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Production of fertile and foliar disease resistant hybrids and backcross progeny between *Arachis hypogaea* and *Synthetic amphidiploids*

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ABSTRACT : Peanut (*Arachis hypogaea* L.) is widely used as a food and cash crop around the world. It is considered to be an allotetraploid (2n = 4x = 40) originated from a single hybridization event between two wild diploids. The utilization of wild germplasm in breeding programs has received little attention due to the reproductive barriers between wild and cultivated species and to the technical difficulties encountered in making large number of crosses. Polyploidy creates severe genetic bottlenecks, contributing to the genetic vulnerability of leading crops. Cultivated peanut is thought to be of monophyletic origin, harboring relatively little genetic diversity. There are only a few reports of successful crosses between cultivated peanut (*Arachis hypogaea* L., section. *Arachis*) and wild species from sections other than section *Arachis*. Many of the wild *Arachis* species harbour important traits necessary for the improvement of peanut. LLS, caused by *Cercosporidium personatum*, is an important fungal disease in Asia and the Americas as well as Africa. To introduce LLS resistance from diploid wild species into tetraploid cultivated *Arachis hypogaea*, a synthetic amphidiploids ISATR 278-18 (*A.duranesis* ICG 8138 x *A.batizocoi* ICG 13160) and ISATGR- 5B (*A.magna* ICG 8966 x *A.batizocoi* ICG 8209) was used as donor parent to generate a backcross population and screened for resistance to LLS. Hybrids in different generations were scored for rust and LLS resistance and found that they were resistant for all components of disease resistance as compared to female parent. Thus crosses with species outside the section *Arachis* may not only confer disease resistance but will also broaden the genetic base of cultivated peanut.

Key Words : Groundnut, Wild species, *Synthetic amphidiploids*, Interspecific hybridization

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Cultivated groundnut, also known as peanut (*Arachis hypogaea* L.), is grown on nearly 24 million hectares between latitudes 408 N and 408 S. Although originating in South America, the vast majority of groundnut is produced in Asia and Africa: Asia 68 per cent (23 Mt), Africa 24 per cent (8 Mt). The remaining 8 per cent (3.5 Mt) comes from North America, the Caribbean, Europe and Oceania. Approximately 94 per cent of groundnut is produced in the developing world, mostly under rainfed conditions. The major groundnut producing countries are China, India, Indonesia, Myanmar and Vietnam in Asia. Groundnut is the principal source of human dietary protein, oil/fat and vitamins such as thiamine, riboflavin and niacin in parts of Asia and Africa (Savage and Keenan, 1994). Groundnut paste is an important source of calories for small children, particularly those being weaned. These children cannot obtain the calories they require from high-bulk cereal grains and depend on groundnut for energy as well as vitamins. Groundnut cake is used as livestock feed and help to maintain livestock productivity. The crop also contributes up to 60 kg/ha nitrogen to the soil, benefiting crops subsequently planted in the same field (Sprent, 1994). Late leaf spots (LLS), caused by *Cercosporidium personatum*, and are an important foliar disease of groundnut in Africa, Asia and the Americas. An estimated global loss in yield of 600 million US$ due to LLS has been reported (Dwivedi *et al*., 2003). Hence, yield losses due to the disease can be a major impediment to groundnut production. Managing the disease through the application of fungicides is not a viable option for resource poor farmers. Besides, the application of fungicides may pollute the environment, including ground water, thus causing greater risk and damage than the loss of the crop due to the disease. Molecular analysis has shown that the crop

Table A : Components of LLS for amphidiploids

Genotypes	Rust	LLS	No. of lesions per leaf	Initiation of sporulation (DAS)	Days to 50% sporulation (DAS)	% leaf infected	lesion diameter (cm)
JL 24(check)	6	7	23.6	20	25	80	0.154
TMV2(check)	5	7	23.2	21	27	60	0.114
ISATGR 278-18	2	3	6.8	41	47	13	0.09
ISATGR 5	2	2	5.6	39	43	22	0.08

has a narrow genetic base (Halward *et al.,* 1991 and Hopkins *et al.,* 1999). A principal reason for this may be that a single hybridization event gave rise to the tetraploid cultivated peanut some 3,500 years ago (Kochert *et al.,* 1996). There is, however, much molecular variation in the nine different sections of *Arachis* (Mallikarjuna, 2005 and Milla *et al.,* 2005). Wild species from the section *Arachis* have been used in the improvement of cultivated species (Stalker *et al.,* 1991 and Mallikarjuna *et al.,* 2004a and b). Wild species in the other eight sections are incompatible with the cultivated peanut and specialized techniques are required for crossing. Synthetic amphidiploids ISATGR 278-18 and ISATGR 5B are resistant to late leaf spot and Rust. Utilization of Synthetic amphidiploids in an *A. hypogaea* improvement programme could contribute resistance to LLS and rust in cultivated varieties and would broaden the genetic base of the crop.

RESEARCH PROCEDURE

Seeds of synthetic amphidiploids ISATR 278-18 (*A.duranesis* ICG 8138 x *A.batizocoi* ICG 13160) and ISATGR-5B (*A.magna* ICG 8966 x *A.batizocoi* ICG 8209) with 2n=2x=40 were obtained from the ICRISAT and grown in a glasshouse. These amphidiploids established in the ring pots were screened for late leaf spot (LLS) resistance by detached leaf technique in laboratory by studying the component traits. Cultivar TMV 2 and JL 24 were used as the susceptible check. Plastic trays with autoclaved sand were used to place tetrafoliate leaves in a randomized block design with 2 replications. LLS spores were harvested with a cyclone spore collector. The concentration of the suspension was 20,000 spores/ml. A few drops of Tween 80 (polyoxyethylene sorbitan mono-oleate) were added. Spore suspension was used to spray inoculate the leaves. Immediately after inoculation, leaves were placed in a growth room at 23–25°C to ensure wetness of the leaf surface during the night. Leaves were observed for damage due to sporulating colonies and time taken to sporulation. Based on these parameters damage due to LLS was calculated at the end of 30 days. The screening was carried out in the glasshouse under unprotected condition. Both amphidiploids were found to be resistant to LLS (Table A, Fig. A). Seeds of *A. hypogaea* cv. ICGV 91114; ICGS 76, ICGV 91278, JL 24 and DH 86 were also grown in the pots. Flowers were emasculated a day before pollination and cross pollination, using *A. hypogaea* as the female parent and synthetic amphidiploids as the pollen donor, was carried out

Fig. A : Showing LLS lesions in check and synthetic amphidiploids

before 10:00 am on the following day. Application of gibberellic acid (GA_3) (0.5 ml; 75 mg/l) by means of a cotton swab impregnated with the hormone and wrapped around the base of pollinated pistils was mandatory for obtaining pods from crosses. Pollination was done up to 30 days and numbers of bud pollinated were recorded. Peg formation was started after 25 days of stopping of pollination. Hybrid pods were harvested 45 days after pegging and per cent crossed pods were calculated in both of the amphidiploids. The F_1 seeds were germinated to raise hybrid plants. Backcross populations, BC_2F_1 were developed by crossing F_1 with 5 cultivated recurrent parents which were selfed to produce BC_2F_2.

RESEARCH ANALYSIS AND REASONING

Seed set in crosses involving ISAT 278-18 as the pollen

Table 1a : Crossability between *A. hypogaea* and synthetic amphidiploids ISATR 278-18

Crosses	No. of buds pollinated	Total no. pods	% crossed pods
JL 24 x ISATGR 278-18	89	11	45
DH 86 x ISATGR 278-18	47	7	15
ICGS 76 x ISATGR 278-18	59	5	29
ICGV 91114 x ISATGR 278-18	78	4	41
ICGV 91278 x ISATGR 278-18	63	8	39

parent was very low (15 to 45%) (Table 1a). Crosses involving ISATR 5 as the pollen parent showed pod formation ranging from 16 to 47 per cent (Table 2). The F_1 seeds were germinated to raise hybrid plants. F_1 hybrid had intermediate morphology with a spreading growth habit. Morphology of the leaves was intermediate between the two parents. The hybrids were similar to male parents also for flower colour and pod morphology. The seeds were germinated to backcross the F_1 hybrids with the respective recurrent parent. F_1s were backcrossed to five cultivated types to raise backcross population (Table 2). Percentage of crossed pod ranged 38 to 50 per cent which was 50 per cent of the no. of bud pollinated. These BC_1F_1s hybrids of five cultivated lines were selected based on disease resistance and then backcrossed again with two recurrent parents to raise BC_2F_1s. Many of the mentioned below criteria were considered for selection of hybrids to produce introgression lines *viz.*, disease resistance, morphology, insect damage score etc. For disease resistance *viz.*, rust and late leaf spot score were taken into consideration, for morphology *viz.*, number of primary and secondary branches, leaf size, leaf shape, growth habit, branching pattern were considered. For insect resistance characters, thrips damage had been used for selection of hybrids. Selected no. of BC_2F_1s hybrids in each of the ten crosses was selfed and screened for resistance to rust and LLS under high disease pressure during *Kharif*. Selection was made based on disease response and morphology. Hybrids in different generations were scored for rust and LLS resistance and found that they were resistant for all components of disease resistance as compared to susceptible female parent (Fig. 1). Number of resistant plants in each cross and generation and there range of disease scores is indicated in Table 3a and b, respectively. Apart from resistant plants, some of the good morphological variants have been found in each cross having characters similar to male parent eg. broad leaves and more

Table 1b : Crossability between *A. hypogaea* and synthetic amphidiploids ISATR 5

Crosses	No. of buds pollinated	Total no. pods	% crossed pods
JL 24 x ISATGR 5	51	13	47
DH 86 x ISATGR 5	49	12	21
ICGS 76 x ISATGR 5	58	4	16
ICGV 91114 x ISATGR 5	60	5	41
ICGV 91278 x ISATGR 5	43	4	23

Table 2 : Backcrossed population

Crosses	No. of true F_1s	No. of buds pollinated (BC_1)	% crossed pods	No. of crossed pod in (BC_1)
JL 24 x (JL 24 x ISATGR 278-18)	6	90	42	54
JL 24 x (JL 24 x ISATGR 5)	13	95	41	16
DH 86 x (Dh 86 x ISATGR 278-18)	7	40	50	5
DH 86 x (DH 86 x ISATGR 5)	5	52	45	6
ICGS 76 x (ICGS 76 x ISATGR 278-18)	2	76	41	13
ICGS 76 x (ICGS 76 x ISATGR 5)	3	83	45	9
ICGV 91114 x (ICGV 91114 x ISATGR 278-18)	4	97	47	23
ICGV 91114 x (ICGV 91114 x ISATGR 5)	5	87	38	17
ICGV 91278 x (ICGV 91278 x ISATGR 278-18)	6	53	47	40

Table 3 a: Number of resistant plants in each cross and generation

Crosses	BC2F2	BC2F3	BC1F3	F3	F4	Total
DH 86 x (Dh 86 x ISATGR 278-18)	5	10	14	-	-	29 R
DH 86 x (DH 86 x ISATGR 5)	-	9	7	2	-	18 R
ICGS 76 x (ICGS 76 x ISATGR 278-18)	15 (MR)	10 (MR)	90 R + 20 MR	11	5	101 R + 45 MR
ICGS 76 x (ICGS 76 x ISATGR 5)	20 (MR)	6 (MR)	-	2	6	8 R + 26 MR
JL 24 x (JL 24 x ISATGR 278-18)	8 (MR)	6	-	-	-	6 R + 8 MR
JL 24 x (JL 24 x ISATGR 5)	5 (MR)	-	-	-	-	5 MR
ICGV 91114 x (ICGV 91114 x ISATGR 278-18)	-	-	2	-	-	2 R
ICGV 91114 x (ICGV 91114 x ISATGR 5)	-	-	-	2	-	2 R
R= Complete resistant						166 R + 84 MR
MR = Moderate resistance						

Table 3 b : Range of disease score (0-9 scale) of resistant plants in each cross and generation

Crosses	Rust (At harvest)	LLS (At harvest)
JL 24 (Female parent)	8	8
JL 24 x (JL 24 x ISATGR 278-18)	2-3	2-3
JL 24 x (JL 24 x ISATGR 5)	4-5 (MR)	4-5 (MR)
DH 86 (Female parent)	7	7
DH 86 x (Dh 86 x ISATGR 278-18)	3	3
DH 86 x (DH 86 x ISATGR 5)	3-4	3-4
ICGS 76 (Female parent)	8	6
ICGS 76 x (ICGS 76 x ISATGR 278-18)	2-3	2-6
ICGS 76 x (ICGS 76 x ISATGR 5)	4-5 (MR)	4-5 (MR)
ICGV 91114 (Female parent)	7	7
ICGV 91114 x (ICGV 91114 x ISATGR 278-18)	2-3	2-3
ISATGR 278-18 (Male parent)	2	3
ISATGR 5 (Male parent)	2	2

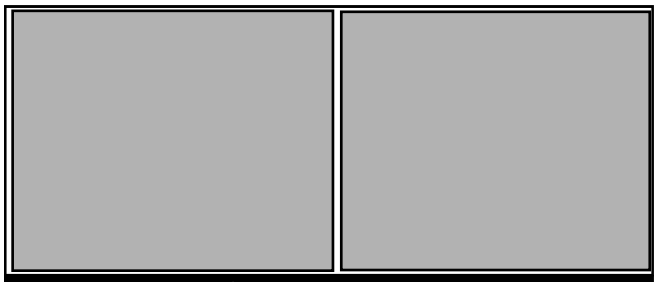

Fig. 1 : Variation for disease resistance in susceptible female parent and resistant BC2F2 hybrids of cross ICGS 76 X ISATGR 278-18

height than female parent, spreading growth habits, more pubescent stem, dark green leaves, more no. of secondary branches, leaf shape variations etc. These morphological variants are not fully resistant but some are moderate resistant and some are susceptible. Many of the wild species from section *Arachis* have been successfully crossed with *A. hypogaea* and hybrids obtained (Stalker *et al.,* 1991 and Mallikarjuna *et al.,* 2004a and b) and various introgression schemes have been used to obtain backcross progeny (Simpson 2001). In the present experiment, F1 hybrid was used as the male parent and crossed with *A. hypogaea*. Synthetic amphidiploids when crossed with *A. hypogaea*, produced fertile plants and the pods resembled those of *A. hypogaea*. *Arachis glabrata* from section Rhizomatosae has been successfully crossed with *A. hypogaea* using *in vitro* techniques (Mallikarjuna and Sastri, 2002) and traits of interest such as resistance to late leaf spot and groundnut viral diseases caused by peanut mottle virus (PMV), peanut stripe virus (PSTV) and peanut bud necrosis virus (PBNV) transferred (Mallikarjuna, 2003). In the present study, it was possible to transfer level of LLS resistance from synthetic amphidiploids. The significance of crossing wild species from other sections is that increased numbers of *Arachis* species become available for the introduction of useful characters into cultivated groundnut. Also, the relationship between different sections will become clearer as the classification of the genus is based on the crossability (Krapovickas and Gregory, 1994). More importantly, such materials broaden the genetic base of the crop. It needs to be seen if the progeny have other desirable traits as crossing with wild relatives reorganizes the whole genome apart from adding exotic genetic material (Hoisington *et al.,* 1999).

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