Chapter 7

MALE MEIOTIC STUDIES IN ABELMOSCHUS SPECIES

Cytogenetical mechanism underlying speciation and evolution of various genera has been a topic of immense importance to geneticists and plant breeders for quite a long time (Bewal et al., 2008). Meiosis is a highly conserved process in eukaryotes and plays a central role in the life cycle of all sexually reproducing organisms (Stace, 2000; Hamant et al., 2006). When estimation of chromosome numbers in mitotic cells become difficult, meiotic studies can be of great help to throw light on species differentiation and diversification. Meiotic analysis dealing with details of both male and female meiosis on the degree of pairing and types of chromosomes associations, chiasma distribution and its frequency as well as chiasma terminalization at diakinesis stage, disjunction of chromosome(s)/chromatids in anaphase I/II can provide an authentic information not only on speciation, but also reveal structural details of genome organization and its inter-relationships (Kumar et al., 2002; Kumar and Rao, 2002a, b, 2003). It is universally known that the meiotic process is primarily influenced by external factors such as temperature, humidity and nutritional aspect while the synaptic behaviour at zygotene (Zickler and Klecner, 1998, 1999) is genetically controlled. Homologous recombination by means of non-sister chromatid exchange is considered to be highly conserved phenomenon in all the taxa ranging from bacteria to higher plants and humans (Hiom, 2001). Disturbance in the synaptic process mediated by gene mutations has also been widely reported in several plant species (Bennett, 1984; John, 1990; Maguire and Riess, 1996; Dave, 1998; Rao and Kumar, 2003).
Family Malvaceae includes many popular plants under cultivation, notably cotton, jute and okra etc. However these economically important plants have received less attention with regard to cytogenetical studies (Youngman, 1927; Davie, 1933; Skovsted, 1935, 1941; Bates, 1967; Bates and Blanchard, 1970; Hazra and Sharma, 1971; Kachecheba, 1972; Bhatt and Dasgupta, 1976). Detailed meiotic studies have not been carried out in many genera and species of the family. Therefore, meiotic studies are often aimed at understanding the basic chromosome numbers, type of ploidy from which the evolution might have progressed and the evaluation of systematic position of different taxa. Thus it is important to study the cytological feature of the putative progenitor species of diploid parents before examining the chromosome behaviour of hybrids.

Only few studies on meiosis are available in the genus *Abelmoschus*. Teshima (1933) studied meiosis of two species *A. esculentus* (syn. *H. esculentus*) with n=36 and *A. manihot* (syn. *H. manihot*) with n=30. Joshi et al., (1974) reported n=65 for *A. esculentus*, n=29 for *A. tuberculatus* and n=36 both for *A. moschatus* and *A. ficulneus*, all the taxa from the Indian sub-continent. Meiotic detailed were also reported by Dasgupta and Bhatt (1980) for *A. manihot* with n=65 as against the n=30 of Teshima (1933). Very recently meiotic chromosome behaviour studies were analysed in *A. moschatus* with the predominant association of 36 II and equal anaphase I distribution of chromosomes by Priyanka et al., (2011).

The comprehensive study about the chromosome associations, chiasma distribution and its frequency and chromosome distribution during anaphase would definitely throw some light on the nature of cytogenetic mechanisms underlying evolution in the genus. In spite of several inherent disadvantages in the material and
scant information available, a concerted attempt has been made presently to bring out
details of male meiosis in nine taxa comprising of seven *Abelmoschus* species
collected from the different parts of India. Such studies will help in determining the
true basic chromosome number of the genus and also to assess the range and
quantum of genetic diversity existing in *Abelmoschus*.

7.1. Observations on associations, chiasmata frequency, anaphase I/II and pollen
    stanibility:

    The meiotic studies in both the wild and cultivated species of nine accessions
belonging to seven *Abelmoschus* species were analysed in the present investigation.
The meiotic details such as total number of PMCs analysed, chromosome association
at metaphase I of nine accessions are summarised in Table 8 and 9. The data on total
and mean number of chiasmata and its range along with number of terminalized
chiasma, terminalization coefficient is summarised in Table 10. The distribution
pattern of bivalents/chromosomes at anaphase I and its percentage pollen stainability
have been detailed in Table 11. The results related to chromosome associations,
chiasma and distribution of univalents/chromatids at anaphase I and anaphase II are
illustrated in Figs. 359-483.

    From the mitotic studies, the ploidy level of *Abelmoschus* species has been
resolved into group A and B species (2n=58, 66 and 72) and group C and D species
(2n=130, 132 and 200). Group A and B are the suggested as true diploid basal
species while group C and D are the derived natural alloplyploids. Hence the meiotic
studies will help to elucidate their accurate ploidy level. The meiotic details of each
accession/species are discussed in detailed in the following:
7.1.1. *A. tuberculatus* (n=29)

Out of 17 cells analysed, 5.88% of the PMCs had shown bivalent associations while the rest showed multivalents associations along with univalents (Figs. 359-369). The mean number of total bivalents was on an average of 23.8 out of which 17.8 were ring and 5.94 were rod bivalents. Quadrivalent associations ranged from 0-4, while univalents ranged from 0-14 in all the cells analyzed (Table 8). The average number of chiasmata per cell ranged from 44-69, mean number being 51.84, out of which on average 37.18 were terminalized giving terminalization coefficient of 0.71 (Table 10). Normal distribution of bivalents/multivalent at anaphase I was recorded in 15 of the cells analysed giving a percentage of 88.24% while two cells had shown unequal distribution of chromosomes with 11.76% and pollen stainibility recorded was 97.67% (Table 11).

7.1.2. *A. ficulneus* (n=36)

The total bivalents observed were on an average of 29.2 out of which 16.3 was ring and 12.7 were rod bivalents (Figs. 370-380). Multivalent associations like quadrivalents were also recorded from a range of 0-7 while trivalents ranged from 0-2 in all the cells analyzed. Likewise univalents was also recorded which range from 0-10 (Table 8). The average number of chiasmata per cell ranged from 43-61, mean number being 54.87, out of which on the average 39.0 were terminalized giving terminalization coefficient of 0.71 (Table 10). Equal anaphase I distribution of chromosomes was recorded in all the PMCs analysed leading to pollen stainibility of 93.81% (Table 11).
7.1.3. *A. moschatus ssp. moschatus* (n=36)

60% of the PMCs analyzed have showed 36 II associations while the rest showed a mixture of trivalents, bivalents and univalents (Figs. 381-391). The mean number of total bivalent associations was estimated as 35.4, which resolved into 19.8 ring and 15.9 rod respectively. Multivalent associations like trivalents was observed with a range from 0-1 and univalents range from 0-6 in all the PMCs analyzed (Table 8). The average number of chiasmata per cell ranged from 49-61, mean number being 55.56, out of which on the average 37.08 were terminalized giving terminalization coefficient of 0.66 (Table 10). 96% of the PMCs had shown equal distribution of chromosome in anaphase and pollen stainability of 59.17% (Table 11).

7.1.4. *A. enbeepeegearensis* (n=36)

A total of 80.95% PMCs had shown to have 36 II associations. The total bivalents observed was on an average of 35.3 out of which 26.0 were ring and 9.2 were rod bivalents (Figs. 392-403). Quadrivalents observed ranged from 0-3 while univalents were estimated to range from 0-4 in all the PMCs analyzed (Table 8). The average number of chiasmata per cell ranged from 54-67, mean number being 62.67, out of which on the average 49.52 were terminalized giving terminalization coefficient of 0.79 (Table 10). Equal anaphase I distribution was recorded in all the PMCs analysed leading to pollen staniability of 96.45% (Table 11).

7.1.5. *A. esculentus*, C 2306 (n=65)

Only 6.67% in all the PMCs analyzed showed 65 II associations. The mean number of total bivalents recorded was 55.8 bivalents, which resolved into 36.4 ring and 19.4 rod bivalents respectively (Figs. 404-419). Quadrivalent associations was
recorded which ranged from 0-8 while univalent range from 0-10 in all the PMCs analyzed (Table 9). The average number of chiasmata per cell ranged from 102-117, mean number being 107.80, out of which on the average 86.67 were terminalized giving terminalization coefficient of 0.80 (Table 10). Only 66.67% of the PMCs had shown equal distribution of chromosomes leading to pollen stainibility of 63.46% (Table 11).

7.1.6. A. esculentus, C 2349 (n=65)

51.61% of the PMCs analyzed showed 65 II associations while the remaining had mixtures of quadrivalents, trivalents, bivalents and univalents (Figs. 420-435). The mean number of total bivalents observed was 63.2, which resolved into 44.3 ring and 18.9 rod bivalents respectively. The quadrivalent associations ranged from 0-7 while that of univalent range from 0-3 in all the PMCs analyzed (Table 9). The average number of chiasmata per cell in A. esculentus, C 2349 (n=65), ranged from 104-141, mean number being 112.97, out of which on the average 95.45 were terminalized giving terminalization coefficient of 0.84 (Table 10). 93.55% of the PMCs has shown the equal distribution chromosomes in anaphase I and pollen stainibility of 80.49% (Table 11).

7.1.7. A. tetraphyllus var. tetraphyllus (n=66)

The mean number of total bivalents associations was recorded as 51.0, out of which 25.4 were ring and 25.6 were rod bivalents (Figs. 436-451). Multivalent association was also recorded in which the quadrivalents ranged from 0-10 while trivalents associations ranged from 0-4 in all the PMCs analyzed. Univalents was also recorded which range from 0-16 in all the cells analyzed (Table 9). The average number of chiasmata per cell ranged from 88-117, mean number being 100.43, out of
which on the average 77.50 were terminalized giving terminalization coefficient of 0.77 (Table 10). Unequal distribution of chromosomes were recorded with only 35.71% showing equal distribution while the rest showed different distribution of chromosomes in anaphase I (Table 11).

7.1.8. A. caillei, C 1366 (n=100)

37.50% of all the PMCs analyzed showed 100 II associations while the rest had mixtures of quadrivalents, trivalents, bivalents and univalents (Figs. 452-467). The mean number of total bivalents observed was 94.0, out of which 71.0 were ring and 23.0 were rod bivalents. Quadrivalents association ranged from 0-6 while trivalent range from 0-1. Univalents ranged from 0-1 in all the PMCs analyzed (Table 9). The average number of chiasmata per cell ranged from 172-181, mean number being 117.0, out of which on the average 138.37 were terminalized giving terminalization coefficient of 0.78 (Table 10). Anaphase I distribution had shown 94.44% of equal distribution of chromosome and the pollen stanibility of 95.84% (Table 11).

7.1.9. A. caillei, C 1349 (n=100)

Only 11.11% PMCs showed the expected 100 II while the remaining had mixtures of quadrivalents, trivalents, bivalents and univalents (Figs. 468-483). The mean number of total bivalents recorded was 82.4, which resolved into 61.7 ring and 20.6 rod bivalents respectively. Quadrivalents associations recorded ranged from 0-15 while trivalents ranged from 0-1. Univalents ranged from 0-7 in all the PMCs analyzed (Table 9). The average number of chiasmata per cell ranged from 166-191, mean number being 178.11, out of which on the average 147.55 were terminalized giving terminalization coefficient of 0.82 (Table 10). 89.47% of the PMCs had
shown the equal distribution of chromosomes and the pollen stainability showed 98.18% (Table 11).

7.2. Discussion:

From the perusal of the published literature, it is amply clear that male meiotic studies in the genus *Abelmoschus* is restricted to only very few species without details on chromosome associations, recombinational frequencies, distributional pattern of bivalents/chromosomes at anaphase I and II. Such information is totally lacking for many of the species in this genus. India being one of the centers of origin, there is an urgent need to evaluate the cytogenetical mechanism(s) underlying speciation and evolution of the genus *Abelmoschus* especially of Indian representative species. In the present investigation, male meiosis has been carried out in nine taxa belonging to seven species of *Abelmoschus*, for their detailed evaluations. Thus this study represents possibly the first report of male meiotic analysis in the some of the important Indian representative species of this genus.

The degree of association of chromosomes in meiosis is determined by the relative homology of the chromosomes which affects the segregation of chromosomes thereby helping to determine their genetic properties (Birchler, 2012). As proposed from the mitotic data, various *Abelmoschus* species has been grouped into four groups. The meiotic division of PMCs in the Group A and B species *viz.*, *A. tuberculatus*, *A. ficulneus*, *A. moschatus* ssp. *moschatus* and *A. enbeepeegearensis* was very regular. The occurrence of distinct bivalents in majority of the Group A and B basal species ranging from 29 II in *A. tuberculatus*, 36 II in *A. ficulneus*, 36 II in *A. moschatus* ssp. *moschatus* and 36 II in *A. enbeepeegearensis*, indicates that they
represent true diploids, which is in accordance with the reported results of Dasgupta and Bhatt (1980). While in case of the Group C and D species *viz.*, *A. esculentus*, *A. tetraphyllus* var. *tetraphyllus* and *A. caillei*, multivalent formation is a regular feature, often found in 48.38 to 99.0% cells, however, their number is low. In all the cases multivalents are either in the form of rings or chains which contain 3 to 6 chromosomes, with predominantly terminal or highly distal chiasmata.

An important observation made in various taxa belonging to the genus *Abelmoschus* is that not a single accession/species belonging to all group C and D was characterized by the presence of its respective bivalents in all the cells analyzed. Rather a mix of bivalents, multivalents association and univalents has been the common feature in all the taxa presently investigated. In case of Group A and B species, 35.38 II (80.95%) were observed in *A. enbeepeegearensis* (n=36), while the least (5.88%) was exhibited by *A. tuberculatus* (n=29). On the other hand, for the Group C and D species, the highest number of expected bivalents i.e. 51.61% was recorded for *A. esculentus*, C 2349 (n=65) and the least (6.67%) was exhibited too by another accession of *A. esculentus*, C 2306 (n=65). In case of Group A and B species, multivalent associations like quadrivalents was recorded with the range of 0-7 as the highest in *A. ficulneus* (n=36) and the least of 0-3 in *A. enbeepeegearensis* (n=36). Trivalent association was recorded only in *A. ficulneus* and *A. moschatus* ssp. *moschatus*. The highest range of 0-10 univalents were encountered in *A. ficulneus* (n=36) while the lowest of 0-4 was recorded in *A. enbeepeegearensis* (n=36). While in the Group C and D species, quadrivalent was recorded the highest of 0-15 and lowest from 0-6 in two accessions of *A. caillei* (n=100) with accession numbers C 1349 and C 1366 respectively. The highest range of 0-10 univalents was encountered
in *A. tetraphyllus* var. *tetraphyllus* (n=66) while the lowest of 0-1 was recorded in *A. caillei*, C 1366 (n=100). Precocious separation of rod bivalents or early separation synapsed homo/or homeologues may be one of the reason for the regular presence of univalents (Kumar, 2000; Singh, 1993). Nevertheless, it may also be due to existence of heterozygosity in genetic makeup in few bivalents, which appears to be the most likely reason. Such phenomena are known to occur in number of plants including several tropical species (Rao and Raina, 2004).

The highest mean value for chiasma frequency for Group A and B species was observed in *A. enbeepeegearensis* as 62.67 while the lowest in *A. tuberculatus* as 51.84. The remaining species had values ranging between these two. Two chiasma per bivalent was the common observation while rod bivalents had just one chiasma, which is mostly found terminalized. On the other hand, the highest mean value of 178.11 was recorded in *A. caillei*, C 1349 (n=100) and the lowest of 107.80 in *A. esculentus*, C 2306 (n=65) for the Group C and D species. Due to extreme small size of the bivalents, locating the exact position of chiasma and counting of chiasmata has been the major obstacle, however, it is mostly observed that the chiasma are distal in position, thereby localizing genetic recombination and enhancing linkage among the constituent genes, only in distal regions (Chua and Roeder, 1998; Moore, 1998; Schnable *et al*., 1998; Sybenga, 1996, 1999; Zickler and Kleckner, 1999; Husband, 2004; Rawat *et al*., 2007). Occurrence of distally localized chiasmata has been attributed to gene control, nature of chromosome pairing and/or interference pattern or even the availability of short segment for crossing over (Kumar, 2000; Stace, 2000; Kumar and Rao, 2002b). It is a phenomenon, which is reported in a large number of plants including several leguminous crops and arid
zone tree species (Sybenga, 1972; Verma, 1981; Kesavacharyulu, 1988; Singh, 1993; Kumar and Rao, 2002b, 2003; Rao and Kumar, 2004). Furthermore, the significant difference in the chiasma frequency between the species with the same chromosome number i.e. n=36 in A. ficulneus, A. moschatus ssp. moschatus and A. enbeepeegearensis might be the indicative nature of their genomic differences, as variation in chiasma frequency and localization is genetically controlled and has been reported in several plant species and crop plant varieties like cotton (Rees and Jones, 1997; Sheidai et al., 2006; Sheidai, 2008). Such variation is considered a means for generating new forms of recombination influencing the variability within natural populations in an adaptive way (Rees and Dale, 1974; Rees and Jones, 1977).

In Group A and B species, the average number of terminalized chiasmata per PMC ranged between 37.08 (A. moschatus ssp. moschatus) to 49.52 (A. enbeepeegearensis) giving the terminalization coefficient a minimum value of 0.71 (A. tuberculatus and A. ficulneus) and a maximum of 0.79 (A. enbeepeegearensis). On the other hand, the average number of terminalized chiasmata per PMC for the group C and D species ranged between 77.50 (A. tetraphyllus var. tetraphyllus) to 147.55 (A. caillei, C 1349) giving the terminalization coefficient a minimum value of 0.77 (A. tetraphyllus var. tetraphyllus) and a maximum of 0.84 (A. esculentus, C 2349).

Of the four species belonging to Group A and B, presently investigated, two of them viz., A. ficulneus and A. enbeepeegearensis and their accessions exhibited normal distribution of chromosomes in all the cells analyzed at anaphase I/or II which is also shown with the pollen stainibility of 93.81% and 96.45% respectively, while A. tuberculatus and A. moschatus ssp. moschatus had at least few cells
showing abnormalities with the univalents distribution at anaphase I and pollen stainibility of 97.67% and 59.17% respectively. However, the presence of univalents in various PMCs, by and large, did not influence the distributional pattern of chromosomes at anaphase I. In case of the Group C and D species *viz.*, *A. esculentus*, *A. tetraphyllus* var. *tetraphyllus* and *A. caillei*, there were unequal distribution of chromosomes at anaphase I/or II with the pollen stanibility ranging from 26.31% to 98.18%. The existence of univalent(s) and (or) multivalents associations is measured to be the main reason for abnormal distribution of chromosomes at anaphase I (Stebbins, 1971; Sybenga, 1972; Arya *et al.*, 1987; Kesavacharyulu, 1988; Rao and Chandel, 1991; Singh, 1993; Gupta, 1995; Kumar *et al.*, 2002; Rao and Kumar, 2003; Rawat *et al.*, 2007).

Metaphase and anaphase chromosome stickiness is a common phenomenon in *Abelmoschus* like in *Gossypium hirsutum* L. The chromosome stickiness was observed in almost all the species and their degree of stickiness ranged from stickiness among two or more chromosomes to involvement of all the metaphase chromosomes forming a complete clump, as reported in cotton (Sheidai, 2008). Genetic-environmental factors and their interaction have been considered as possible reasons for the occurrence of chromosome stickiness in different plant species (Baptista-Giacomelli *et al.*, 2000).

In ascertaining the true basic number of a genus, it is essential to have informations regarding gametic and/or zygotic number of as many species as possible. Taking mitotic data into consideration it becomes clear that the basic number of *x*=29 is in agreement to as proposed by Darlington and Wylie (1955) of *x*=9, 11, 12, 17, 18, 19, 29 and 39. Therefore it is imperative to propose *x*=6 and 10
in addition to \( x=29 \) as the sole basic number for this genus. These different basic numbers in a genus could be recognized as the basis of evolution originated from a relatively primitive basic number as reported in many other genera such as *Vigna* (Rao and Raina, 2004) and *Tribulus* (Rawat *et al*., 2006). Therefore \( x=6, 10 \) or 29 could be proposed as the probable basic numbers of the genus, is which is reasonably justified from the present observations.

Thus the meiotic behaviour of chromosomes at prophase I of meiosis can elucidate some idea about the extent and nature of homology of chromosomes of a taxon i.e. allo- or autopolyploid. The occurrence of maximum number of bivalent associations within groups of A (AA) and B (BB) species suggests their probable true diploid nature while bivalent associations along with the multivalent associations like quadrivals in group C (AABB) suggest their segmental allopolyploid nature which is the resultant of fusion of two different genomes, one from group A and the other from group B. Such ploidy was observed in resynthesized *Brassica napus* derived from the diploid progenitors, *B. oleracea* and *B. rapa* (Xiong *et al*., 2011) and naturally occurring tetraploid, *Tragopogon miscellus* (Chester *et al*., 2012). Differences in chiasma frequency may be the reason for the higher frequency of quadrivalents among the larger chromosomes and lower in the small ones (Heilborn, 1924) while the absence of true quadrivalents may be due to limited chiasma frequency as seen in *Ulmus americana*, an autotetraploid (Sax, 1933). While the group D (AAAABB) is suggested to be an allohexaploid with the involvement of three distinct genomes i.e. group B (AABB) and group A (AA). Chromosome pairing in allohexaploids shows diploid-like meiosis which is under genetic control, and is a common occurrence in many allohexaploids (Kihara, 1919;
Lilienfeld, 1951; Dvorak et al., 1988) of several crop plants like *Triticum aestivum* (Okamoto, 1957; Sears and Okamoto, 1958; Riley and Chapman, 1958), *Avena sativa* (Rajhathy and Thomas, 1972; Jauhar, 1977), *Festuca arundinacea* (Jauhar, 1975) and *Hordeum parodii* (Subrahmanyam, 1978). Polyploid evolution among many angiosperm genera is an ongoing process and not a rare, macroevolutionary event. Therefore, polyploids often comprise of novel physiological characteristics not present in their progenitor cytotypes, thereby allowing a plant to enter a new ecological niche (Levin, 1983; Lumaret, 1988). Since plants of different ploidies are often reproductively isolated by strong post-zygotic barriers, polyploidy is also one of the major mechanisms by which plants evolve reproductive isolation (Darlington, 1963; Grant, 1981). The success of newly formed angiosperm polyploids is partly ascribed to their highly-plastic genome structure, as manifested by adaptation to changing chromosome numbers (aneuploidy and polyploidy), genome size, (retro) transposable element mobility, insertions, deletions and epigenome restructuring (Leitch and Leitch, 2008). The cell and pollen size increases with ploidy and the plants typically take on a “stocky” appearances with at times deranged physiology (Blakeslee, 1941; Randolph, 1942; Rhoades and Dempsey, 1966; d’Erfurth et al., 2009; Yao et al., 2011). Surprisingly, it is known that duration of meiosis is shorter in polyploid plants than in their diploid progenitors (Bennett and Smith, 1972) which is due to the presence of more chromosome sets, hence shorter the time required to sort them (Martinez-Perez et al., 2000). Certain phenomena such as gene silencing, neofunctionalization and subfunctionalization of homoeologues are common consequence of polyploidy (Lynch and Conery, 2000; Adams and Wendel, 2005; Veitia, 2005; Adams, 2007). These events usually render greater variation at the gene
level, particularly in higher polyploids (Wendel and Doyle, 2005) and can be used as sources of phylogenetic signal for identifying origins and parental lineages in diploid-polyploid complexes.

Heterochromatin plays an important role in chromosome pairing and chromosome alignment. The heterochromatin of centromeric regions, supernumerary chromosomes (B-chromosomes or accessory chromosomes), nucleolus organizer regions and knobs (Knob 10 in maize) consists of highly repetitive DNA (Singh, 1992). Heterochromatin of homologous chromosomes remains associated throughout prophase until metaphase I regardless of whether they undergo exchange, suggesting that homologous recognition can lead to segregation even in the absence of chiasmata. Chromosomal processes, including transcription, chromosome segregation and long-range chromatin interactions (Grewal and Jia, 2007). Several reports show that heterochromatin (highest degree of DNA synthesis-S phase) increases chiasma frequency (Rees and Evans, 1966), increases the frequency of non-homologous association at diplonema (Church, 1974), increases crossing over (Nel, 1973) and lengthens meiotic prophase. Thus, heterochromatin prolongs the time during which crossing over takes place (Rhoades and Dempsey, 1972). Disruption in heterochromatin formation leads to centromeres malfunction (Allshire et al., 1995; Kellum and Alberts, 1995). Defects in heterochromatin formation, especially those affecting the pericentromeric regions, result in mis-segregation of chromosomes (Allshire et al., 1995; Kellum and Alberts, 1995; Grewal et al., 1998; Peters et al., 2001).

The concept of segmented allopolyplploid nature of the derivative species belonging to group C (AABB) and allohexaploid nature of group D (AAAABB)
derives support from our observations on both chromosome associations and their disjunction at anaphase I and II. Newly formed allopolyploids are known to exhibit considerable meiotic complexity, including multivalent pairing, multisomic inheritance and production of unbalanced gametes. Such complexity has been recorded in colchicines-induced tetraploids of *Cyamopsis tetragonoloba* (Bewal *et al.*, 2009).

To shed further light on group C and D polyploid species origin, it is essential to have more molecular and cytogenetical data. More accessions should be cytologically studied and a directed analysis through GISH and multicolour FISH technique are essential (Heslop-Harrison, 2000). Molecular methods so far have verified the genomic relationships established by cytogenetics in the genus *Arachis, Avena, Glycine, Gossypium, Hordeum, Lycopersicon, Oryza, Phaseolus, Pisum, Secale, Triticum, Vigna, Zea* and many more (Katsiotis *et al.*, 1997; Moscone *et al.*, 1999; Robledo and Seijo, 2008; Gan *et al.*, 2013).

Workers like Davie (1933), Skovsted (1935), Bates and Blanchard (1970) and many others believe that polyphyletic course of evolution might have taken place in the family Malvaceae. Allopolyploid forms through inter-specific hybridization and whole genome duplication. While allopolyploids may display increased vigor relative to their progenitors, they can also face challenges to fertility following hybridization. Genetic changes in allopolyploids result from recombination between the hybridized subgenomes, which can influence phenotype and ultimately determine fitness of future generations. All these observations implies speciation by successive amphidiploidy within the species complex linked to the reduction of variability in the genus.