible than most of the wild species accessions in this study. *Arachis monticola*, an allotetraploid wild species, considered by some authors to be the ancestor or, alternatively, a derivative of *A. hypogaea*, also showed susceptibility to *P. arachidis*. *Arachis ipaënsis*, one of the original diploid ancestors of *A. hypogaea* (Fávero et al., 2006), also showed susceptibility to *P. arachidis*. The variation coefficient was 71.43%.

A sum of points was done to determine the most resistant and most susceptible genotypes and the added values for each accession are shown in Table 2 (Seventh column). Some data were missed, so with these data, the ranking may change in some cases. For *Cercospora arachidicola*, the letters from Scott & Knott test varied from “a” to “f”, and some genotypes were not included in the variance analysis as they presented no lesion, therefore earning a zero score. To rank all the lesions and all the genotypes, the categorization by letters was converted to numbers: where it varied from zero to “f”, the ranking varied from zero to six. For *Cercosporidium personatum* the values were from zero to “d”, so they became zero to four. For *P. arachidis*, group one included the categories zero to “d”, changing to 0 to 4; for group two, “a” and “b” groups were replaced by, respectively, scores 5 and 6. So, all test values obtained for each genotype were added, according to the conversions above. Most of the accessions of wild species were more resistant than the accessions of *A. hypogaea*. The allotetraploid *A. monticola*, also has been more susceptible to *Cercosporidium personatum* and *P. arachidis* when compared with most of the wild species. *Arachis monticola* was not tested for *C. personatum* because the leaves were not in appropriate condition for the analysis, nor were other accessions without group letters. *Arachis ipaënsis*, one of the diploid ancestors of *A. hypogaea*, was also shown to be more susceptible to *Cercospora arachidicola* and *P. arachidis* when compared to many of the wild species.

For *Cercospora arachidicola*, *Cercosporidium personatum* and *P. arachidis*, the results presented by Stalker & Moss (1987) in a table of species and respective resistance results for several diseases and pests show a marked similarity to those found in the present study.

The resistance to late leaf spot and rust were studied by Pande & Rao (2001) in 74 accessions of wild species of *Arachis* under greenhouse conditions. The accession KG 30006 of *A. hoehnei* did not show symptoms of either of the two diseases. Twenty-six accessions were classified as resistant to late leaf spot.

Table 3 - Comparison among A and "non-A" genome wild species and *A. hypogaea* and between A and "non-A" genome species for *Cercospora arachidicola*, *Cercospora arachidicola* and *Puccinia arachidis* based on P-values obtained by the use of the t test. Values lower than 0.05 indicate difference between genomes.

Genomes	<i>Cercospora arachidicola</i>	<i>Cercospora arachidicola</i>	<i>Puccinia arachidis</i>
A × AB (<i>A. hypogaea</i>)	0.0012	0.0015	0.0000
"Non-A" × AB (<i>A. hypogaea</i>)	0.0008	0.0014	0.0001
A × "Non-A"	0.7461	0.9049	0.0913

Sixty-eight accessions were considered rust resistant. Although most of the accessions appraised by Pande & Rao (2001) are not the same as those used in the present work, results can be extrapolated in some cases, as there are several common sites for the collections. Our accession of *A. monticola* (V 14165), although collected more recently, but from the same site of Pande & Rao's accession, and the coincident accession of *A. ipaënsis* (K 30076) were also susceptible to rust, corroborating the results of Pande & Rao (2001).

There is resistance to *Cercosporidium personatum*, *Cercospora arachidicola* and *Puccinia arachidis* in many accessions of wild species and these resistances may be different among accessions of the same species.

Species with A genome are more resistant than *A. hypogaea* under the conditions of the present *Cercospora arachidicola*, *Cercosporidium personatum* and *Puccinia arachidis* bioassays (Table 3). The same was observed for species that have "non-A" genome. On the other hand, species with A genome were not significantly different from species with "non-A" genome, showing that resistance genes for the three fungal diseases are at both genomes, and it is possible to introgress them from the both genomes, doing the gene pyramidization.

ACKNOWLEDGMENTS

To the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for providing the funds and fellowships used in this work.

REFERENCES

- COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB. Available at: <http://www.conab.gov.br/download/safra/safra20022003Lev06.pdf>. Accessed 23 Sept. 2003.
- FÁVERO, A.P.; SIMPSON, C.E.; VALLS, J.F.M.; VELLO, N.A. Study of the evolution of cultivated peanut through crossability studies among *Arachis ipaënsis*, *A. duranensis*, and *A. hypogaea*. *Crop Science*, v.46, p.1546-1552, 2006.
- FERNÁNDEZ, A.; KRAPOVICKAS, A. Cromosomas y evolución en *Arachis* (Leguminosae). *Bonplandia*, v.8, p.187-220, 1994.

- FOSTER, D.J.; STALKER, H.T.; WYNNE, J.C.; BEUTE, M.K. Resistance of *Arachis hypogaea* L. and wild relatives to *Cercospora arachidicola* Hori. *Oléagineux*, v.36, p.139-143, 1981.
- GODOY, I. J.; MORAES, S.A.; ZANOTTO, M.D.; SANTOS, R.C. Melhoramento do amendoim. In: BORÉM, A. (Ed.) **Melhoramento de espécies cultivadas**. Viçosa: Editora UFV, 1999. p.51-94.
- KRAPOVICKAS, A.; GREGORY, W.C. Taxonomía del género *Arachis* (Leguminosae). *Bonplandia*, v.8, p.1-186, 1994.
- LAVIA, G.I. Karyotypes of *Arachis palustris* and *A. praecox* (section *Arachis*), two species with basic chromosome number x=9. *Cytologia*, v.63, p.177-181, 1998.
- LAVIA, G.I. Relación entre cromosomas "A" y ciclo de vida en especies de la sección *Arachis*. In: ENCUESTRO DE ESPECIALISTAS EN *ARACHIS* SPP. DE AMERICA LATINA, 2., Córdoba, 1999. *Anais*. Córdoba: INTA, 1999. p.21.
- MORAES, S.A.; SALGADO, C.L. Esporulação de *Cercospora arachidicola* Hori em meio de cultura. *Summa Phytopathologica*, v.5, p.65-74, 1979.
- MORAES, S.A.; SALGADO, C.L. Utilização da técnica de folhas destacadas de amendoim (*Arachis hypogaea* L.) para inoculações com *Cercospora arachidicola* Hori e *Cercospora personata* (Bert. & Curt.) Ell. & Ev. *Summa Phytopathologica*, v.8, p.39-55, 1982.
- MULAMBA, N.N.; MOCK, J.J. Improvement of yield potential of the Eto Blanco maize (*Zea mays* L.) population by breeding for plant traits. *Egyptian Journal of Genetic and Cytology*, v.7, p.40-51, 1978.
- PANDE, S.; RAO, J.N. Resistance of wild *Arachis* species to late leaf spot and rust in greenhouse trials. *Plant Disease*, v.85, p.851-855, 2001.
- PEÑALOZA, A.P.S.; VALLS, J.F.M. Contagem do número cromossômico em acessos de *Arachis decora* (Leguminosae). In: SIMPÓSIO LATINO-AMERICANO DE RECURSOS GENÉTICOS VEGETAIS, 1., Campinas, 1997. **Programa e Resumos**. Campinas: IAC, 1997. p.39.
- PEÑALOZA, A.P.S.; VALLS, J.F.M. Chromosome number and satellited chromosome morphology of eleven species of *Arachis* (Leguminosae). *Bonplandia*, v.14, p.65-72, 2005.
- SCOTT, A.J.; KNOTT, M. A cluster analysis method for grouping means in the analysis of variance. *Biometrics*, v.30, p.507-512, 1974.
- SMITH, O.D.; SIMPSON, C.E.; BLACK, M.C.; BESSLER, B.A. Registration of 'Tamrun 96' peanut. *Crop Science*, v.38, p.1403, 1998.
- STALKER, H.T.; MOSS, J.P. Speciation, cytogenetics and utilization of *Arachis* species. *Advances in Agronomy*, v.41, p.1-40, 1987.
- SUBRAHMANYAM, P.; MOSS, J.P.; RAO, V.R. Resistance to peanut rust in wild *Arachis* species. *Plant Disease*, v.67, p.209-212, 1983.
- VALLS, J.F.M.; SIMPSON, C.E. New species of *Arachis* L. (Leguminosae) from Brazil, Paraguay and Bolivia. *Bonplandia*, v.14, p.35-63, 2005.

Received September 03, 2007

Accepted June 11, 2008