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RESEARCH ARTICLE

Development of new sources of tetraploid *Arachis* to broaden the genetic base of cultivated groundnut (*Arachis hypogaea* L.)

Nalini Mallikarjuna · S. Senthilvel · David Hoisington

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Abstract Groundnut, an important crop of many countries of the world, is susceptible to a range of diseases and pests. High levels of resistances are not available in the cultivated gene pool as the crop is said to have a narrow genetic base. Narrow genetic base is attributed to the evolution of the crop which took place by the combination of A and B genome species, and later doubling their chromosome number, giving rise to tetraploid cultivated groundnut. Direct utilization of cross-compatible wild relatives, which are diploids, to broaden the genetic base and introduction of useful traits, is not a straight-forward process due to ploidy differences between the cultivated species and wild relatives. Hence amphiploids and autotetraploids were created by not only combining the putative genomes, but many other A and B genome species, thus producing a highly variable population of tetraploid groundnuts also called new sources of *Arachis hypogaea*. This study describes the development and characterization of newly generated tetraploid groundnuts and the level of molecular diversity as assessed by DArT markers.

Keywords *Arachis* · Amphiploids · Autotetraploids · A and B genome species · Groundnut · Peanut · SSR markers · Tetraploid

N. Mallikarjuna (✉) · S. Senthilvel · D. Hoisington
International Crops Research Institute for Semi Arid Tropics, Patancheru 502 324, Andhra Pradesh, India
e-mail: N.Mallikarjuna@CGIAR.ORG

Introduction

Groundnut (*Arachis hypogaea* L.) is one of the most 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, early leaf spot, 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 is lacking in the cultivated species. The lack of resistances in the cultivated germplasm is attributed to its narrow genetic base (Kochert et al. 1991). Various bottlenecks have narrowed the genetic base, such as the origin of the crop. Tetraploid groundnut originated from one to few hybridization events, thus narrowing the genetic base. There is evidence regarding the A genome species as *A. duranensis* Krapov. et W.C. Gregory (Kochert et al. 1991, 1996; Seijo et al. 2007) and B genome species as *A. ipaensis* Krapov. et W.C. Gregory (Kochert et al. 1991, 1996) which gave rise to tetraploid groundnut. This is again a bottleneck as only two founder species gave rise to the crop. It is known in literature that polyploidy causes genetic bottlenecks (Sanford 1983),

the change from diploidy to polyploidy has been another main cause of the narrow genetic base of groundnut. The crop originated in South America and it was not until the beginning of fifteenth century that groundnut spread to other countries, and became an important crop in the rest of the world. A few tetraploid plants might have played a major role in spreading the crop to the rest of the world, thus paving way for the narrow origin. The self pollinating nature of the crop could be another major reason for the narrow genetic base.

Groundnut improvement can take place if the genetic base is broadened. Various studies have shown that closely related wild relatives of crop plants are diverse with many traits necessary for the improvement of cultivated species. It is an underexploited sustainable resource, with a potential to enrich the genetic base of cultivated plants with novel alleles, thus improving productivity and adaptation (McCouch 2004; Tanksley and Fulton 2007). Studies so far have clearly demonstrated that certain genomic regions derived from wild relatives improved yield in tomato, barley and rice (Gur and Zamir 2004; Fernie et al. 2006; Nevo and Chen 2010; Fu et al. 2010). Wild *Arachis* species exhibit many of the traits related to disease, pest, insect resistance and drought tolerance, hence the approach of creating tetraploid groundnuts and crossing them with cultivated groundnut would unravel variation for many useful traits not observed in the parental species. Such a phenomenon has been observed in wheat and rice wide crosses (Hoisington et al. 1999; McCouch et al. 2005).

Tremendous progress has been made in wheat and brassica, two similar polyploid genera in the development and utilization of synthetics, by combining the putative genome donors of the cultivated species. This triggered the development of tetraploids/amphidiploids (synthetics) in groundnut. There are three sources of amphiploids or new sources of *A. hypogaea* available in public domain. The first one originated from a cross between *A. cardenasii* Krapov. et W.C. Gregory, *A. diogoi* Hoehne and *A. batizocoi* Krapov. et W.C. Gregory and it was utilized to develop backcross progeny (Simpson et al. 1993). Two groundnut cultivars namely Coan and NemaTAM 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 Brazil and Senegal to develop backcross population. Preliminary mapping data indicated low level of marker segregation distortion in F_2 population utilizing amphidiploid (Dwivedi et al. 2008). The third is cross between *A. gregoryi* and *A. linearifolium* (GCP 2005).

Cultivated amphidiploid peanut is presumed to be of recent origin, about 3,500 years old (Singh and Simpson 1994). The genus *Arachis* is divided into nine sections based on morphology and crossability studies (Krapovickas and Gregory 1994). Species in section *Arachis* have A, B and D genomes (Stalker 1991). The lone D genome species exhibits unstable meiosis. Wild species in the other nine sections have been assigned different genome designations (Stalker 1991). Genome relationships between the section *Arachis* and the other eight sections will become clearer once the hybrids between the sections are available and detailed molecular analysis is conducted, as of now, they all come under incompatible gene pool.

Considering the potential uses of new sources of tetraploid groundnuts, ICRISAT has developed a set of amphiploid and autotetraploid groundnuts. A number of *Arachis* species with A and B genome were used in the crossing program and diploid hybrids were created. Diploid hybrids were treated with colchicine to develop tetraploid plants, in diploid hybrids with 2n pollen, they were self pollinated to obtain tetraploid plants. These amphiploids and autotetraploids developed are in fact new sources of *A. hypogaea* or in other words synthetic *A. hypogaea*. As these have not been selected for any of the constraints, neither have they undergone the evolutionary or plant breeding selection, they would contain broad based variation with broader genetic base. The 17 new sources of tetraploid groundnuts developed at ICRISAT are being reported.

Materials and methods

Diploid *Arachis* species with A genome namely *A. duranensis*, *A. cardenasii*, *A. diogoi*, *A. kempff-mercadoi* Krapov. W.C. Gregory et C.E. Simpson and *A. stenosperma* Krapov. et W.C. Gregory and B genome species *A. batozocoi*, *A. valida* Krapov. et W.C. Gregory, *A. magna* Krapov. W.C. Gregory et C.E. Simpson and *A. ipaensis* were grown in a

glasshouse. Emasculations, pollinations and growth regulator treatments were as described in Mallikarjuna and Sastri (2002). Although wild relatives in section *Arachis* are cross compatible, application of growth regulators increased the number of pegs and pods formed. Seed set also varied according to the male and female species used in the crossing program. There were a few combinations where seed set was either not observed or was better than the combination of the putative genome donor species.

Diploid hybrids were analyzed for their hybridity using simple sequence repeat (SSR) markers. Total DNA was extracted from fresh leaf tissues of individual plants by CTAB method. The extracted DNA was dissolved in TE (Tris–HCl 10 mM and EDTA 1 mM) and quantified on 0.8% (w/v) agarose gels with a known concentration of DNA as standards.

For SSR analysis, PCR amplification of microsatellite loci using 14 fluorescent-dye-labeled primer pairs was carried out in 15 µl volume. The reaction mixture contained 10 mM Tris–HCl, 50 mM KCl, 10 ng of genomic DNA, 2–4 mM $MgCl_2$, 300–400 µM of dNTP, 1 unit of Taq DNA polymerase. Amplified products were size-separated on an ABI 3700 DNA analyzer.

Diploid hybrids were grown in a glasshouse and the apical shoot buds were treated with 0.25% colchicine to double their chromosome number. Colchicine treatment was given for two consecutive days; the apical shoot buds were washed with sterilized distilled water and allowed to grow under high humidity (>60–65%). Care was taken to allow only the apical bud to grow. Diploid hybrids with 2n pollen were identified through tetrad analysis. They were self pollinated to encourage pod set. Pods larger in size than the pods in the diploid hybrid were selected.

Tetraploid F_1 hybrids, the amphidiploids and autotetraploids, were confirmed by chromosome counting. To count the number of chromosomes in tetraploid plants, flower buds were fixed in carnoy's fixative and stained 2.0% acetocarmine. Chromosome counts were made from well-spread preparations. Pollen stainability with 2.0% acetocarmine was calculated by counting well stained pollen grains as fertile grains and partially stained or grains devoid of stain were counted as sterile. The details of the procedure are as given in the paper by Mallikarjuna and Sastri (2002).

Diversity between cultivated *A. hypogaea* and newly created tetraploid *A. hypogaea* was analysed using DArT markers. DArT was performed essentially as reported by Jaccoud et al. (2001) and Wenzl et al. (2004) and is briefly described below.

A genomic representation was generated from a mixture of genomic DNA from six groundnut genotypes viz., ICGS 44, ICGS 76, CSHG 84-1, TAG 24, ICGV 86031 and GPBD 4, which are parental lines of mapping populations available at ICRISAT, using the *Pst*I-based complexity reduction method as described by Wenzl et al. (2004). The procedure involved digestion of approximately 100 ng of DNA with 1.4U of rare cutter enzyme *Pst*I and frequent cutter *Hpa*II, ligation of a *Pst*I adapter with T4 DNA ligase (NEB) and amplification of small adapter-l ligated fragments. For amplification, 1 µl aliquot of the ligation product was used as a template in 50 µl amplification reaction with *Pst*I adapter specific primer and a PCR program: 94°C for 4 min, followed by 30 cycles of 94°C for 20 s, 58°C for 40 s, 72°C for 1 min, and 72°C for 7 min.

The amplicons from representations were ligated into the PCR2.1-TOPO vector using the TOPO cloning kit and transformed into electro-competent *E. coli* strain TOP 10 according to the manufacturer's instructions. Transformants were selected on medium containing ampicillin and X-gal. Individual white colonies (containing recombinant plasmids) were picked by toothpicks and grown in 384-well plates containing LB medium supplemented with 100 mg/ml ampicillin and a freezing mix and thus a library of 5,760 clones was developed. The inserts were amplified in a 15 µl reaction containing 0.2 pm each of forward and reverse M13 primers and 0.3 U of Taq polymerase using program: 95°C for 3 min, 57°C for 35 s, 72°C for 1 min, followed by 40 cycles of 94°C for 35 s, 52°C for 35 s, 72°C for 1 min. After amplification, the PCR products were precipitated with 1 vol of isopropanol at room temperature and washed once with 100 µl of 77% ethanol. Ethanol was removed and the products were dried as per Jaccoud et al. 2001. The DNA was re-suspended in a spotting buffer. The fragments were arrayed with two replicates per fragment onto polylysine-coated slides using a microarrayer. After printing, slides were left at room temperature for at least 1 day and then processed by keeping in boiling water at 92°C for 2 min and dried by centrifugation.

Table 1 Details of diploid and tetraploid ISATGR newly synthesized groundnuts

S. no	ICRISAT symbol	Origin	Cross	Pollen fertility (%)—2n	Pollen fertility (%)—4n
1	ISATGR 1212	Synthetic amphidiploid	*A. duranenesis* ICG 8123 × *A. ipaensis* ICG 8206	0.9	90
2	ISATGR 1	Synthetic autotetraploid	*A. magna* ICG 8960 × *A. valida* ICG 13230	55	85
3	ISATGR 5B	Synthetic autotetraploid	*A. magna* × *A. batizocoi* ICG 8209	2	90
4	ISATGR 9A	Synthetic amphidiploid	*A. batizocoi* ICG 8124 × *A. cardenasii* ICG 8216	0.3	91
5	ISATGR 11A	Synthetic autotetraploid	*A. magna* ICG 8960 × *A. valida*, ICG 13230	53	88
6	ISATGR 40A	Synthetic amphidiploid	*A. ipaensis* ICG 8206 × *A. duranensis* ICG 8123	6	94
7	ISATGR 90B	Synthetic autotetraploid	*A. kempff-mercadoi* ICG 8959 × *A. stenosperma* ICG 15160	43	88
8	ISATGR 99B	Autotetraploid	*A. diogoi* ICG 4983 × *A. cardenasii* ICG 8216	71	98
9	ISATGR 168B	Synthetic amphidiploid	*A. valida* ICG 11548 × *A. duranensis* ICG 8123	13	80
10	ISATGR 278-18	Synthetic amphidiploid	*A. duranensis* ICG 8138 × *A. batizocoi* ICG 13160	0.4	95
11	ISATGR 265-5	Synthetic amphidiploid	*A. kempff-mercadoi* ICG 8164 × *A. hoehnei* ICG 8190	14	89
12	ISATGR 160	Synthetic autotetraploid	*A. diogoi* ICG 4983 × *A. cardenasii* ICG 8216	72	90
13	ISATGR 268-5	Synthetic amphidiploid	*A. batizocoi* ICG 8124 × *A. cardenasii* ICG 8216	24	86
14	ISATGR 10B	Synthetic autotetraploid	*A. magna* ICG 8960 × *A. valida* ICG 13230	30	79
15	ISATGR 35A	Synthetic amphidiploid	*A. batizocoi* × *A. duranensis* ICG 8138	0.3	97
16	ISATGR 80A	Synthetic amphidiploid	*A. kempff-mercadoi* ICG 8959 × *A. hoehnei* ICG 8190	23	NF[2]
17	ISATGR 206	Synthetic amphidiploid	*A. duranensis* ICG 8123 × *A. valida* ICG 11548	56	96
19	DH 86	Cultivated	Cultivated	–	84
19	ICGV 91114	Cultivated	Cultivated	–	88
20	ICGS 44	Cultivated	Cultivated	–	87
21	TMV2	Cultivated	Cultivated	–	83

Genomic representations were generated from 21 selected genotypes (Table 1) using the same complexity reduction method used for library construction (*Pst*I/*Hpa*II). Representations were precipitated with one volume of isopropanol, denatured at 95°C for 3 min and labelled using 0.1 µl of cy3/cy5—dUTP and the exo-Klenow fragment of *E.coli* DNA polymerase I. Labelled representations, also called targets, were mixed with the 6-FAM labelled polylinker fragment of the plasmid used for library construction and hybridized to slides as per Wenzl et al. (2004). After overnight hybridization at 65°C, the slides were washed in 1 × SSC + 0.1% SDS for 5 min; in 1 × SSC for 5 min; in 0.2 × SSC for 2 min and in 0.02 × SSC for 30 s. Slides were centrifuged and dried in vacuum desiccators. Slides were scanned using a fluorescent microarray scanner (Tecan LS300 scanner) and images were generated

for each of the fluorescent dyes using the appropriate laser/filter combination. DArTsoft, a software package developed by DArT P/L, Canberra, Australia was used to automatically analyze each batch of TIF image pairs generated in an experiment. The software localized spots, rejected those with a weak reference signal, computed and normalized the relative hybridization intensities [= log (cy3target/cy5 reference) or log (cy3target/FAM reference)], calculated the median value for replicate spots, identified polymorphic clones by using a combination of ANOVA and fuzzy K-means clustering at a fuzziness level of 1.5 and classified polymorphic clones as being present ("1") or absent ("0") in the representation hybridized to a slide.

The presence (1) and absence (0) scores were used to generate Jaccard dissimilarity matrix and a dendrogram based on Un-weighted Pair Group Method with Algorithmic Mean (UPGMA) was constructed using DARWin. The Jaccard distance matrix was also used as input for Principal Coordinate Analysis (PCO).

Results

Diploid hybrids were generated as a result of crossing different A and B genome species. Both inter and intra genomic hybrids were generated, which are presented in Table 1. Morphologically all the hybrids had trailing growth habit as seen in the diploid wild *Arachis* species, except for the hybrids between two B genome species *A. magna* and *A. valida* (ISATGR 1A; ISATGR 11A). These F_1 hybrids had erect growth habit (Fig. 1e). Many of the cultivated *A. hypogaea* have erect growth habit. Hybrids between *A. magna* and *A. duranensis* or vice versa had small bushy growth habit, which was not seen in any other combinations used in the present investigation (Fig. 2e, f). Diploid F_1 hybrid plant between *A. batizocoi* × *A. cardenasii* (ISATGR 9A) flowered for a short period of time and the number of flowers produced was also low. In the hybrid between *A. kempff-mercadoi* and *A. hoennei* (ISATGR 265-5), flowers were of three colors: orange, yellow and pale white (Fig. 2a, b). The color of the flower was not restricted to any particular branch. In yet another F_1 plant from the same cross *A. kempff-mercadoi* × *A. hoehnei*, flowers were abnormal and clasmogamous (Fig. 2c). The tetraploid (ISATGR 265-5) of the diploid hybrid from the cross *A. kempff-mercadoi* × *A. hoehnei* did not produce multi-colored flowers. The F_1 hybrid (2×) between *A. duranensis* ICG 8138 and *A. cardenasii* ICG 8216 also produced orange and yellow colored flowers and here too the color of the flower was not restricted to any particular branch. The F_1 hybrid between *A. trinitensis* and *A. hoehnei* did not reach reproductive stage and did not set any flowers neither did the tetraploids generated from the diploid plants set flowers; both 2× and 4× hybrids are being maintained by vegetative propagation. A form of somaclonal variation was observed in the diploid hybrid plants raised through cuttings, between *A. batizocoi* ICG 8124 × *A. cardenasii* ICG 8216 (ISATGR 9A). Six cuttings were taken from the hybrid plant and were treated with colchicine. Amongst them, two had doubled chromosome number and only one flowered and set seeds. The seeds were germinated to raise F_2 plants. In the 6 F_2 plants obtained, only two reached the flowering stage, the remaining four did not flower in spite of profuse vegetative growth.

Although ISATGR 1A, ISATGR 10B and ISATGR 11A have the same parental combination of two B genome species, they were generated from different F_1 hybrid seeds. Both the diploid F_1 hybrid and the tetraploid derivatives had crinkled leaves. The diploid F_1 hybrid plants had erect growth habit, gave rise to large number of flowers, which were tripped and hormone aided, formed a large number of pegs. None of the pegs formed pods in the diploid hybrid (Fig. 1e) which gave rise to tetraploid ISATGR 1A (Fig. 1f), whereas the diploid hybrid which gave rise to ISATGR 11A produced a few pods. Both the tetraploid derivatives produced less number of flowers, they were self-pollinated and hormone aided, as a result pods were obtained (Figs. 3b).

Pollen fertility in the diploid hybrids were less than 1% in ISATGR 9A, ISATGR 35A, ISATGR 278-18, and ISATGR 1212, about 5–25% in ISATGR 168B, ISATGR 40A, ISATGR 80A, ISATGR 268-5 and more than 40% in ISATGR 1A and ISATGR 90B (Table 1). Highly fertile diploid hybrid was an intra-genomic hybrid between *A. diogoi* and *A. cardenasii* (ISATGR 160 and ISATGR 99B). Most of the diploid hybrids between A and B genome species had low pollen fertility (Table 1), except for intra-genomic hybrids within A and B genome, whose pollen

Fig. 1 Diploid hybrids and newly synthesized tetraploid *A. hypogaea.* **a** Diploid ISATGR-5B; **b** Tetraploid ISATGR-5B; **c** Diploid ISATRGR-9A; **d** Tetraploid ISATGR-9A; **e** Diploid ISATGR-1; **f** Tetraploid ISATGR-1A; **g** Diploid ISATGR-40A; **h** Tetraploid ISATGR-40A; **i** Diploid ISATGR-160; **j** Tetraploid ISATGR-160; **k** Tetraploid ISATGR 80A; **l** Diploid ISATGR-35A; **m** Tetraploid ISATGR-35A; **n** Diploid ISATGR 90B; **o** Tetraploid ISATGR 90B; **p** Tetraploid ISATR 265-5; **q** Tetraploid ISATR 268-5; **r** Tetraploid ISATR 1212; **s** Diploid ISATGR 168B; **t** Tetraploid ISATGR 168B; **u** Tetraploid ISATR 278-18

fertility was above 25%, but not as high as in the tetraploids. The only inter-genomic diploid hybrid with higher pollen fertility was ISAT GR 206 (*A. duranensis* × *A. valida*). Chromosome doubled tetraploids, irrespective of the genome combination had high pollen fertility (Table 1).

Brief descriptions of synthetic *A. hypogaea*

ISATGR 1212

It is an amphidiploid between *A. duranensis* and *A. ipaensis*. These species are the putative A and B

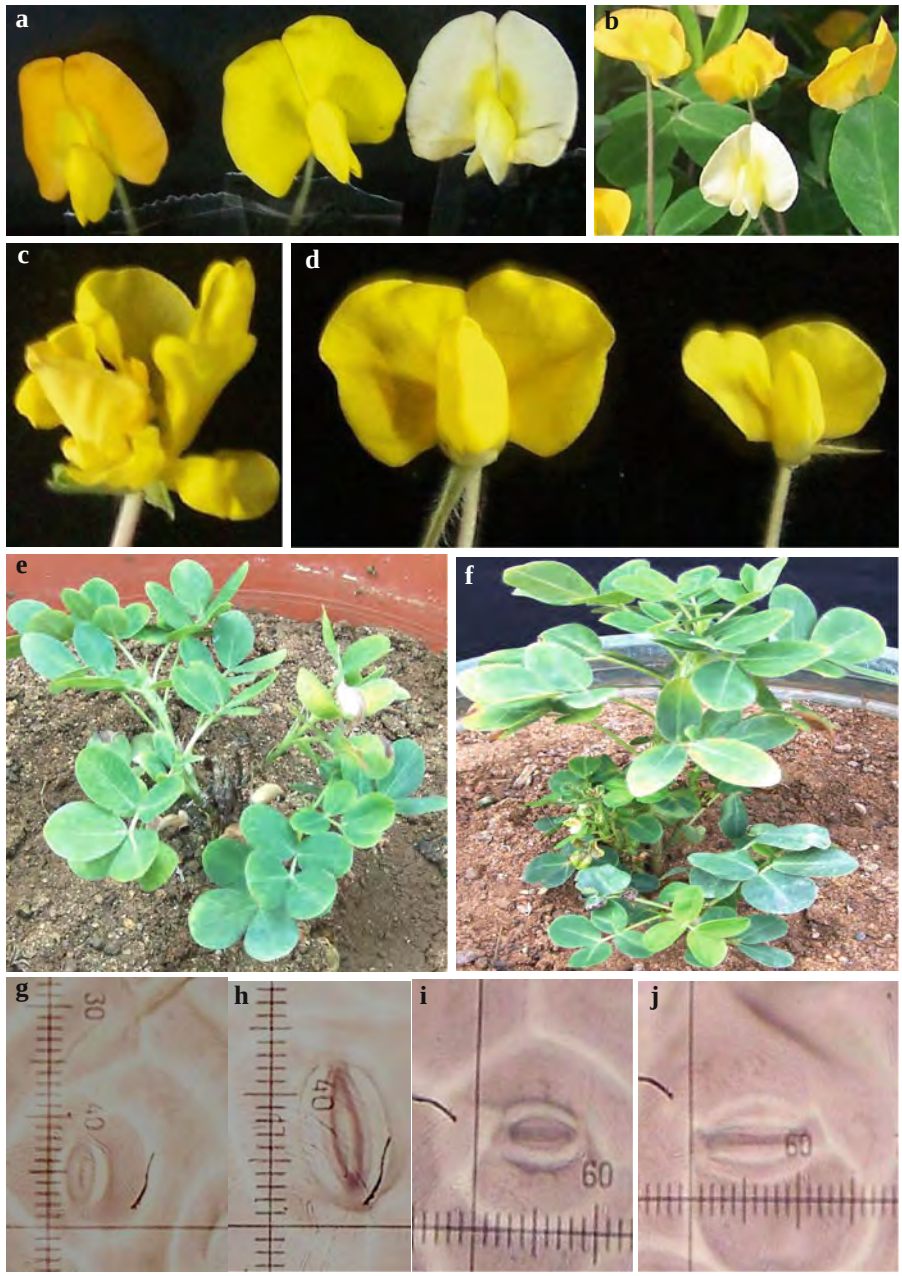

Fig. 2 Morphological characters of some hybrids between *Arachis* species. **a** ISATGR 265-7 showing *orange*, *yellow* and *pale white flowers*; **b** ISATGR 265-5 showing *yellow* and *white flowers*; **c** ISATGR 265-4 showing two keeled, *open* and *abnormal flower*; **d** *right*: tetraploid flower of ISATGR 5B; *left*: diploid flower of ISATGR 5B; **e**, **f** Short statured plant of ISATGR 407 and ISATGR 408; **g**, **h** Stomatal sizes of diploid (**g**) and tetraploid ISATGR 5B (**h**); **i**, **j** Comparision of stomatal sizes of diplopid ISATGR 9A and tetraploid ISATGR 9A

genome donors of cultivated groundnut *A. hypogaea*. Pollen fertility in the diploid hybrid was less than 1%, and it increased to 90% after doubling its chromosome number (Table 1). Both the diploid and tetraploid hybrids had trailing growth habit (Fig. 1r). Flower color was orange in both. The diploid hybrid set very few pods only after the tripping the flowers with application of growth regulators to the pollinated

Fig. 3 Pods of some new sources of *A. hypogaea* developed at ICRISAT. **a** Pods of ISAT GR 278-18; **b** Pods of ISAT GR1; **c** Pods of ISAT GR206B; **d** Pods of ISAT GR265-5; **e** Pods of ISAT GR160; **f** pods of ISAT GR40A; **g** Pods from diploid ISAT GR5B (*left*) and pods from tetraploid ISAT GR5B; **h** Pods of ISAT GR90B

pistils. The tetraploid plant set pegs and seeds through natural hybridization. It was not necessary to apply growth regulators to induce peg and pod formation. At present a large number of tetraploid seeds are available. According to Jaccard dissimilarity index (Table 2), ISATGR 1212 showed closer relationship with ISATGR 278-18 and ISATGR 268-5 and was distant from ISATGR 40A, ISATGR 90B, ISATGR 206 and cultivated *A. hypogaea* (Fig. 5).

ISATGR 1A

This is an autotetraploid between two B genome species *A. magna* and *A. valida* (Table 1). The plant had erect growth habit, unlike any of the hybrid combinations generated. Even the secondary branches were erect. Leaves were crinkled in both diploid and tetraploid plants (Fig. 1e, f). Flower was orange colored in both the plants. It was essential to apply growth regulators to obtain pegs. Although a large number of pegs were observed on the diploid hybrid, none of them set pods/seeds whereas the tetraploid plant set a few pods (Fig. 3b). The number of pegs to pod formation was 11%. Attempts are underway to obtain more number of pods/seeds from the tetraploid hybrid. ISATGR 1A showed a dissimilarity matrix value of 0.402–0.429 with cultivated groundnuts (Table 2). ISATGR 1 and ISATGR 40A showed a greater dissimilarity matrix value of 0.701, but showed closer relationship with the hybrids with the same parental combination (ISATGR 10B and ISATGR 11A) (Fig. 5), and showed a dissimilarity matrix value of greater than 0.3 with all other tetraploid groundnuts.

ISATGR 9A

An amphidiploid between *A. batizocoi* ICG 8124 × *A. cardenasii* ICG 8216 (Fig. 1d) had 91% pollen fertility (Table 1). The diploid hybrid (Figs. 1c, 4k)

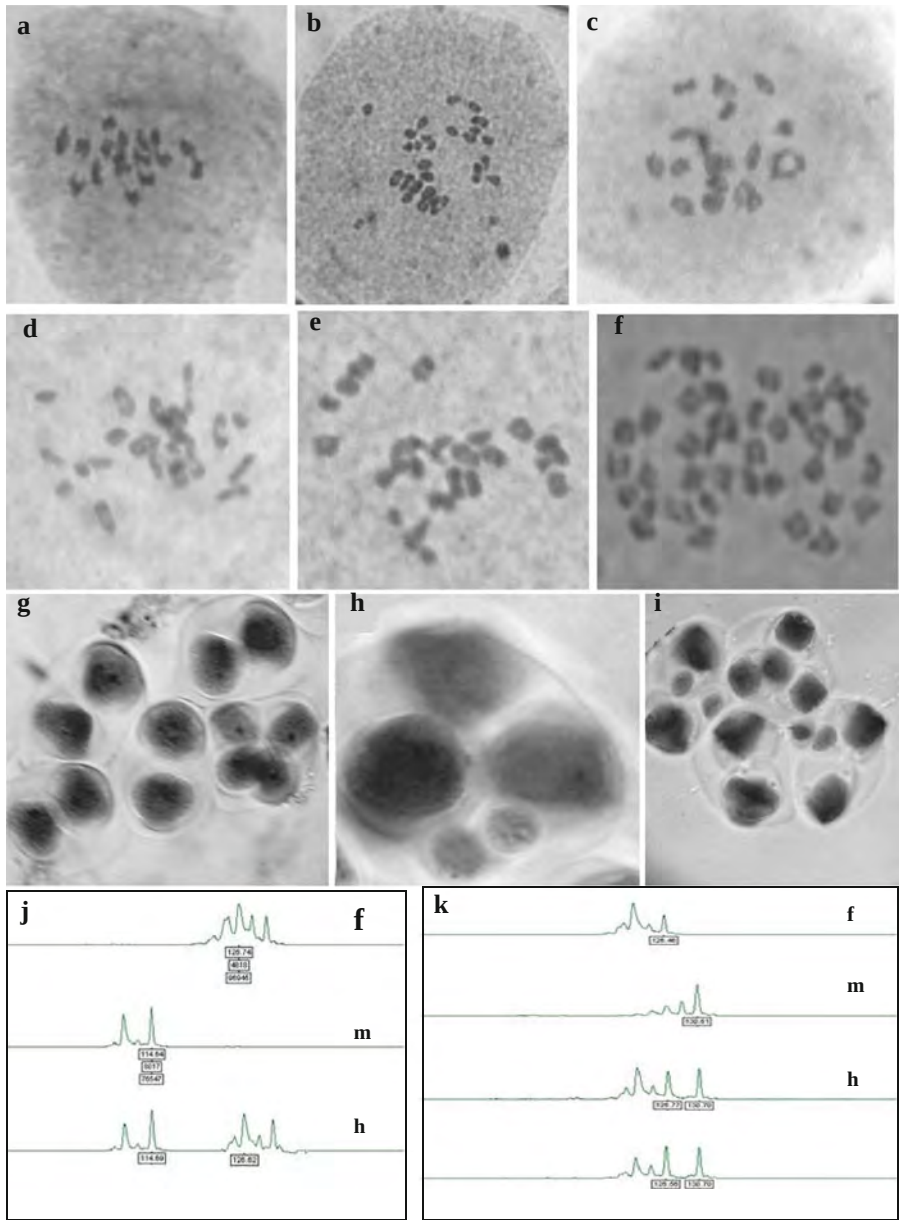

Fig. 4 Chromosome number and molecular profile of some tetraploid *A. hypogaea*. **a** Metaphase showing 20 chromosome of diploid ISATGR-5B; **b** Metaphase showing 40 chromosomes of tetraploid ISATGR 5B; **c** Metaphase of ISATGR 11A showing 40 chromosomes of ISATGR 11A; **d** metaphase of ISATGR 206B showing 40 chromosomes; **e** Metaphase of ISATGR 9A showing 40 chromosomes; **f** Anaphase of ISATGR 40A showing two groups of 20 chromosomes; **g** Diads seen in ISATGR 10; **h** Tetrads of ISATGR 278-18 with micronuclei; **i** Diads and some with micronuclei in diploid ISATGR 5B; **j** Snapshot of SSR analysis of ISAT GR40A: f = *A.* ipaensis, m = *A.* duranensis, h = ISAT GR40A; **k** Snapshot of SSR analysis of ISAT GR 9A: f = *A.* batizocoi, m = *A.* cardenasii, h = ISAT GR9A

set very few flowers and no seeds. The number of flowers in the tetraploid (Fig. 4e showing 2n = 40) was also low, and a few pods were obtained. The diploid plant had 0.3% fertile pollen grains. The flowers were tripped to encourage self pollination. All the pollinated pistils were treated with growth regulators to encourage pod formation. Six F_2 seedlings were raised from the tetraploid plant. Amongst the 6, two flowered and set pegs, rest of the four, in spite of profuse vegetative growth, did not flower. Efforts are underway to generate a large number of seeds. The tetraploid had the highest dissimilarity matrix value of 0.582 with ISATGR 90B, and with the rest of the tetraploids it varied from 0.320 to 0.521 (Table 2). In the UPGMA dendrogram (Fig. 5), all the cultivated genotypes clustered together while the tetraploids were spread around and ISAT GR 9A showed closer relationship with ISAT GR 11A, ISAT GR 99B and ISAT GR 160. Principal coordinate analysis (Fig. 6) did not show any of the tetraploid and cultivated groundnut showing closer relationship and the grouping of

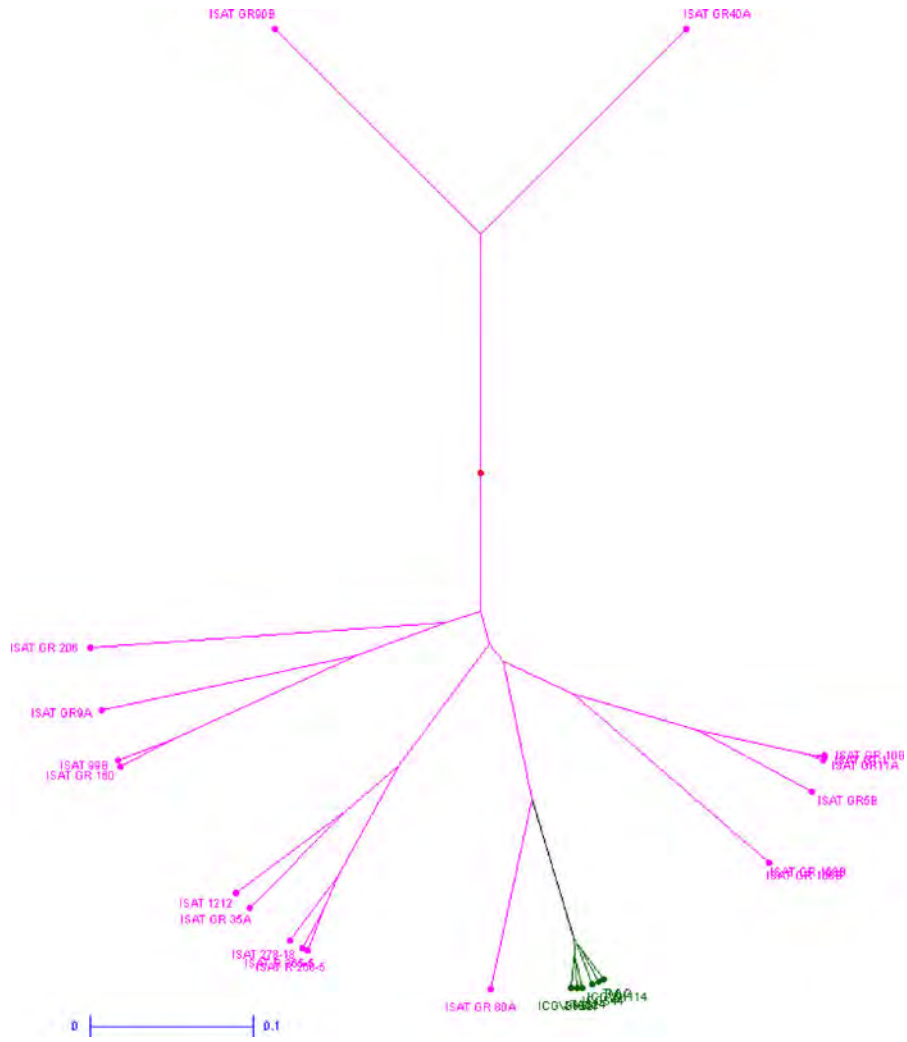

Fig. 5 UPGMA dendrogram of cultivated and newly synthesized tetraploid groundnuts based on Jaccard dissimilarity coefficient derived from DArT data

ISAT GR 9B with the other new sources of tetraploid *A. hypogaea*.

ISATGR 5B

It is an autotetraploid between diploid wild species *A. magna* ICG 8960 and *A. batizocoi* ICG 8209 (Fig. 1b). Although it is hybrid between two B genome species, pollen fertility was low. The diploid hybrid (Figs. 1a, 4a) had 2% pollen fertility. Flowers were small and yellow in color (Fig. 2d). Meiotic analysis of the F_1 diploid hybrid showed the formation of dyads in large numbers (45%). Dyads were the result of restitution nucleus in anaphase II. Here the wall formation between two anaphase bundles did not take place, thus giving rise to dyads with 2n chromosomes. Some of the dyads had microleucli (Fig. 4i). The flowers of the diploid F_1 hybrid were self-pollinated and growth regulator was applied to encourage the formation of pegs. The F_1 hybrid gave rise to tetraploid hybrid (Fig. 1b) which was confirmed by cytological analysis (Fig. 4b) and stomatal diameter studies (Fig. 2g, h). Pollen fertility in the tetraploid increased to 90%. The flowers were large, and retained the yellow color (Fig. 2d). Pod set was observed both in the diploid and tetraploid hybrids (Fig. 3g). ISATGR 5B showed a low dissimilarity matrix index value of 0.152–0.162 with ISATGR 1A, ISATGR 10B and ISATGR 11A, as all the three tetraploids share the same maternal *Arachis* species *A. magna*, showing a closer relationship between these tetraploids (Figs. 5, 6) but showed the largest

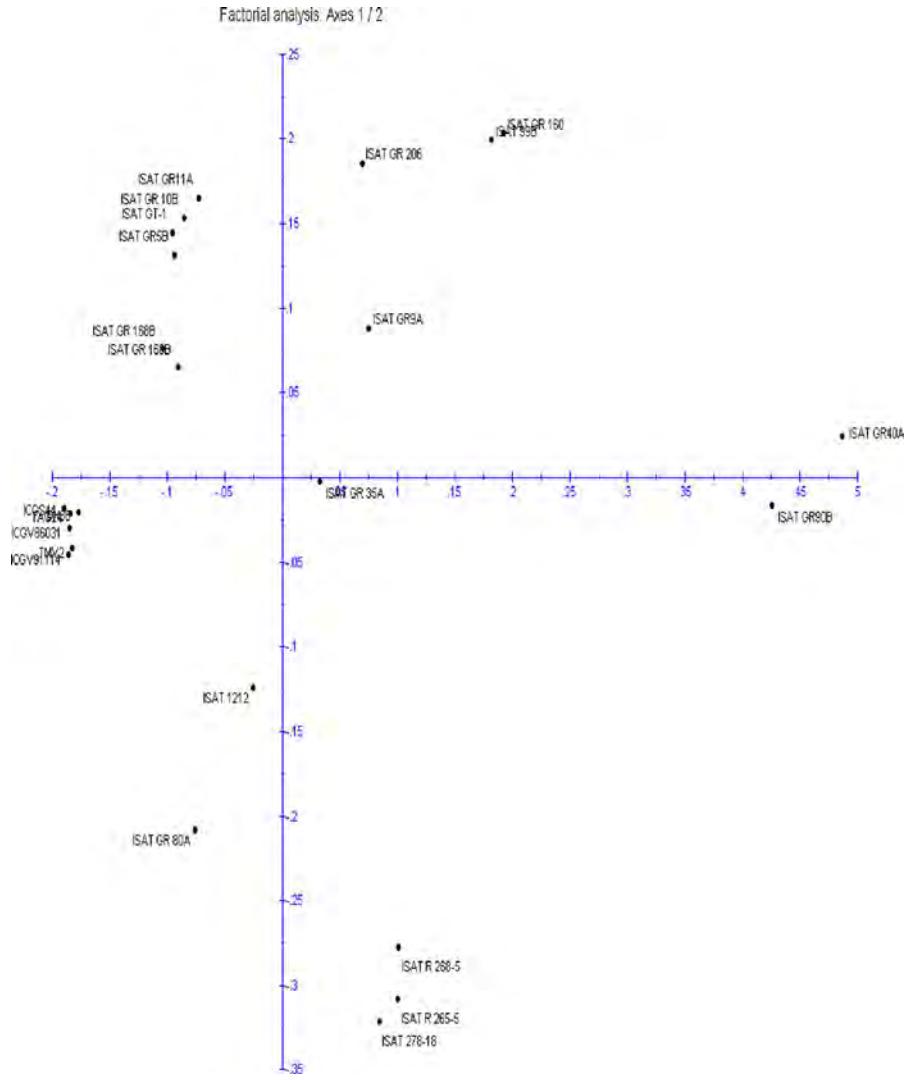

Fig. 6 Principal coordinates analysis of cultivated and newly synthesized tetraploid groundnuts based on Jaccard dissimilarity coefficient derived from DArT marker data

dissimilarity index value of 0.708 with ISATGR 40A, as both the tetraploids do not have any common parental *Arachis* species. Jaccard dissimilarity matrix index showed distant relationship between ISATGR 5B and cultivated *A. hypogaea* (0.405–0.446).

ISATGR 11A

This is an autotetraploid between *A. magna* and *A. valida* (Table 1). This is from a different F_1 diploid plant than the one which gave rise to ISATGR 1A. The diploid hybrid had erect growth habit, with crinkled leaves, as seen in ISATGT 1A. The colchicine treated tetraploid (Fig. 4c) was larger than the diploid hybrid. The color of the flower in both diploid and tetraploid was orange, but the size of the flower was larger in the tetraploid hybrid than in the diploid. The number of flowers was low both in diploid and tetraploid plant. Flowers were tripped and growth regulator was applied in the diploid hybrid to encourage peg and pod set. Peg to pod formation ratio was low (8%). The number of flowers set and the pegs formed was very low in the tetraploid plant too. Efforts are underway to trip every flower and apply growth regulators to encourage peg and pod formation in the tetraploid plant. As described under ISATGR 1A, the tetraploid showed closer relationship with other *A. magna* × *A. valida* hybrids (ISATGR 10B and ISATGR 1A; Fig. 5). Highest dissimilarity index value was observed for ISATGR

Table 2 Jacaard dissimilarity index among the cultivated and newly synthesized groundnuts

	ISAT 1212	ISAT GT-1	ISAT GR5B	ISAT GR9A	ISAT GR11A	ISAT GR40A	ISAT GR90B	ISAT 99B	ISAT GR 168B	ISAT 278-18	ISAT R 265-5	ISAT GR 160	ISAT R 268-5	ISAT GR 10B	ISAT GR 35A	ISAT GR 80A	ISAT GR 206	DH86	ICGV 91114	ICGS 44	TMV 2	TAG 24
ISAT GT-1	0.351																					
ISAT GR5B	0.329	0.152																				
ISAT GR9A	0.274	0.369	0.331																			
ISAT GR11A	0.364	0.022	0.162	0.363																		
ISAT GR40A	0.671	0.701	0.708	0.626	0.688																	
ISAT GR90B	0.616	0.668	0.679	0.582	0.654	0.357																
ISAT 99B	0.436	0.484	0.483	0.321	0.472	0.561	0.514															
ISAT GR168B	0.286	0.319	0.296	0.348	0.323	0.715	0.676	0.469														
ISAT 278-18	0.191	0.550	0.525	0.452	0.560	0.676	0.621	0.588	0.484													
ISAT R 265-5	0.175	0.540	0.525	0.449	0.553	0.656	0.601	0.573	0.476	0.082												
ISAT GR 160	0.439	0.491	0.482	0.323	0.479	0.572	0.514	0.069	0.465	0.594	0.570											
ISAT R 268-5	0.169	0.510	0.494	0.421	0.519	0.631	0.584	0.556	0.454	0.112	0.054	0.554										
ISAT GR 10B	0.361	0.013	0.162	0.363	0.020	0.696	0.664	0.472	0.318	0.550	0.543	0.481	0.514									
ISAT GR 35A	0.166	0.340	0.312	0.240	0.344	0.640	0.601	0.363	0.298	0.341	0.337	0.354	0.311	0.349								
ISAT GR 80A	0.360	0.461	0.475	0.521	0.477	0.640	0.565	0.563	0.445	0.383	0.372	0.579	0.361	0.462	0.450							
ISAT GR168B	0.279	0.327	0.298	0.357	0.329	0.698	0.664	0.467	0.002	0.477	0.469	0.465	0.441	0.329	0.302	0.440						
ISAT GR 206	0.502	0.574	0.557	0.491	0.569	0.653	0.644	0.406	0.425	0.650	0.626	0.412	0.608	0.570	0.457	0.626						
DH86	0.342	0.411	0.405	0.440	0.426	0.712	0.644	0.489	0.360	0.514	0.510	0.504	0.492	0.417	0.395	0.231	0.481					
ICGV91114	0.342	0.440	0.446	0.469	0.457	0.724	0.660	0.501	0.380	0.512	0.506	0.526	0.499	0.445	0.411	0.242	0.488	0.072				
ICGS44	0.351	0.422	0.417	0.460	0.437	0.725	0.660	0.491	0.369	0.527	0.519	0.507	0.503	0.427	0.415	0.256	0.489	0.067	0.055			
TMV2	0.336	0.427	0.429	0.455	0.443	0.721	0.651	0.496	0.369	0.506	0.502	0.516	0.486	0.431	0.413	0.246	0.496	0.069	0.049	0.051		
TAG24	0.330	0.402	0.405	0.436	0.419	0.720	0.652	0.488	0.348	0.509	0.504	0.502	0.485	0.409	0.383	0.235	0.478	0.041	0.063	0.065	0.061	
ICGV86031	0.330	0.411	0.413	0.448	0.429	0.723	0.655	0.486	0.363	0.504	0.501	0.508	0.487	0.419	0.399	0.232	0.478	0.056	0.060	0.063	0.049	0.042

40A and ISATGR 90B (Table 2), and an index value ranging from 0.419 to 0.457 was observed for *A. hypogaea* cultivars.

ISATGR 40A

This is an amphidiploid between A and B genome species *A. ipaensis* × *A. duranensis* (Table 1). The reverse of the cross that gave rise to cultivated *A. hypogaea*. Both diplopid and tetraploid had the similar morphology, except for marginally larger leaves (Fig. 1g, h) and larger pods (Fig. 3f) in the tetraploid. Pollen fertility in diploid hybrid was 6 and 94% in the tetraploid plant (Table 1). Thirty percent of the pegs formed pods in the diploid hybrid and pod formation was observed due to natural self fertilization. Cytological analysis showed that the plants were tetraploids (Fig. 4f). ISATGR 40A showed greatest dissimilarity index value ranging from 0.561 to 0.725 with all the other tetraploid *A. hypogaea* as well as with cultivated *A. hypogaea*, except for ISATGR 90B with a index value of 0.357, although both the hybrids do not have any common *Arachis* species as parents. UPGMA dendrogram placed ISATGR 40A away from rest of the material used in the study (Fig. 5).

ISATGR 90B

Pollen fertility in the diploid hybrid between two A genome species *A. kempff-mercadoi* and *A. stenosperma* was 43% and its autotetraploid plant had 88% fertile pollen (Table 1). Diploid hybrid was treated with colchicine to obtain tetraploid plant (Figs. 1n, o, 3h). Both the diploid and tetraploid plants had orange colored flowers. Morphologically the diploid was smaller in size than the tetraploid plant. Cytological analysis confirmed tetraploid nature of the plant. With the rest of the synthetics dissimilarity index value ranged from 0.514 to 0.644 and from 0.644 to 0.660 with the cultivated groundnut (Table 2).

ISATGR 99B

The diploid hybrid between two A genome species namely *A. diogoi* and *A. cardenasii* had the higher pollen fertility of 71%, higher than any intraspecifc diploid hybrid plant. The autotetraploid had 98% pollen fertility. The diploid hybrid grew profusely setting a large number of yellow flowers. Peg and pod formation occurred naturally without the application of growth regulators. Numbers of pegs set was also large. Percent peg to pod set was 40%, the largest in any diploid hybrid used in the present study. The colchicine treated tetraploid plant had larger leaves, with yellow flowers. Percent peg to pod formation was 14%. Attempts are underway to collect more number of pods. ISATGR 99B showed close relationship with ISATGR 160 as both share the same parental species combination. The marginal difference of 0.069 may be due to the difference between different F_1 seeds (Table 2). With the rest of the synthetics, dissimilarity index value varied from 0.363 to 0.588 and with cultivated groundnut the index value varied from 0.486 to 0.501.

ISATGR 168B

This is an amphidiploid between A and B genome species *A. valida* and *A. duranensis*. The diploid plant was large with trailing growth habit, with small yellow flowers (Fig. 1s). The hybrid had 13% pollen fertility. Flowers were tripped to encourage self pollination and such flowers were growth regulators aided. Diploid plant was colchicine treated to double its chromosome number. The tetraploid (amphidiploid) plant had few flowers (Fig. 1t). Flowers were tripped and growth regulators were applied to the base of the pistil to encourage pod formation. Percent peg to pod formation was 19%. Jaccard dissimilarity index did not show closer relationship between ISATGR 168B and any of the tetraploid *A. hypogaea*, and the index value varied from 0.279 to 0.484, and between 0.348 and 0.380, with cultivated groundnut.

ISATGR 278-18

This is an amphidiploid between A and B genome species *A. duranensis* and *A. batizocoi* (Fig. 1u). Pollen fertility in the diploid was 0.4%, but the tetraploid had 95% fertile pollen. In the initial generations flowers were tripped and growth regulator aided to encourage pod formation. The advance generation plants set pods in large numbers without the aid of growth regulator or the necessity to trip the flowers. Chromosome configuration in the tetraploid was normal with 20 bivalents, but a few tetrads had micronuclei (Fig. 4h). A large number of pods are

available from the tetraploid plant (Fig. 3a). Low Jaccard dissimilarity index value of 0.082, 0.112 and 0.191 was seen between ISATGR 278-18 and ISATGR 265-5, ISATGR 268-5 and ISATGR 1212 respectively. This shows closer relationship between these four tetraploids than the rest of them (0.341–0.676) or with cultivated groundnuts (0.506 to 0.514).

ISATGR 265-5

This is an amphidiploid between *A. kempff-mercadoi* and *A. hoehnei,* a different F_1 plant than the diploid hybrid that gave rise to ISATGR 80A. The plant had trailing growth habit. The flowers were of two colors, orange and pale white (Fig. 2b). Orange and yellow flowers have been observed in wild *Arachis* species, but pale white flower has not been observed in any of the species used to generate diploid hybrids. Particular color was not restricted to any branch or part of the plant. The tetraploid plant (Fig. 1p) derived from the diploid had only orange colored flowers. Pollen fertility was 14 and 89% in the diploid hybrid and its tetraploid plant respectively. Flowers were tripped followed by growth regulator application in the diploid plant. As a result a large number of pods (peg to pod formation was 70%) were obtained (Fig. 3d). Initially in the tetraploid, flowers were tripped but in the later generation (>F4) pods formed from natural self pollinations. As observed in the ISATGR 278-18, ISATGR 265-5 showed closer relationship with ISAT 1212, ISAT 278-18, and ISATGR 268-5, but a greater dissimilarity index value between the rest of the tetraploids and cultivated groundnuts (Table 2).

ISATGR 10B

This is an autotetraploid between two B genome species *A. magna* and *A. valida* with 30% pollen fertility in the diploid hybrid. Both male and female parents were B genome species and none of them were putative parental species of cultivated groundnut. The diploid hybrid produced a large number of flowers and pegs. Cytological analysis of the diploid hybrid showed the presence of a large number of diads (40%). The flowers were tripped and growth regulator aided, as a result a few pods were obtained. Seedlings were obtained by germinating mature seeds. One of the seedlings showed large leaves and flowers. Chromosome count of the plant showed 40 chromosomes, confirming the tetraploidy of the plant. The tetraploid plant had 79% pollen fertility. Efforts are underway to obtain seeds in large numbers. ISATGR 10B showed close homology with other *A. magna* × *A. valida* hybrids and a closer relationship with ISATGR 5B (Fig. 5), which has the same maternal parent in the cross. With the rest of the tetraploids dissimilarity index value varied from 0.318 to 0.696 and the index value varied from 0.409 to 0.445 with cultivated groundnuts (Table 2).

ISATGR 35A

The amphidiploid between *A. batizocoi* and *A. duranensis*, called ISATGR 35A, had 0.3% pollen fertility in the diploid hybrid. In the colchicine treated tetraploid (amphidiploid), pollen fertility rose to 97% (Table 1). Morphologically the tetraploid had larger stature with larger leaves and flowers. The difference in pod size was observed in the diploid and tetraploid plants. ISATGR 35B is the reciprocal of the cross ISATGR 278-18, and pollen fertility in the diploid hybrids in both the combinations was less than 1%, which rose to 95% or more in their tetraploids. A large number of seeds are available. Dissimilarity index values between other synthetics and ISATGR 35A varied from 0.166 (ISATGR 1212) to 0.640 (ISATGR 40A), the value being 0.341 with ISATGR 278-18 and from 0.383 to 0.415 with cultivated groundnuts (Table 2).

ISATGR 80A

This is a hybrid between A and B genome species *A. kempff-mercadoi* and *A. hoehnei*. The plant had spreading growth habit. Although both the parents had orange flowers, the diploid hybrid had orange, yellow and white flowers, as seen in another diploid hybrid ISATGR 265-7 (Fig. 2a). The color of the flower was not restricted to any particular branch or part of the plant. Pollen fertility in the diploid hybrid was 23%. In spite of tripping the flowers and applying growth regulators, pegs and pods have not been obtained. Colchicine treated tetraploid plant had massive vegetative growth (Fig. 1k) but did not reach the flowering stage. Another F_1 hybrid with the same cross combination ISATGR 265-5 flowered both in

its diploid and tetraploid hybrids. The plant is being maintained by vegetative multiplication.

ISATGR 206B

This is an amphidiploid between A genome species *A. duranensis* ICG 8123 and B genome species *A. valida* ICG 11548. Although it is a hybrid between A and B genome species the diploid hybrid had a higher pollen fertility of 56%. In the tetraploid hybrid, 96% of the pollen grains were fertile. Although the A genome species is the same as in the cultivated species, the B genome species is *A. valida*, a different B genome species than the putative B genome donor of cultivated groundnut. Efforts are underway to obtain large number of pods (Fig. 3c). Dissimilarity index value between other hybrids with the same maternal parent ISATGR 1212 and ISATGR 278-18 were 0.502 and 0.650 respectively showing larger variation. With other tetraploids too it varied from 0.412 to 0.653 and from 0.478 to 0.489 with cultivated groundnuts (Table 2).

ISATGR 268-5

This is an amphidiploid between B genome species *A. batizocoi* and A genome species *A. cardenasii*. Pollen fertility in the diploid hybrid was 24 and 86% in the tetraploid plant (Fig. 1q). Although this is an interspecific hybrid, pollen fertility in the diploid hybrid was higher than the diploid hybrid between the putative genome donors of *A. hypogaea*. In the diploid hybrid, peg and pod formation was observed with out tripping the flowers or the application of growth regulators. The tetraploid plant set a large number of pods. It showed closer relationship with a dissimilarity index value of 0.054 with ISATGR 265-5, 0.169 with ISATGR 1212 and the index value of 0.112 with ISATGR 278-18 (Table 2). With the rest of the synthetics the value varied from 0.361 to 0.631 and from 0.504 to 0.526 with cultivated groundnut.

ISATGR 160

This is an autotetraploid between *A. diogoi* and *A. cardenasii*. Although the parents are the same as in ISATGR 99B, it is from a different F_1 seed. Pollen fertility in the diploid hybrid was 72%, the highest observed in any intraspecific hybrid. Pollen fertility in the tetraploid plant was above 90%. It was not necessary to apply growth regulators to obtain pegs or pods in the diploid hybrid. The tetraploid hybrid too set pods through natural hybridization (Fig. 3e). The plant showed closer relationship with another tetraploid ISATGR 99B with the same combination. With the rest of the tetraploids dissimilarity index value varied from 0.323 to 0.594. Dissimilarity index value ranged from0.502 to 0.526 with cultivated *A. hypogaea*.

Genetic diversity studies

Genetic relatedness of the synthetics and cultivated lines was assed using DArT. Among c5700 features in the DArT array, 803 were scored polymorphic for the genotyping panel consisting of 17 synthetics and 5 cultivated lines. The level of diversity was very low among the cultivated lines studied and very high among the synthetics developed in this study. The Jaccard dissimilarity index among cultivated groundnut cultivars ranged from 0.041 to 0.073, whereas it was 0.020–0.725 among synthetics (Table 2). The UPGMA clustering resulted in five distinct groups (Fig. 5) of which four were formed only by synthetics. The fifth group consisted of all cultivated lines along with a lone synthetic, ISATGR 80A, which was however placed distantly from the cultivated lines in the same group. ISATGR 80A was derived from the cross *A. kempff-mercadoi* × *A. hoehnei* both of which are not considered as the putative genome donor of *A. hypogaea*. ISATGR 80A cannot be used in the crosses with cultivated groundnuts as it has not flowered, and hence not available for the improvement of cultivated groundnut. Interestingly, the other synthetic, ISAT GR 265-5 which was derived from the same cross combination was placed apart from ISATGR 80A into a separate cluster). Also ISATGR 265-5 and cultivated groundnut showed a dissimilarity index value ranging from 0.502 to 0.510, showing distant relationship between the two. The synthetics ISATGT-1, ISAT GR 11A and ISATGR 10B which were derived from the same cross combination (*A. magna* × *A. valida*) showed very high similarity and were grouped together with other synthetics having *A. valida* as one of the parents. The same was the case with the synthetics ISAT 99B and ISAT GR 160 which were derived from *A. diogoi* and *A. cardenasii*. ISAT GR40A (*A. ipaensis* × *A. duranensis*) and

ISAT GR 90B (*A. kempff-mercadoi* × *A. cardenasii*) were highly diverged from other synthetics and cultivated lines forming a separate cluster. However moderate level of diversity was observed between them (Dissimilarity index = 0.37). The synthetic ISATGR 1212 derived from the putative parental species of cultivated groundnut (*A. duranensis* and *A. ipaensis*) was placed along with cultivated lines and ISATGR 80A in the same quadrant (Axes 1/2) in the principal coordinate analysis (Fig. 6).

Amongst the tetraploids, ISATGR 1212 and ISATGR 168B showing closer relationship with cultivated groundnuts, but diverse enough to be used in the crossing program. Most diverse tetraploids were ISATGR 40A, ISATGR 90B with a dissimilarity index value ranging from 0.644 to 0.723 (Table 2). In summary, all the tetraploids showed distant relationship with cultivated *A. hypoagae* and exhibited wide variability within them.

Discussion

Amongst the 17 new sources of tetraploid *A. hypogaea* generated, only one (ISATGR 1212) had parental genome combination of cultivated *A. hypogaea* and another amphiploid/tetraploid (ISATGR-40A) had the reverse of the parental genome combination of cultivated *A. hypogaea*. In the remaining 15 new tetraploid *A. hypogaea*, five of them had *A. duranensis* as one of the parent (ISATGR-40A; ISATGR-168B; ISATGR-278-18; ISATGR-35A and ISATGR-206), but the other parental genome in these hybrids was different than the putative genome donor combination (*A. duranensis* and *A. ipaensis*). These are new combinations compared to the putative genome donors of cultivated *A. hypogaea*. In 11 tetraploid groundnuts both the parents were not putative genome donor species, hence they can called as totally new sources of *A. hypogaea*.

Amongst the 17 tetraploid groundnuts, one of them (ISATGR 80A) did not reach the reproductive stage (flowering stage) although its diploid form flowered. The other F_1 hybrid plant from the same cross (ISATGR 265-5) flowered both in its diploid and tetraploid status. Although non-flowering synthetic groundnuts are genetic dead-ends, this observation would be restricted to that particular F_1 hybrid (diploid or tetraploid) as another F_1 hybrid from the same cross ISATGR 265-5 flowered both in the diploid and tetraploid forms. ISATGR 9A, a cross between *A. batizocoi* × *A. cardenasii*, set very few flowers and pegs, but no seeds, in the diploid hybrid, but tetraploid derived through colchicine treatment flowered and set a few seeds. This combination would have been a genetic dead end and would not have been available as another source of tetraploid groundnut, had it not been raised to the tetraploid level. Similarly diploid ISATGR 1 gave rise to only pegs with no pod formation. Without generating tetraploid plants, this hybrid again would have been a genetic dead-end as it did not produce any pods. For some unknown reason some of the diploid combinations do not set seeds in spite of moderate levels of pollen fertility, but when raised to the tetraploid level, they set seeds.

Pollen fertility did not vary much (<1%) when *A. duranensis* was used as the male (ISATGR 278-18) or female (ISATGR 35A) parent, in combination with *A. batizocoi*. Whereas, marginal increase in pollen fertility was observed in the reciprocal of the cross *A. duranensis* × *A. ipaensis* (Table 1). In the diploid hybrid ISATGR 5B (*A. magna* × *A.batizocoi*), 2% of the pollen grains were fertile. By changing the pollen parent to another B genome species *A. valida*, pollen fertility increased to 50% (ISATGR 11A).

The female parent of the cross ISATGR 206 was *A. duranensis*, same as that of ISATGR 1212 but the pollen donor was different, a different B genome species namely *A. valida*. The B genome species in cross ISATGR 1212 was *A. ipaensis*. The pollen fertility in diploid ISAT 1212 was less than 1% whereas pollen fertility in ISATGR 206 was 56%. If putative B genome donor was *A. valida* instead of being *A. ipaensis*, probably groundnut would have evolved as a diploid crop, as the diploid was fertile enough to perpetuate the species!

There are two methods by which tetraploidy can be achieved in diploid groundnut hybrids. In the first and a more feasible method, diploid hybrids are treated with colchicine to double the chromosome number. The tetraploid plant obtained is confirmed through cytology. In the second method, the restitution nucleus produced by diploid hybrid is exploited to obtain tetraploid plants, which is again confirmed through chromosome counts. Obtaining unreduced gametes although not a universal phenomenon in diploid hybrid, is not new in groundnut. 2n gametes

were observed in triploid hybrids between *A. duranensis* and *A. glabrata* and *A. diogoi* × *A. glabrata* (Mallikarjuna and Tandra 2006).

The eight F_1 hybrid plants produced between *A. magna* and *A. duranensis* had dwarf growth habit. Replacing the female parent *A. magna* (B genome) with other B genome species such as *A. batizocoi* or *A. valida* or *A. ipaensis* gave rise to F_1 hybrids normal spreading growth habit. The reason for dwarfness may due to the interaction between the cytoplasmic genome of *A. magna* with the nuclear genome of *A. duranensis*.

None of the tetraploid groundnut showed close homology with the cultivated *A. hypogaea*. The purpose of generating tetraploid groundnuts was achieved as they were very distinct from the cultivated species, thus providing the sources for broadening the genetic base of *A. hypogaea*. Results from initial screening of some of the synthetic *A. hypogaea* showed resistant reaction to late leaf spot disease of groundnut (data not shown). This shows that the synthetics are very different from susceptible cultivated *A. hypogaea*.

A dissimilarity index of 0.330–0.351 was observed between ISATGR 1212 and cultivated *A. hypogaea*. The variation can be attributed to evolutionary and plant breeding effects. It was interesting to observe that in the reciprocal (ISATGR 90B) of the cross *A. duranensis* × *A. ipaensis* (ISATGR 1212), higher dissimilarity (0.671) was observed. This means the cytoplasmic genome has a major role to play in the genetic relationships. It would be interesting to check for variation in the synthetics after a few generations of turn-over as molecular variation was observed in brassica synthetics after four generations of selfing (Song et al. 1995).

Results of crossing tetraploid groundnuts with cultivated groundnut have been encouraging as low level of marker segregation distortion was observed in the F_2 population (Dwivedi et al. 2008). Preliminary results of utilizing ISATGR 1212 and ISATGR 278-18 in the crossing program with *A. hypogaea* have produced progeny with pollen fertility ranging from 70 to 90% and higher number of bivalents (18–19) in meiotic metaphase. This shows that there is good recombination between cultivated and newly generated tetraploid *A. hypogaea*.

Modern bread wheat, just like groundnut, was born out of a chance crossing of just a few individuals from three grass species about 10,000 years ago. Many other grass species that were not part of this cross are still around and are being used by scientists to provide modern wheat with a boost of useful genetic diversity. Similarly modern groundnut was born out of a chance doubling of a diploid hybrid between A and B genome *Arachis* species some 3,500 years ago. With the development of tetraploid groundnuts, with the putative genome combination, which has not been selected for any constraints, as well as eight other *Arachis* species in different combinations, there is ample scope to broaden the genetic base of groundnut. Efforts are underway to create more number of tetraploid groundnuts in different cross combinations.

Plant breeding and for that matter even domestication narrows the genetic base of crop plants as selections are made for traits of interest or survival, thus eliminating vast genetic variation during the process. Creation of tetraploid/amphiploid groundnuts is an important and viable way of getting back variation in the form easily available for the improvement of the crop.

A systematic evaluation and documentation of newly synthesized tetraploid *A. hypogaea* is essential as changes which were seen in polyploids of brassica, wheat, cotton to name a few, due to deletion events (Ozkan et al. 2001; Ma and Gustafson 2006), gene conversion events (Wendel et al. 1995; Kovarik et al. 2005), rDNA changes (Joly et al. 2004; Pontes et al. 2004), transposon activation (Kashkush et al. 2002; Madlung et al. 2005), chromosome rearrangements (Pires et al. 2004; Udall et al. 2005) and epigenetic phenomena (Adams et al. 2003; Lukens et al. 2006). If similar events can bring about changes in synthetic groundnut, it can be identified and variation exploited for groundnut improvement.

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