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Meiotic analysis of the hybrids between cultivated and synthetic tetraploid groundnuts

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With 1 figure and 1 table

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Abstract

Groundnut is susceptible to a range of diseases and pests owing to its narrow genetic base. Closely related wild relatives of groundnut are diploid, and crossability between diploid wild relatives and tetraploid cultivated groundnut is difficult. Amphidiploid and autotetraploid groundnuts, which are tetraploids (also called synthetic groundnut), were developed at ICRISAT. Crossability between cultivated tetraploid groundnut and synthetic tetraploid groundnut was a straightforward process. Crosses between cultivated and synthetic tetraploid groundnuts yielded mature seeds. The resultant F_1 hybrids were cytologically analysed to study the relationship between the chromosomes/genomes of the parents i.e. between *Arachis hypogaea* cultivars and synthetic tetraploid groundnut. Meiotic study showed that there was good chromosome pairing between the parental species resulting in high pollen fertility. Thus, development of synthetic groundnut is a feasible way of utilizing important wild species gene pool of *Arachis*, closely related to cultivated groundnut, for the improvement to the crop.

Key words: *Arachis* species — cultivated groundnut — meiosis — pollen fertility — recombination

Groundnut (*Arachis hypogaea* Linn.) is an important legume crop, grown globally in more than 100 countries of the world, encompassing major regions of Asia and Africa. The crop is particularly important to small-holder farmers who grow groundnuts under low-input conditions for food, oil, feed and confectionary purposes. Various biotic stresses such as foliar fungal diseases, namely late leaf spot caused by *Phaeoisariopsis personata* (Berk. & Curt.) Van Arx, early leaf spot caused by *Cercospora arachidicola* Hori, other fungal diseases such as *Aspergillus flavus* Link ex Fries producing aflatoxin, diseases caused by viruses such as groundnut bud and stem necrosis, groundnut rosette and abiotic stresses such as drought and salinity affect the productivity of the crop. Unfortunately, adequate sources of resistance to various stresses are lacking in the cultivated species.

There is evidence to show that cultivated groundnut has a narrow genetic base (Grieshammer 1989, Knauft and Gorbet 1989, Kochert et al. 1991, Varshney et al. 2009). As a result, the crop is susceptible to a range of diseases caused by fungi, viruses and insect pests, without desirable levels of resistance in cultivated groundnut. Although utilization of landraces has made some difference, there is ample scope to improve the crop, utilizing other sources of germplasm. Various evaluation studies have shown that the wild relatives in the secondary gene pool have high levels of resistance to many diseases (Subramanyam et al. 1985; Pande and Rao 2001). This has opened up renewed interest to utilize wild relatives closely related to the cultigen to introgress/introduce traits of interest. A study of the wild relatives of groundnut shows that all the closely related *Arachis* species are diploid (except *A. monticola*), whereas cultivated groundnut is tetraploid (Krapovickas and Gregory 1994). The ploidy differences can impede successful transfer of desirable traits, and an elaborate backcross programme can slow down the base-broadening efforts and introduction of useful traits.

A review on the origin of the crop has shown that groundnut originated by the combination of two diploid *Arachis* species *A. duranensis* (A genome; Kochert et al. 1991; Kochert et al. 1996; Seijo et al. 2007) and *A. ipaensis* (B genome; Kochert et al. 1991, Kochert et al. 1996). The resultant diploid hybrid/s was sterile, and chance doubling of the chromosome number resulted in highly fertile tetraploid groundnut. Molecular analysis has shown that such events did not occur too often, resulting in narrow genetic base for cultivated groundnut (Burow et al. 2001; Kochert et al., 1996).

A programme was initiated at ICRISAT to develop synthetic groundnut by combining the putative genome donors as well as many other closely related A and B genome species. As a result, there is a range of tetraploid (amphidiploid and autotetraploid) groundnut (also called synthetic groundnut) available for the improvement to cultivated species. Recombination between the parental genomes is essential for the transfer of useful traits from the parents to the offspring. This paper reports meiotic studies showing higher number of bivalent formation with higher pollen fertility. Good chromosome homology is an indication of higher recombination between cultivated and newly synthesized tetraploid groundnut.

Materials and Methods

New sources of tetraploid groundnut namely ISATGR 1212 (*A. duranensis* × *A. ipaensis*), ISATGR 278-18 (*A. duranensis* × *A. batizocoi*), ISATGR 265-5 (*A. kempff mercadoi* × *A. hoehnei*), ISATGR 268-5 (*A. batizocoi* × *A. cardenasii*) and ISATGR 72B (*A. duranensis* × *A. cardenasii*) were synthesized at ICRISAT by combining different diploid *Arachis* species from section *Arachis*. The resultant diploid hybrids were treated with colchicine to double their chromosome number. Tetraploid hybrids, also called synthetic

groundnut, were developed by combining A with A genome (autotetraploid) and A with B genome or vive versa (amphidiploids). Many of the synthetic groundnuts had similar genome constitution but not necessarily the same *Arachis* species combination. Only ISATGR 1212 had the genome combination similar to that of *A. hypogaea*, i.e. putative genome donors *A. duranensis* × *A. ipaensis*. Molecular analysis has shown that there is a Jaccard dissimilarity diversity index value of 0.330 between cultivated *A. hypogaea* and ISATGR 1212 (Mallikarjuna et al. 2011). ISATGR 72B was synthesized by combining two A genome species. Hence, this is an autotetraploid, and the remaining three are amphiploids. To avoid using two terms, a generic term of tetraploids will be used for synthetic tetraploids.

Five new sources of tetraploid groundnut were crossed with cultivated *A. hypogaea* cultivar 'TMV 2', used as the female parent. F_1 hybrids obtained were raised in a glasshouse, and flower buds were collected at the appropriate time and stage to study meiosis. Flower buds were squashed in 2% acetocarmine, and well-spread preparations were examined. To summarize different stages of meiosis, namely metaphase, anaphase and tetrad, at least 20 pollen mother cells (PMCs) were examined. Pollen fertility analysis was carried out by staining mature pollen grains in 2% acetocarmine. Well-stained grains were counted as fertile grains, and partial-to-unstained grains were counted as sterile.

Results

Table 1 summarizes the results of meiotic studies of the F_1 hybrids between *A. hypogaea* and synthetic tetraploids. It is clear from Table 1 that the highest recombination was between *A. hypogaea* and ISATGR 1212. The number of bivalents ranged from 18 to 20, with 70% of the PMCs showing 20 bivalents (Fig. 1, top row, left), and 15% of the PMCs had 19 bivalents. Presence of 20 bivalents indicates homology between the parental genomes. Presence of 19 bivalents and two univalents shows that except for one chromosome in *A. hypogaea* and in ISATGR 1212, the other 19 chromosomes were homologous to each other. The number of univalents in the cross was also small, ranging from 1 to 2. Presence of two univalents shows non-homology between one chromosome each in *A. hypogaea* and ISATGR 1212 (Fig. 1, top row, centre). Analysis did not show abnormalities except for the presence of univalents. A high degree of homology between *A. hypogaea* and ISATGR 1212 was also reflected in higher pollen fertility, which was above 90%. In the tetrad stage, micronuclei were noticed in less than 1% of the PMCs (Fig. 1, top row, right).

Table 1: Meiotic configuration of F_1 hybrids of *A. hypogaea* cv 'TMV 2' and four new amphidiploids and a autotetraploid of groundnut synthesized at ICRISAT

Cross	PMC (%)	1	2	3	4
Cv TMV2 × ISATGR 1212	14 (70)	20	0	0	0
	3 (15)	19	2	0	0
	2 (10)	18	1	1	0
	1 (5)	17	0	2	0
Cv TMV2 × ISATGR 278-18	12 (60)	20	0	0	0
	3 (15)	19	2	0	0
	2 (10)	18	1	1	0
	2 (10)	17	0	2	0
	1 (5)	16	2	2	0
Cv TMV-2 × ISATGR 265-5A	7 (35)	20	0	0	0
	4 (20)	19	2	0	0
	3 (15)	18	4	0	0
	3 (15)	18	1	1	0
	2 (10)	17	3	1	0
	1 (5)	16	2	2	0
Cv TMV2 × ISATGR 268-5	12 (60)	20	0	0	0
	2 (10)	19	2	0	0
	1 (5)	18	4	0	0
	3 (15)	18	1	1	0
	1 (5)	17	3	1	0
	1 (5)	16	2	2	0
Cv TMV2 × ISATGR 72B	4 (20)	20	0	0	0
	8 (40)	19	2	0	0
	2 (10)	18	1	1	0
	3 15)	17	3	1	0
	3 (15)	15	4	2	0

1, Bivalents; 2, Univalents; 3, Trivalents; 4, Quadrivalents; PMC, pollen mother cell.

ISATGR 278-18 was synthesized using *A. duranensis* and *A. batizocoi*. *Arachis duranensis* is one of the putative genome donors of *A. hypogaea* but not *A. batizocoi*. Crosses between *A. hypogaea* and ISATGR 278-18 gave rise to 1–2 univalents and 17–20 bivalents (Fig. 1, second row) and 0–2 trivalents. There were 20 bivalents in 60% of the PMCs and 19 bivalents in 15% of the PMCs in the cross. Although pollen fertility in the hybrids ranged between 71 and 99 per cent, in most of the plants it was 99%. Presence of 20 bivalents shows high degree of homology between the parental genomes. In tetrad analysis, micronuclei were not noticed in any PMCs.

Crosses between *A. hypogaea* and ISATGR 265-5 showed 1–3 univalents. Presence of 1–3 univalents shows that there were two chromosomes in one of the parent which did not show homology with any of the chromosomes of the other parent. ISATGR 265-5 was synthesized by combining *A. kempff mercadoi* and *A. hoehnei*. Neither of the species is putative genome donors of cultivated *A. hypogaea*. This was also reflected in the formation of 20 bivalents in 35% of the PMC (Table 1). Pollen fertility in the F_1 hybrids ranged between 60% and 76%, showing that there was recombination between the two parental species but non-homology in two chromosomes, resulted in decreased pollen fertility. One micronucleus was observed in 2% of the tetrads, and two micronuclei were also observed (Fig. 1, third row).

Bivalents in the crosses between *A. hypogaea* and ISATGR 268-5 ranged between 16 and 20 (Table 1; Fig. 1, fourth row). Similarly, the number of univalents ranged from 0 to 4. Presence of three univalents shows that in one parent, there were two chromosomes without partners. This is not surprising as ISATGR 268-5 was synthesized utilizing *A. batizocoi* and *A. cardenasii*. Neither of the species is putative genome donor of *Arachis hypogaea*. Pollen fertility ranged between 65% and 74%, showing recombination between the parental genomes. One micronucleus was observed in 3% of the tetrads.

Arachis duranensis and *A. cardenasii*, two A genome species, were used to synthesize ISATGR 72B. *Arachis duranensis* is a putative genome donor of cultivated *A. hypogaea*. Twenty bivalents were seen in 20% of the PMCs, the lowest compared to the other four hybrids. Nineteen bivalents were seen in 40% of the PMCs (Table 1). Pollen fertility in the hybrids ranged between 70% and 78%, showing good recombination between parental genomes. One to two micronuclei were observed in 4% of the PMCs.

Mature pod formation in F_1 plants varied from 13% to 57% in all cross-combinations put together, and there were no significant differences between different cross-combinations. Pod formation was more when cultivated *A. hypogaea* was crossed with ISATGR 1212 (55–65%) compared to other cross-combinations. A similar trend in pod set was observed in

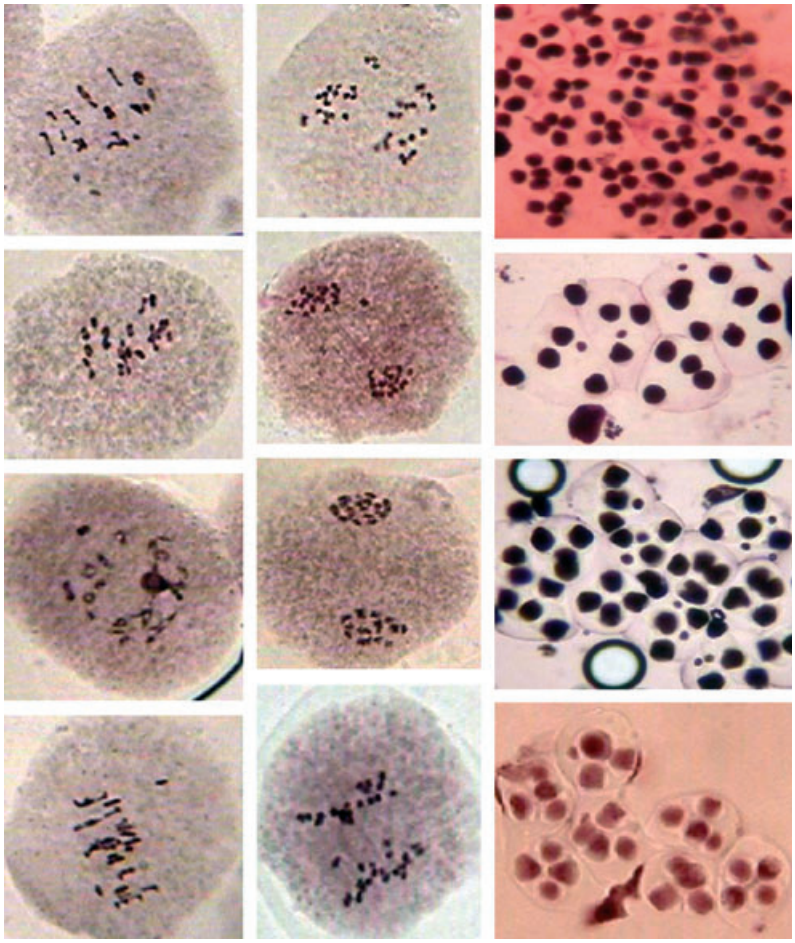

Fig. 1: Meiotic analysis of F_1 hybrids between cultivated *A. hypogaea* and new sources of *A. hygogaea*. First row *A. hypogaea* × ISATGR 1212: left-metaphase; center-anaphase; right-tetrads. Second row *A. hypogaea* × ISATGR 278-18: left-metaphases; center-anaphase; right-tetrads. Third row *A. hypogaea* × ISATGR 265-5A: left-metaphase; center-anaphase; right-tetrads. Fourth row *A. hypogaea* × ISATGR 268-5: left-metaphase; center-anaphase; right-tetrads

BC_1 plants. F_1 pod set in other crosses ranged from 3% to 44%, which was lower in BC_I crosses, but the maximum of 44% was observed in *A. hypogaea* × ISATGR 1212.

Discussion

Utilization of diploid *Arachis* species directly in the crossing programme invariably delayed the introgression of traits owing to ploidy differences (Mallikarjuna et al., 2004). F_1 pod formation between diploid *Arachis* species and tetraploid cultivated groundnut did not exceed 10–15%. As F_1 hybrids were triploids, a detailed backcross programme was essential to obtain tetraploid progeny (Simpson 2001). A detailed backcross programme would be time- and labour-intensive with a chance to lose the trait of interest. Utilization of amphidiploids and tetraploids has curtailed the necessity for detailed and multiple backcrosses as tetraploids were obtained in the F_1 generation itself; nevertheless, 1–2 backcrosses are necessary to recover progeny resembling cultivated groundnut (N. Mallikarjuna, unpublished results). Company et al. (1982) reported 4–12 univalents in the crosses between *A. hypogaea* × *Arachis* species (2n = 20) triploids. Simpson and Davies (1983) reported 8–14 univalents in triploid hybrids. Univalents are usually lost during meiosis, which means loss of traits/genetic diversity. Presence of large number of univalents reduces pollen fertility of the hybrid plant. In the present investigation, there were not more than three univalents in the tetraploid hybrids. This may be responsible for highly fertile F_1 hybrids. Double-seeded pods were observed even after one backcross or three selfed generations. To obtain such seeds utilizing diploid *Arachis* species, at least four to five backcrosses were essential (N. Mallikarjuna, unpublished results).

Observation of higher number of bivalent formation, which in turn reflects recombination between the parental species, ensures that traits present in *Arachis* species can be recovered in *A. hypogaea*. This was confirmed with high pollen fertility in the hybrids between the parental genomes and by the formation of 18–20 bivalents.

Micronuclei were observed in many of the hybrids, and it can be presumed that univalents contribute to the formation of micronuclei but it is not known how many univalents are necessary to form a micronuclei. Formation of micronuclei shows that a few chromosomes are not transferred to the progeny. These chromosomes are lost by the time pollen grains are formed. As micronuclei were present in a small number of tetrads (in 2% of the tetrads in the cross *A. hypogaea* × ISATGR 265-5), it may not be a matter of concern in the crosses utilizing synthetic tetraploids. Although univalents were noticed in the cross *A. hypogaea* × ISATGR 278-18, micronuclei were not noticed in the tetrad stage. This means either the micronuclei later moved to one of the poles or they were lost in metaphase and did not reach the pole. Either way, micronuclei are not desirable as they are lost in meiosis and do not form viable pollen grains.

New sources of tetraploid groundnut were generated to circumvent the ploidy differences between cultivated groundnut and *Arachis* species. Presence of high number of bivalents and high pollen fertility between cultivated *A. hypogaea* and

ISATGR 1212 showed that the genomes of two have major regions of similarity and with more homologous regions compared with other three new sources of *A. hypogaea* used in the crossing programme.

Recombination between the other four new sources of tetraploid groundnut (ISATGR 265-5, ISATGR 268-5, ISATGR 278-18 and ISATGR 72B) and cultivated groundnut was also high, owing to high bivalent formation. This shows that synthetic tetraploid groundnut can be successfully used to broaden the genetic base of cultivated groundnut with the introduction of useful traits.

As *A. duranensis* and *A. ipaensis* are putative genome donors of cultivated groundnut, they are closely related to *A. hypogaea* than any other *Arachis* species. *Arachis batizocoi* is the B genome donor of ISATGR 278-18 and is genetically different than *A. ipaensis*. This distant relationship was reflected in lesser number of bivalent formation with *A. hypogaea*. *Arachis kempff mercadoi* (A genome donor of ISATGR 265-5) is not closely related to either *A. duranensis* or *A. hypogaea* (N. Mallikarjuna, unpublished data), and *A. hoehnei* (B genome donor of ISATGR 265-5) is not closely related to *A. ipaensis* (N. Mallikarjuna, unpublished data); this was reflected in lower bivalent formation with *A. hypogaea*, thus showing that the species are not closely related. Although *A. duranensis* is the A genome donor of *A. hypogaea* and hence closely related to it than many other A genome species, ISATGR 72B did not form as large number of bivalents with *A. hypogaea* as the other genome donor was *A. cardenasii*, an A genome species and distantly related to both *A. duranensis* and *A. hypogaea* (N. Mallikarjuna, unpublished data). Moreover, a synthetic amphidiploid with AABB genome has the same genome combination as *A. hypogaea* than an autotetraploid with AAAA genome. Based on the studies on bivalent formation with *A. hypogaea*, it can be concluded that *A. duranensis*, *A. ipaensis* and *A. batizocoi* have closer relationship with *A. hypogaea* than *A. kempff mercadoi*, *A. cardenasii* and *A. hoehnei*.

The present paper summarizes the utilization of five new sources of tetraploid groundnut synthesized utilizing diploid *Arachis* species. There are twenty more new sources of tetraploid groundnut at ICRISAT meant for sharing with interested groundnut improvement scientists and their utilization in the crossing programme to broaden the genetic base of groundnut. There are only two new sources of synthetic tetraploid groundnut in public domain, which were used to broaden the genetic base of groundnut. The first synthetic groundnut originated from a cross between *A. cardenasii*, *A. diogoi* and *A. batizocoi*, and it was utilized to develop backcross progeny (Simpson et al. 1993). Two groundnut cultivars namely 'Coan' (Simpson and Starr 2001) and 'NemaTAM' (Simpson et al. 2003) were released utilizing this source. More recently, an amphidiploid was constructed utilizing *A. ipaensis* and *A. duranensis* (Fávero et al. 2006). This amphidiploid is being used by ICRISAT, Brazil and Senegal to develop backcross population. Preliminary mapping data indicated low level of marker segregation distortion in F_2 population utilizing this amphidiploid (Dwivedi et al. 2008).

Majority of the closely related wild *Arachis* species are diploids. *Arachis* species in other seven sections (except in section *Rhizomatosae*) are distantly related and are diploids too. Cultivated groundnut is tetraploid. The only effective way how they can be utilized for the improvement in cultivated groundnut is by the development of tetraploids through either amphidiploidy or autotetraploidy. Hence, the development of new sources of tetraploid groundnut will generate renewed interest to utilize *Arachis* species for accelerated introgression/transfer of useful traits and broaden the genetic base.

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