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Research Article

Cytomorphological investigations in wide cross of *Arachis* involving *Arachis hypogaea* and an amphidiploid (*Arachis correntina* x *Arachis helodes)*

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Abstract
Inter-specific cross of *Arachis hypogaea* (2n=4x=40) and *Arachis correntina x Arachis helodes* (2n=2x=20) yielded the unstable triploid and sterile derivatives. The ploidy level of this triploid (3x) was then doubled with colchicine to get an unstable hexaploid derivative. The paper presents the cyto-morphological features of the produced unstable derivatives so as to frame a suitable breeding strategy to evolve the stable culture tolerant to major foliar diseases. Phenotypic attributes like leaf colour, length and width of calyx tube, poor pollen fertility and abortive ovarian system were found to be dominant in the triploid. Hexaploid progeny possessed leaves with larger veins, semi spreading habit, slow growth, shriveled anthers and improved pollen fertility as compared to triploid. The normal meiotic behaviour was witnessed among diploid and tetraploid parents while abnormal meiotic system as evidenced from the formation of trivalents and quadrivalents was noticed among triploid and hexaploid derivatives. Abnormal sporads also recorded among triploid and hexaploid leading to formation of sterile pollen grain.

Key words: *Arachis correntina, Arachis helodes, Arachis hypogaea* , Triploid, Colchiploidy, Hexaploid

Groundnut (*Arachis hypogaea* L.) is an important legume and oilseed crop grown globally over 100 countries encompassing regions of Asia and Africa. Groundnut is a native of South America brought to Asia from Brazil by Portuguese early in the 16th century when they established regular communication with Indian sub continent (Simpson, 2001). The crop is considered as a gift for small and marginal farmers who grow groundnut under low input conditions for food, oil, cattle feed and confectionery purposes. However, the crop suffers from many diseases and pests that cause serious yield reduction because of narrow genetic base (Varshney *et al.,* 2009). Wild relatives of groundnut have reported to be the potential sources for number of diseases such as rust, late leaf spot, early leaf spot, bud and stem necrosis besides their tolerance to insect pests such as thrips and aphids (Pande and Narayana Rao, 2001). *Arachis hypogaea* L. belongs to the section Arachis and hence several other wild species such as *Arachis villosa* Benth., *Arachis cardenasii*, *Arachis correntina*, *Arachis diogoi*, *Arachis stenosperma HLK.*, *Arachis duranensis* and *Arachis batizocoi* belong to section Arachis have more compatibility with *Arachis hypogaea* (Stalkar and Simpson, 1995). The genome introgression of wild *Arachis* into cultivated *Arachis* would greatly aid in retrieval of some cultures tolerant of major disease / pest besides restored agronomic superiority (Milla, 2003). Hence, utilization of wild *Arachis* for genetic improvement of groundnut along with their tolerance of major biotic stresses forms a key factor for groundnut research considering that those are lacking in cultivated *Arachis hypogaea* L.

Material and methods

VG 0505 belongs to *Arachis hypogaea* var (2n=4x=40) used as a female parent in cross with *Arachis correntina (PI 338312)* x *Arachis helodes* (2n=2x=20) to obtain triploids. The triploid (2n=3x=30) being sterile was then doubled with colchicine to get hexaploid (2n=6x=60). The triploid and hexaploid obtained were studied for traits *viz.*, flower colour, leaf shape, length-width of calyx tube and pollen stainability to establish the hybridity. For cytological investigation, young flower buds of these plants and their respective parents were fixed in Carnoy's fluid (6:3:1) and squashed in 1 per cent aceto-carmine. The cytological analysis of chromosome behaviour in PMCs was made from temporary mounts (Stalkar, 1992). Pollen fertility was tested with differential stain. Seedlings with age of 3 to 5 days old were treated with 0.2 per cent colchicine solution following cotton swab method adopting 6 and 8 hours treatments for 2 to 4 days (Singh, 1986).

Results and discussion

The comparative phenological characters of triploid and hexaploid derivatives of *Arachis hypogaea* x (*Arachis correntina* x *Arachis helodes)* was given in Table 1. Significant differences in leaf shape, flower colour, calyx length, length width ratio of petal, pollen fertility and nature of ovary were noticed among parents (diploids and tetraploid groundnut), triploid and colchicine induced hexaploid derivative. Triploids differed from their parents in habit, leaf texture, leaf shape, bract shape, bract size, length of staminal column and pollen fertility. Larger leaves, flowers, enlarged size of petals, petal spot, longer

staminal column, fertile anthers and increased pollen fertility than triploids were the distinguishing traits for colchicine-induced hexaploid.

In all the pollen mother cell studied, the course of meiosis was normal and regular in parents. There were 20 and 10 bivalents, respectively in VG 0505 and *Arachis correntina* x *Arachis helodes* at metaphase I of meiosis. During anaphase I, the chromosome separation was equal and normal and tetrad analysis also showed normal behaviour. Cytological interpretation of triploid hybrid revealed the genomic introgression from wild to cultivated *Arachis* species. Triploid hybrid resulting from the cross *Arachis hypogaea x (Arachis correntina* x *Arachis helodes)* showed 2n=3x=30 and is generally sterile setting seeds occasionally. The pollen fertility ranged between 5 to 27 per cent.

At metaphase I, the formation of univalents, trivalents and quadrivalents along with bivalents was noticed (Figure 2). The mean chromosome association was $0.85_{IV} + 2.75_{III} + 5.85_{II} + 5.00_{I}$ with a maximum and minimum association of $2_{IV}+2_{III}+4_{II}+8_{I}$ and $3_{III}+7_{II}+3_{I}$ respectively. The bivalents ranged 4 and 7 at metaphase I of meiosis in triploid progeny (Table 2)**.** The anaphase I analysis revealed the unequal and irregular chromosome distribution to opposite poles as 12+12+6 along with laggards in the 3x progeny (Table 3). Normal tetrad formation was also influenced by the formations of diad and triad formation (Table 4). Similar reports were also made by Singh (1986) and Mallikarjuna and Sastri (2002).

The findings of the present study agree with the reports of Simpson (2001), Mallikarjuna *et al. (*2004). Mallikarjuna *et al. (*2004) reported that the ploidy differences among wild and cultivated *Arachis* species delayed the introgression of traits upon crossing. Simpson and Davis (1983) reported 8 to 14 univalents in triploid hybrids.

The studies on meiotic behaviour in hexaploid progeny[2n=6x=60] revealed the abnormal and irregular chromosome behaviour with formation of varied number of bivalents ranging from 18 to 21 along with univalents, trivalents and quadrivalents. The maximum and minimum chromosomal association was recorded as $3_{IV} +1_{III} + 21_{II} +3_{I}$ and $1_{IV} +5_{III} + 20_{II} +1_{I}$ respectively with mean chromosomal association of $2.00_{IV} + 3.29_{III} + 19.79_{II} + 2.57_{I}$ at metaphase I of meiosis (Table 2). Further, formation of dyad, triad, pentad and unequal chromosomal distribution during anaphase I (25+25+10) along with laggards confirm chromosomal instability (Table 3 & 4; Figure 3). The formation of higher number of bivalents reflects the recombination of genetic system and retrieval of more of *Arachis hypogaea* genome in the hybrid derivative (Mallikarjuna et al., 2004).

The *Arachis* section of groundnut harbor many of diploid wild species which shows more compatibility with *Arachis hypogaea*. The diploid wild groundnut does have broader genetic mechanism enabling higher buffering capacity against the fungal and insect activity. The introgression of genetic system from wild tocultivated *Arachis* species adjudged as better way in the development of newer tetraploid groundnut that strengthen the retrieval of stable tetraploid groundnut cultures tolerant of major foliar diseases.

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Table 1. Morphological features of triploid and hexaploid derivatives

Trait	*Arachis hypogaea* x (*Arachis correntina* x *Arachis helodes)* (3x)	*Arachis hypogaea* x (*Arachis correntina* x *Arachis helodes)* (6x)
Length of primary branch (cm)	55-69	80-115
Length of secondary branch (cm)	34-48	67-105
Leaf area (cm^2)	8.4-10.6	10.0-14.2
Length of calyx tube (cm)	4.5-5.2	4.8-5.4
Length of standard petal (cm)	1.20-1.36	1.30-1.32
Width of standard petal (cm)	1.46-1.64	1.50-1.74
Pollen fertility (%)	12-27	30-45

Table 2. Details of chromosomal association in derivatives involving *Arachis hypogaea* x (*Arachis correntina* x *Arachis helodes)*

Cytostudy	Chromosomal association				Number of pollen mother cells (PMC's)			
	IV	III	II	I	*his hypogaea* x *chis correntina* *chis helodes)* (3x)	*his hypogaea* x *chis correntina* *chis helodes)* (6x)	*Arachis* *ypogaea* (4x)	*.correntina* x *A. helodes)* (2x)
I	2	2	4	8	6	-	-	-
II	-	3	7	3	9	-	-	-
III	1	3	6	5	5	-	-	-
Total	17	54	117	100	**20**	-	-	-
Mean	**0.85**	**2.70**	**5.85**	**5.00**	-	-	-	-
I	2	4	18	4	-	4		
II	1	5	20	1	-	5		
III	3	1	21	3	-	5		
Total	28	46	277	36	-	**14**		
Mean	**2.00**	**3.29**	**19.79**	**2.57**	-	-		
I	-	-	20	-	-		7	
I	-	-	10	-	-	-	-	8

Table 3. Tripolar separation of chromosome during anaphase I in triploid and hexaploid progeny developed involving *Arachis hypogaea x (Arachis correntina* x *Arachis helodes)*

Entries / Groups	Number of PMC's				
	10+10	20+20	13+13+4	12+12+6	25+25+10
Arachis hypogaea (4x)		14	-		
Arachis correntina x Arachis helodes (2x)	12		-		
Arachis hypogaea x (*Arachis correntina* x *Arachis helodes)* (3x)	-	-	3	5	
Arachis hypogaea x (*Arachis correntina* x *Arachis helodes)* (6x)	-	-	-		5

Table 4. Status of abnormal sporads at telophase 1 in triploid and hexaploid progeny developed involving *Arachis hypogaea* var VG 0505 and *Arachis correntina,* PI 338312 x *Arachis helodes*

Entries / Abnormal sporads	Number of PMC's			
	Dyad	Triad	Tetrad	Pentad
Arachis hypogaea (4x)	-	-	10	-
Arachis correntina x Arachis helodes (2x)	-	-	5	-
Arachis hypogaea x (*Arachis correntina* x *Arachis helodes)* (3x)	4	3	3	-
Arachis hypogaea x (*Arachis correntina* x *Arachis helodes)* (6x)	7	4	6	2

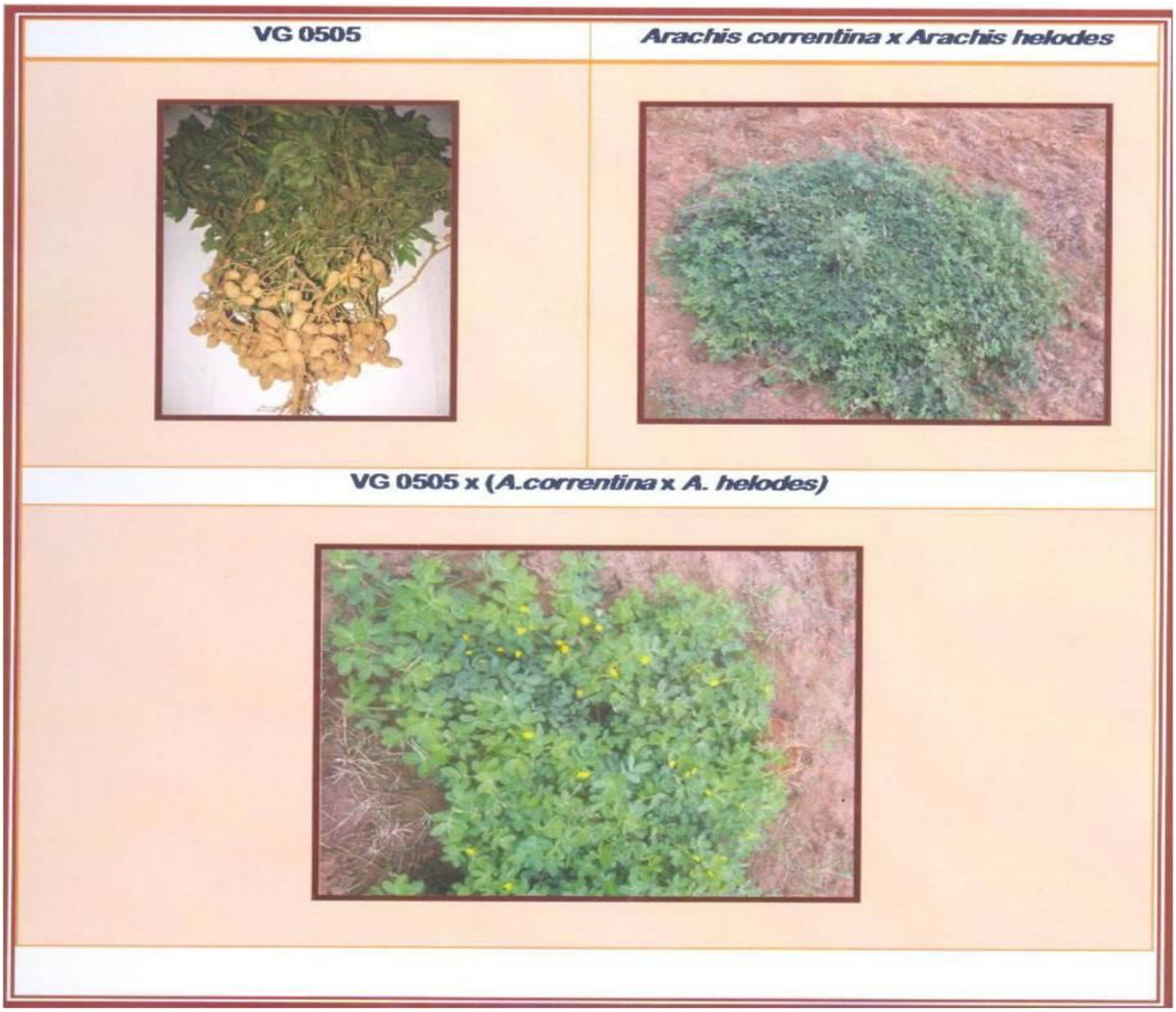

Figure 1 *Arachis hypogaea* x (*Arachis correntina* x *Arachis helodes)* (3x)

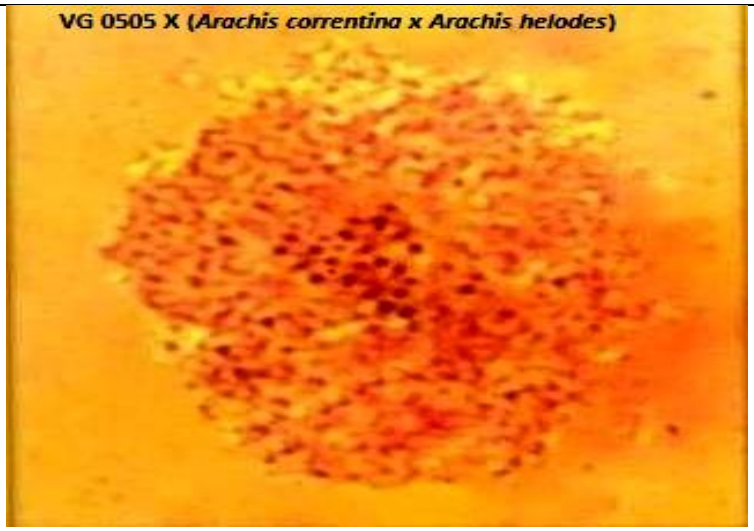

Figure 2 Metaphase stage in *Arachis hypogaea* x (*Arachis correntina* x *Arachis helodes)* (3x)

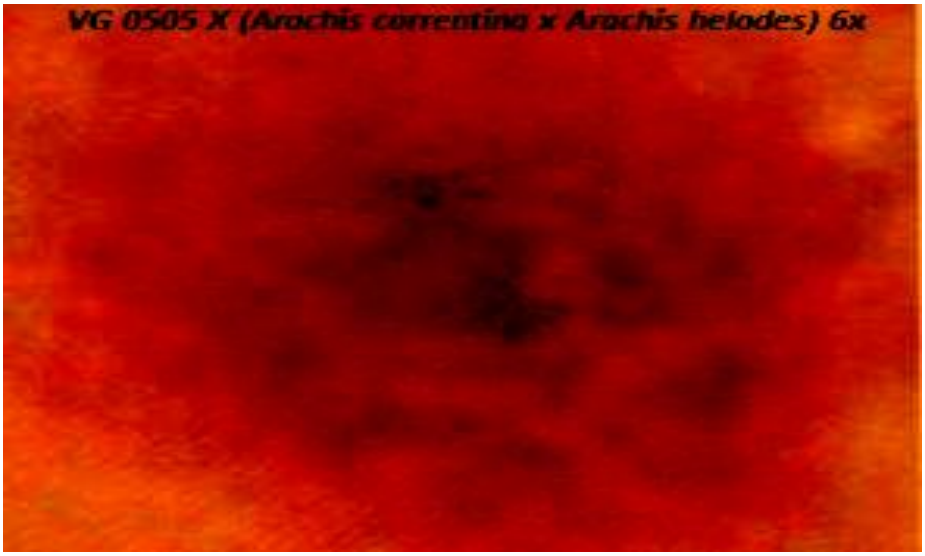

Figure 3 Anaphase stage in *Arachis hypogaea* x (*Arachis correntina* x *Arachis helodes)* (3x)