Indian J. Genet., 72(3): 332-335 (2012)

Meiotic study of three synthesized tetraploid groundnut

Sandhya Srikanth, Krishna Shilpa, Deepak Jadhav, T. Satyanarayana and Nalini Mallikarjuna*

International Crops Research Institute for Semi Arid Tropics, Patancheru 502 324

(Received : March 2012; Revised : July 2012; Accepted: August 2012)

Abstract

Closely related wild relatives (diploid) of groundnut (*Arachis hypogaea* L.), a (tetraploid), have broad based resistance to a range of diseases and pests. Direct crosses between diploid relatives and tetraploid groundnut gives rise to undesirable triploids. There are no tetraploid relatives (except *A. monticold*) which can be easily crossed to transfer desirable traits. Hence amphidiploids were synthesized by combining A and B genome of *Arachis* species and the autotetraploid was synthesized by combining two B genome species. Diploid and tetraploid hybrids were cytologically analyzed to study the relationship between the chromosomes/genomes of the parents. The study showed that in the diploid hybrids, pollen fertility varied but in the tetraploids there was good recombination between the parental genomes resulting in high pollen fertility.

Key words: *Arachis* species, amphidiploid, autotetraploid, meiosis, recombination, pollen fertility.

Introduction

Groundnut (*Arachis hypogaea* L.), the second important oil seed crop after soybean, is grown globally in 109 countries of the world and, Asia is the largest producer of groundnut. 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* 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 cultivated groundnut. The lack of resistances in the cultivated germplasm is attributed to its narrow genetic base.

Groundnut improvement necessitates the genetic base to be 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 [1, 2]. Wild *Arachis* exhibit many of the traits related to disease, pest, insect resistance and drought tolerance, hence the approach of creating synthetic tetraploid (amphidiploid and autotetraploids) groundnut 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 pigeonpea wide crosses [3].

Considering the potential uses of synthetics, ICRISAT has developed a set of synthetic groundnuts which are new sources of tetraploid groundnut. *Arachis* species with A and B genome namely *A. diogoi*, *A. valida* and *A. magna* which are not putative genome donors of cultivated groundnut, together with *A. duranensis*, which is the putative A genome donor, were used in the crossing program and diploid hybrids were created. Diploid hybrids were treated with colchicine to develop tetraploid plants. These were created to develop new sources of tetraploid groundnut or in other words synthetic groundnut. 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. In the present paper cytological analysis of three diverse synthetic groundnuts are discussed.

*Corresponding author's e-mail: n.mallikarjuna@cgiar.org
Published by Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com

Materials and methods

Diploid *Arachis* species with A genome namely *A. duranensis* and *A. diogoi,* and B genome species *A. valida* and *A. magna* were grown in a glasshouse. Emasculations, pollinations and growth regulator treatments were as described in literature [4]. Although wild relatives in section *Arachis* are cross compatible, application of gibberellic acid (GA_3) increased the number of peg and pod formation. Pod/seed set also varied according to the male and female species used in the crossing program.

Diploid hybrids with the following parental combinations *A. valida* x *A. diogoi*, *A. magna* x *A. valida* and *A. valida* x *A. duranensis* 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.

Tetraploid F_1 hybrids were confirmed by chromosome counting. To count the number of chromosomes and their association 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 elsewhere [4].

Results and discussion

None of the three new sources of tetraploids had putative parental genome combination of cultivated *A. hypogaea.* It is known in literature that *A. duranensis* is the contributor of the A genome and the female parent of *A. hypogaea* and *A. ipaensis* is the B genome contributor and the male parent of *A. hypogaea.* In all the three tetraploids, *A. valida* was a common parent and two of them had *A. valida* as the female parent and in all the three hybrids the other parent was a different *Arachis* species. Two of the tetraploids were amphidiploids (*A. valida* x *A. diogoi; A. valida* x *A. duranensis*) and one was a autotetraploid (*A. magna* and *A. valida*). Immense differences were observed in the three tetraploids synthesized. Differences were also observed between the two amphidiploids. The three tetraploids are discussed below.

Amphidiploid between A. valida and A. diogoi (ISATGR 23A)

The diploid hybrid between *A. valida* and *A. diogoi,* a B and A genome species, had high pollen fertility of 65%. It set a large number of pegs and pods. It was essential to treat the diploid hybrid with colchicine to obtain the tetraploid, which grew profusely and set a large number of flowers. Pollen fertility in the hybrids was above 90% (Fig. 1 f). Sixty percent of the meiocytes at metaphase showed 20 bivalent formation (Fig. 1 a), and 10% of the meiocytes had 19 bivalents and 20% of the meiocytes had 18 bivalents. Formation of 18 to 20 bivalents in 90 % of the meiocytes (Table 1) shows homology between the parental genomes. Anaphase analysis too showed the presence of a few laggards (Fig. 1 b, c and d). In spite of high" degree of chromosomal homology and high pollen fertility the tetraploid plant did not set pegs or pods/seeds. Flowers were self pollinated and GAs was applied to promote peg formation and pod growth, in spite of the treatment the plant did not produce pegs and pods.

Amphidiploid between A. valida and A. duranensis (ISATGR 65B)

Thirteen percent pollen fertility was observed in the diploid hybrid between *A. valida* and *A. duranensis,* a B and A genome species. Pod set in the diploid was meager. Colchicine treatment was essential to obtain tetraploids, which had higher pollen fertility which was above 95 % (Fig. 2. f). Metaphase analysis showed 40% of the pollen mother cells with 20 bivalents (Fig. 2. a)

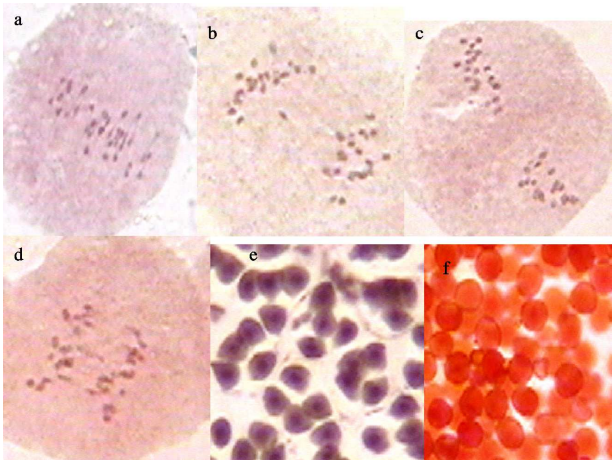

Fig. 1. Meiotic analysis of ISATGR 23A. a: Metaphase showing the formation of 20 bivalents; b, c, d: Chromosome configuration in anaphase; e: tetrads; f: Pollen grains

and 20 % each with 19 and 18 bivalents (Fig. 2. b). Thus 80 % of the meiocytes (Table 1) showed good homology between the parental genomes. Application of GAs was not essential to obtain pegs and pods and the amphidiploid set a large number of pegs and pods.

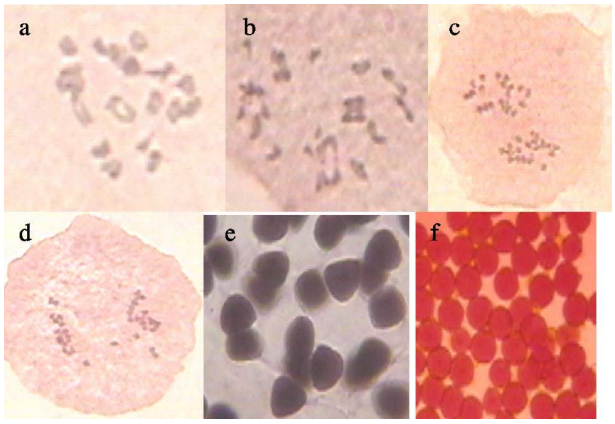

Fig. 2. Meiosis in ISATGR 65B. a: Metaphase showing the formation of 20 bivalents; b: Tetravalents seen with bivalents; c: Early anaphase; d: Anaphase with one laggard; e: Tetrads; f: Pollen fertility

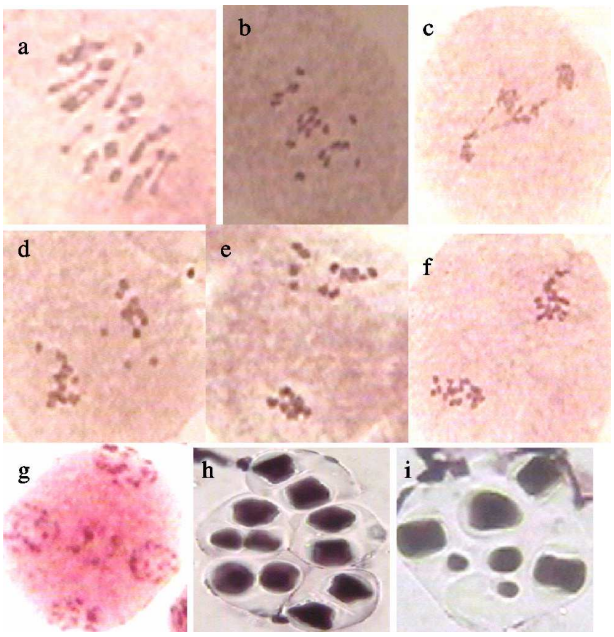

Fig. 3. Study of meiosis in tetraploid ISATGR 10B. a: 20 bivalents in tetraploid ISATGR 10B; b: 20 chromosome in diploid ISATGR 10B; c: Anaphase with laggards; d: As in c; e and f: normal anaphase; g: Abnormal late anaphase showing five poles; h: Dyads and triads in the diploid hybrid; i: Tetrad with micronuclei

Table 1. Chromosome configuration of synthesized tetraploid groundnut

Identity	Bivalents	Univalents	Trivalents	Tetravalents	% Meiocytes
A. valida x *A. duranensis*	20	0	0	0	40
(ISATGR 6 SB)	19	2	0	0	20
BA genome combination	18	0	1	0	20
	17	3	1	0	10
	17	2	0	1	5
	16	0	0	2	5
A. valida x *A. diogoi*	20	0	0	0	60
(ISATGR 23 A)	19	2	0	0	10
BA genome combination	18	1	1	0	10
	18	0	0	2	10
	17	2	0	1	5
	15	0	2	1	5
A. magna x *A. valida*	20	0	0	0	40
(ISATGR 10B)	19	2	0	0	10
BB genome combination	18	0	0	1	10
	17	1	0	1	20
	16	5	1	0	10
	16	1	1	1	10

Autotetraploid between A. magna and A. valida (ISATGR 10B)

Pollen fertility in the diploid hybrid between *A. magna* and *A. valida* (Fig. 3. b), the two B genome species, was 35 %. The hybrids produced a large number of flowers and pegs. Very few pods were obtained. The size of the pods obtained was generally large compared to other diploid hybrids. Similarly the size of the seeds was also large. Cytological analysis of F_1 hybrid confirmed the tetraploidy (Fig. 3. a) of the plant with 40 chromosomes. The diploid hybrid showed 20 chromosomes (Fig. 3. b) but dyads and triads were predominantly observed (Fig. 3. h). Dyads are a result of restitution nucleus in meiotic division. Hence tetraploid plant was obtained by the formation of restitution nucleus and not by the application of colchicines. Some of the meiocytes in anaphase (Fig. 3. e and f) showed the presence of laggards (Fig. 3. c and d). Other than dyads and triads, tetrads and rarely pentads (Fig. 3. g) were also observed. Pollen fertility in the tetraploid plant was 79 %. Twenty bivalents were observed in 40 % of the meiocytes, showing homology between parental genomes. 20 % of the meiocytes showed 19-18 bivalents. 20 % of the meiocytes showed 16 bivalents and such meiocytes had one trivalent (Table 1). All these chromosome configurations show that the parental genomes have a large degree of homology.

According to Holbrook and Stalker [5], the observation that autotetraploids are generally weak and do not survive more than one generation was not observed in the present study. Autotetraploid ISATGR 10B and a few others (Mallikarjuna *et al*., 2011) have been stable over generations and produced pods in larger number in subsequent generations. One autotetraploid between *A. magna* and *A. batizocoi* had robust vegetative growth and has set the largest number of pods in the F_1 and subsequent generations (Mallikarjuna N, unpublished). This shows that each combination forming a synthetic tetraploid groundnut is unique.

To confirm tetraploidy, apart from chromosome number, stomatal diameter studies were also conducted. There was clear difference between the diploid stomata (Fig. 4 a, c and e) and the stomata from tetraploids (Fig. 4. b, d and f). Thus stomatal diameter can be used as a quick check to study the ploidy of the synthetic groundnut plants. The study also shows that the three sources of synthetic tetraploids are stable and ready for use in broadening the genetic base of groundnut.

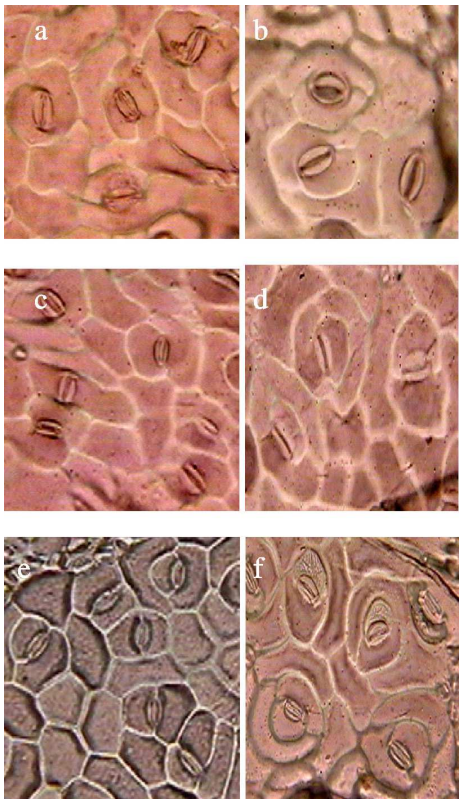

Fig. 4. Comparison of stomatal size in diploid hybrids and tetraploids synthesized from them. a & b: ISATGR 65B; stomata of diploid (a) and tetraploid (b) plant; c & d: ISATGR 10B; stomata of diploid (c) and tetraploid (d) plant; e & f: ISATGR 23A; stomata of diploid (e) and tetraploid (f) plant

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