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Screening new *Arachis* amphidiploids, and autotetraploids for resistance to late leaf spot by detached leaf technique

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Abstract Cultivated groundnut is susceptible to late leaf spot (LLS) caused by *Phaeoisariopsis personata* [(Berk. & M.A. Curtis) Aex] and resistance is low to moderate in the primary gene pool of groundnut. Closely related wild species in the secondary gene pool are highly resistant to the disease. All the closely related *Arachis* species are diploid and cultivated groundnut is a tetraploid. Utilization of diploid *Arachis* species to transfer LLS resistance is time consuming and cumbersome. New sources of *Arachis hypogaea* (also called synthetic groundnut) were developed at ICRISAT. These are tetraploids and the present investigation has shown that they are resistant to LLS.

Keywords *Arachis* species · *Arachis hypogaea* · Amphidiploids · Autotetraploids · Late leaf spot resistance

Late leaf spot (LLS) caused by *Phaeoisariopsis personata* [(Berk. & M.A. Curtis) Aex] = *Cercosporidium personatum* [(Berk. & M.A. Curtis) Deighton] is an important foliar fungal disease of groundnut [*Arachis hypogaea* (L.)]. Global losses due to the disease are estimated to be 600 US$ m, and it is estimated that a potential yield gain of 300 US$ m can be made if host plant resistance can be introduced (Dwivedi et al. 2003). LLS causes severe defoliation and reduces both haulm and pod yields by more than 50% (McDonald et al. 1985). LLS spot can occur in all groundnut growing regions. Chemical control of foliar fungal diseases of groundnut is common in many parts of the world, but use of fungicides is not widespread in many tropical regions where the crop is grown by resource-poor farmers. Indiscriminate use of fungicides is not desirable as the fungus may develop resistance to the fungicide, which may also pollute the environment.

Resistance to LLS in cultivated germplasm is moderate to low (Dwivedi et al. 2002). Low level of resistance to LLS in the primary gene pool of groundnut is attributed to the narrow genetic base of the crop. The major cause for the narrow genetic base is due to the nature of the origin of the crop. A single hybridization event gave rise to amphidiploid groundnut, thus creating a very narrow window of origin (Burrow et al. 2001).

Wild *Arachis* species in the secondary gene pool, closely related to cultivated groundnut, have shown immune to resistant reactions to LLS (Subrahmanyam et al. 1985) Components of resistance such as incubation period, number of leaf spots, and leaf area damage play an important role in calculating infection frequency. In the present report the study of the components of resistance of LLS disease in some species of *Arachis* species, diploid hybrids generated

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by crossing *Arachis* species, autotetraploids and amphiploids developed from diploid hybrids, are presented. Autotetraploids were synthesized by crossing one A (or B) genome species with another A (or B) genome species and amphidiploids were synthesized by crossing A genome species with B genome species or vice-versa, were especially advantageous as they have the same ploidy (2n=40) as the cultivated groundnut.

Wild species of groundnut (*Arachis* species) were grown in 30-cm diameter pots in a glasshouse. Healthy plants were maintained in the screen house without the use of pesticides. Diploid hybrids were generating by crossing wild *Arachis* species in different cross combinations. Gibberellic acid (GA_3) was applied to pollinated pistils irrespective of the cross combination. Application of gibberellic acid was advantageous to retain pistils after cross pollinations (Mallikarjuna and Sastri 2002). Amphiploids and autotetraploids, also called synthetic groundnut, were generated by the application of 0.025% colchicine to the apical buds of diploid hybrid shoots for three consecutive days to double the chromosome number (Mallikarjuna et al. 2011). Ploidy of the plants was cytologically checked (Mallikarjuna et al. 2011).

To study the components of LLS resistance in *Arachis* species, diploid hybrids, autotetraploids and amphidiploids (details of the material in Tables 1, 2 and 3), a detached leaf technique was used. Cultivar TMV 2 was used as a susceptible control as it is highly susceptible to the disease and is commonly used as a susceptible check (Pande and Narayan Rao 2001). For the bioassay, quadrifoliate, mature and healthy leaves, using either the second or third fully expanded leaf with the pulvinus on the main stem was excised. The leaves were thoroughly washed in sterilized distilled water to remove surface dirt and arranged in a randomized block design in plastic trays (55 cm long × 27.5 cm wide × 5 cm deep) containing sterilized river sand. There were five leaves per row and five rows per tray. Trays were covered with clear polyethylene bags (62×38 cm) with the open ends folded to maintain high relative humidity. Each genotype was replicated thrice and each replicate consisted of one leaf having four leaflets. The trays were kept in incubators at 25°C with 95% relative humidity at 12 h photoperiod (54.04 micromole/m^2/s). Inoculum (isolated from local fields) was prepared by suspending conidia in sterile distilled water containing the surfactant Tween 80 (polyoxythylene sorbitan monoleate) (10 drops l^{-1}). The spore suspension was stirred well using a magnetic stirrer to make a uniform inoculum. Spore concentration was adjusted to 50,000 conidia ml^{-1} using a haemocytometer. After 24 h, the trays were removed from the incubator and the leaves

Table 1 Screening for components of resistance to late leaf spot in *Arachis* species

Arachis species	IF	IP	LAD	LD	LN	LP
A. valida ICG-13256	0.00 a	16.24 a	0.00 a	0.00a	0.00 b	12a
A. duranensis ICG-8123	0.17 a	16.95 a	0.33 a	0.00 a	1.70 b	12 a
A. batizocoi ICG-8124	0.33 a	16.67 a	1.67 a	0.95a	3.00b	12a
A. hoehnei ICG-8190	0.00 a	16.24 a	0.00a	0.00a	0.00b	12a
A. kempffmercadoi ICG-8959	0.08 a	18.17a	0.67a	0.42 a	0.70 b	12a
A. batizocoi ICG-8209	0.00 a	16.24a	0.00a	0.00a	0.00b	12a
A. diogoi ICG-4983	0.00a	16.24a	0.00a	0.00a	0.00b	12a
A. duranensis ICG-8139	0.00a	16.24a	0.00a	0.00a	0.00b	12a
A. ipaensis ICG-8206	0.00a	16.24a	0.00a	0.00a	0.00b	12a
A. cardenasii ICG-8216	0.00a	16.24a	0.00a	0.00a	0.00b	12a
TMV 2	8.33b	1.84ab	78.33b	3.78ab	57.70a	12a
Mean	0.49	16.24	21.17	5.15	11.8	12
Ese	0.19	1.34	2.33	1.22	4.47	0
Sed	0.27	1.89	3.3	1.73	6.3	0
LSD	0.55	4.36	6.8	3.5	13.11	0
F.Prob	< 0.001	0.004	<0.001	<0.001	<0.001	0
Cv	14.30	66.90	19.10	165	65.70	0

IF Infection frequency; *IP* Incubation period; *LAD* Leaf area damage; *LD* Lesion diameter; *LN* Lesion number; *LP* Latent period calculated; TMV 2-Susceptible control; *Ese* Standard error; *Sed* Standard error of differences; *LSD* Least significant difference; *F.Prob* F-probability; *CV* Critical variance

Means followed by the same letter are not significantly different at P0.05

Table 2 Screening for components of resistance to late leaf spot in diploid *Arachis* species hybrids

S. no.	Genotype	Parental details	Collector numbers for the parents	IF	IP	LAD	LD	LN	LP
1	R-235	*A. duranensis* × *A. hoehnei*	GKP 10038 × KG 30006	0.00 cd	18.83 cdef	0.00 d	0.00 e	0.00 e	23.22 c
2	R-239	*A. kempffmercadoi* × *A. diogoi*	KGBSPSc2 30085 × GK 10602	0.00 cd	18.83cdef	0.00 d	0.00 e	0.00 e	23.22 c
3	R-240	*A. valida* × *A. diogoi*	KG 30011 × GK 10602	0.00 cd	18.83cdef	0.00 d	0.00e	0.00 e	23.22 c
4	R-248	*A. valida* × *A. duranensis*	KG 30011 × GKP 10038	0.00 cd	18.83cdef	0.00 d	0.00 e	0.00 e	23.22 c
5	R-257	*A. ipaensis* × *A. duranensis*	……× K 7988	0.00 cd	18.83cdef	0.00 d	0.00 e	0.00 e	23.22c
6	R-265	*A. kempffmercadoi* × *A. hoehnei*	KGBSPSc2 30085 × KG 30006	0.36 c	14.00 fg	4.67 b	1.63 bcd	6.67 bcd	21.00 d
7	R-303	*A. trinitensis* × *A. hoehnei*	Grif 14278 (DEW 1117) × KG 30006	0.00 cd	18.83 cdef	0.00 d	0.00 e	0.00 e	23.22 c
8	R-384	*A. diogoi* × *A. hoenhei*	GK 10602 × KG 30006	0.00 cd	18.83 cdef	0.00 d	0.00e	0.00e	23.22 c
9	R-391	*A. valida* × *A. batizocoi*	……× KGBSPSc 30079	0.00 cd	18.83 cdef	0.00 d	0.00 e	0.00 e	23.22 c
10	R-396	*A. duranensis* × *A. diogoi* ICG8962	K 7988 × …..	0.09 cd	15.75def	1.33 cd	0.83cde	2.00e	25.33c
11	R-401	*A. valida* × *A. duranensis*	…….× K 7988	0.00 cd	18.83 cdef	0.00 d	0.00e	0.00e	23.22c
12	R-402	*A. duranensis* × *A. valida*	K 7988 × …..	0.14 cd	24.40abc	0.33 d	0.33de	0.67e	23.22c
13	R-405	*A. magna* × *A. batizocoi*	KGSSc 30092 × KGBSPSc 30079	0.12 cd	13.40 g	1.00 cd	0.56de	2.00e	24.50c
14	R-407	*A. magna* × *A. duranensis*	KGSSc 30092 × K 7988	0.22 cd	21.30 cd	1.33 cd	0.92cde	2.00 e	23.16 c
15	R-412	*A. valida* × *A. duranensis*	VPoBi 9153 × K 7988	0.00 cd	18.83cdef	0.00 d	0.00e	0.00e	23.22c
16	R-413	*A. magna* × *A. valida*	KGSSc 30092 × VPoBi 9157	0.00 cd	18.83cdef	0.00 d	0.00e	0.00 e	23.22c
17	R-420	*A. cardenensis* × *A. diogoi*	GKP 10017 × GK 10602	0.05 cd	27.40ab	0.33 d	1.33bcde	1.00e	23.22c
18	R-425	*A. batizocoi* × *A. duranensis*	K 9484 × K 7988	0.00 cd	18.83cdef	0.00 d	0.00e	0.00e	23.22c
19	R-414	*A. valida* × *A. magna*	VPoBi 9157 × KGSSc 30092	0.27 cd	16.00 def	5.00b	2.20 bc	7.00bc	28.16a
20	R-415	*A. duranensis* × *A. cardenasii*	KGBSPSc 30061 × GKP 10017	0.00 cd	18.83cdef	0.00 d	0.00 e	0.00 e	23.22c
21	R-419	*A. diogoi* × *A. cardenasii*	GK 10602 × GKP 10017	0.35c	20.00 cdef	3.00bc	2.47b	7.00bc	27.83 a
22	R-255	*A. kempffmercadoi* × *A. stenosperma*	KGSPSc 35001 × VMiSv 10229	0.00 cd	18.83cdef	0.00 d	0.00 e	0.00 e	23.22c
	TMV 2	Cultivated		1.48 a	8.33 g	90.00 a	4.53 a	55.33 a	14.66 e
	Mean			0.14	18.44	4.6517	0.643	3.638	23.31
	F. pro			<.001	0.002	<.001	<.001	<.001	0.001
	Ese			0.08	1.60	0.95	0.42	1.20	0.30
	Sed			0.14	2.79	1.64	0.73	2.09	0.52
	LSD			0.29	6.04	3.20	1.47	4.18	1.60
	Cv%			161.6	21.00	63.30	170.30	93.70	3.20

IF Infection frequency; *IP* Incubation period; *LAD* Leaf area damage; *LD* Lesion diameter; *LN* Lesion number; *LP* Latent period; *Ese* Standard error; *Sed* Standard error difference; *LSD* Least significant difference; *CV* Critical variance; *F.pro* F. probability; TMV 2-Susceptible control; *Ese* Standard error; *Sed* Standard error of differences; *LSD* Least significant difference; *F-Prob* F- probability; *CV* critical variance

Means followed by the same letter are not significantly different at P0.05

were sprayed on both surfaces with spore suspension using a plastic atomizer.

The components for disease resistance were defined as: (1) Incubation period (IP), recorded by counting number of days from inoculation to appearance of first symptoms; (2) Latent period (LP), the number of days from inoculation to the appearance of first sporulating lesion with the help of 20 × magnifying lens; (3) Lesion number (LN), the average number of lesions on the examined leaf surfaces; (4) Leaf area damage (LAD), the percentage of leaf area which was infected; (5) Lesion diameter (LD), corresponded to the average diameter of four lesions measured at 30 days after inoculation; (6) Infection frequency (IF), the number of lesions per cm^2 at 30 days after inoculation and obtained by measuring total number of lesions on each

Table 3 Screening for components of resistance to late leaf spot in newly synthesized tetraploid groundnut

Identity	Parental details	IF	IP	LAD	LD	LN	LP
ISATGR 43A	*A. valida* × *A. diogoi*	0.00 e	18.00 b	0.00 d	0.00 c	0.00 d	19.67 b
ISATGR 51B	*A. valida* × *A. duranensis*	0.00 e	18.00 b	0.00 d	0.00 c	0.00 d	19.67 b
ISATGR 90B	*A. kempffmercadoi* × *A. stenosperma*	0.00 e	18.00 b	0.00 d	0.00 c	0.00 d	19.67 b
ISATGR 1212	*A. duranensis* × *A. ipaensis*	0.18 cd	22.00 a	2.33 bc	1.07 b	5.00 bc	18.00 c
ISATGR 268 5A	*A. batizocoi* × *A. cardenasii*	0.00 e	18.00 b	0.00 b	0.00 c	0.00 d	19.67 b
ISATGR 265 5	*A. kempffmercadoi* × *A. hoehnei*	0.45 a	18.00 b	3.33 b	1.20 b	7.30 b	23.00 a
ISATGR 278-18	*A. duranensis* × *A. batizocoi*	0.25 bc	16.00 c	1.00 cd	1.42 b	2.00 cd	19.67 b
ISATGR 5B	*A. magna* × *A. batizocoi*	0.38 ab	16.00 c	5.00 a	3.07 a	16.00 a	18.00 c
TMV 2	Cultivated	1.48	8.33	90.00	4.53	55.33	14.66
Mean		0.16	18.00	1.45	0.84	3.78	19.66
Ese		0.19	2.83	1.60	0.79	5.00	1.30
Sed		0.27	4.00	2.30	1.30	7.07	2.21
LSD		0.53	5.83	5.50	2.40	15.17	1.40
F.Prob.		<0.001	0.002	<.0.001	<0.001	<0.001	<0.001
Cv		208.00	27.20	198.00	164.00	228.00	2.51

IF Infection frequency; *IP* Incubation period; *LAD* Leaf area damage; *LD* Lesion diameter; *LN* Lesion number; *LP* Latent period; TMV 2- susceptible control, *Ese* Standard error; *Sed* Standard error of difference; *LSD* Least significant difference; *F-Prob* F-probability; *CV* critical variance

Means followed by the same letter are not significantly different at P0.05

leaf and estimating the total leaf area using a leaf area meter. The ratio of number of lesions to the area of a leaf gave the infection frequency for that leaf. In experiments involving *Arachis* species, diploid hybrids and tetraploids, two replicates were combined to give the mean. Data were analyzed using PROC GLM SAS 9.2 version, 2008.

Compared to cultivated groundnut, all the *Arachis* species used in the present investigation showed resistance to the disease. Lesion number at 30 days after inoculation was 0 to 1.7% compared to 58% at 30 days after inoculation in cultivar TMV 2. Other cultivars also showed 38 to 40% lesions on the leaves. Leaf area damage varied between 78.3% in the cultivar compared to a maximum of 1.7% in *Arachis* species *A. batizocoi*. In *A. diogoi*, *A. cardenasii*, *A. valida*, *A. hoehnei* and one accession of *A. duranensis*, there were no lesions on the leaves even after 30 days from inoculation. These species can be categorized as immune to the disease. There were differences between accessions of the same species to the disease. *A. duranensis* ICG 8123 and *A. batizocoi* ICG 8124 showed some lesions (1.7 and 3.0 respectively) on the leaves compared to other accessions (ICG 8139 and ICG 8209) of the same species which had no lesions. Incubation period was shorter in cultivars between 8 and 19 d, compared to a minimum of 16 d on *Arachis* species. Infection frequency varied from 1.5 to 2.0 in the susceptible cultivars compared to an infection frequency of 0.0 to 0.33 in *Arachis* species. All the *Arachis* species used in the study were characterized as immune to resistant to the disease (Table 1).

A range of reactions were observed in diploid *Arachis* hybrids. No lesions were observed on ISATR 239, ISATR 240, ISATR 255, ISATR 235, ISATR 248, ISATR 257, ISATR 303, ISATR 384, ISATR 391, ISATR 401, ISATR 412, ISATR 413, and ISATR 425 (Table 2). These diploid hybrids were immune to the disease. Some of the diploid hybrids such as ISATR 396, ISATR 405, ISATR 419, ISATR 265, ISATR 407, ISATR 420, and ISATR 402 had less than 7 lesions per leaf compared to 55 lesions in susceptible cultivar TMV 2. Leaf area damage varied from 0.0 to 4.7% in the diploid hybrids, compared to 90% damage in cultivar TMV 2. Days after incubation to the first symptom of the disease varied from 21 to 28 days compared to 14 days in cultivar TMV 2. Infection frequency varied from 0.0 to 0.37 in diploid hybrids compared to 1.5 on TMV 2. It was concluded that

diploid hybrids were immune to resistant to the disease (Table 2).

Amphidiploids ISATGR 43A, ISATGR 51B, and ISATGR 268–5 did not have any lesions even after 30 days from inoculation. Another amphidiploid namely ISATGR 265–5 had 7 lesions on the leaves, compared to 55 on TMV 2. Autotetraploid ISATGR 90B did not have lesions even after 30 d post-inoculation, but ISATGR 5B had 16 lesions. Even though a few lesions were observed on one amphidiploid and one autotetraploid, leaf area damage ranged from 1 to 5% compared to 90% on TMV 2. In all the synthetics with some leaf area damage, first symptoms began to appear by 18 to 23 d post inoculation, compared to 15 d for TMV 2. Based on components of resistance to LLS, ISATGR 43A, ISATGR 51B, ISATGR 90B, and ISATGR 268–5 were classified as immune while ISATGR 1212, ISATGR 278–18, and ISATGR 5B were classified as resistant (Table 3).

Many of the *Arachis* species are known to be either immune or resistant and hence good sources of LLS resistance. Introgression of LLS resistance is not a straightforward process due to ploidy differences between cultivated tetraploid groundnut and diploid *Arachis* species. In recent years at ICRISAT, utilization of diploid (2n) pollen from the triploids has accelerated the process of obtaining tetraploids from F_1 triploid hybrids (Mallikarjuna and Tandra 2006).

More recently, diploid hybrids were created by crossing various wild *Arachis* species and intragenomic and intergenomic hybrids were generated. Tetraploids (autotetraploids and amphidiploids) were generated from diploid hybrids either through colchicine treatment or by the exploitation of diploid gametes (Mallikarjuna, N., unpublished). Tetraploids are new sources of *A. hypogaea* (also called synthetics) which have not undergone selection. Hence these would have broadly based variation and carry many useful traits necessary for the improvement of groundnut; traits which might have been lost during evolution, domestication and plant breeding.

Diploid hybrids were screened for resistance to LLS to determine if the process of hybridization would have any effect on LLS resistance as these are the basic stock to develop tetraploid hybrids. It is known that hybridization can cause variations in the hybrid genome (Hoisington et al. 1999). Since many of the diploid hybrids and the tetraploids generated from them showed immune to highly resistant reactions, they are valuable genetic stocks which can be used to transfer not only LLS resistance per se, but to broaden the genetic base of cultivated groundnut. Currently, the only effective way diploid wild relatives of *Arachis* can be used to improve cultivated groundnut, is through the development and utilization of tetraploid (synthetic) groundnut.

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