

Meiosis in Pollen Mother Cells (PMC) and Pollen Fertility in *Allium cepa* and its Crosses with *Allium fistulosum***Solanki Bal**

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SUMMARY

Within the genus *Allium*, interspecific hybridization has been an important aspect in alleviating genetic variation by transferring useful traits from wild relatives and in creating new varieties. However such hybridisation techniques have been very often accompanied by pre- and post-fertilization barriers, which results in limited number of F₁ hybrids as well as back-crosses. Studies on meiosis pertaining to hybrids and back-cross progenies within *Allium* opens up interesting facts about nature of alien introgression. In addition, an understanding on knowledge of transmission of 'foreign' chromatin into the cultivated species can be acquired. The purpose of this article is to present a concise view on meiosis in pollen mother cells (PMC) and pollen fertility in *Allium cepa* and its crosses with *Allium fistulosum*.

INTRODUCTION

Onion (*Allium cepa* L.) and Japanese bunching onion (*Allium fistulosum*) are among the most economically important vegetables within genus *Allium*. Both of these species have got great medicinal values. Species hybridization has been used as a means of accelerating genetic variation which compels in generating new varieties within a short span of time and transferring useful agronomic traits from one species to another and from wild relatives. However, such hybridisation techniques have been very often accompanied by pre- and post-fertilization barriers, which results in limited number of F₁ hybrids as well as back-crosses. Meiotic studies within *Allium* especially in hybrids and its back-cross progenies presents a broader picture about the perspective of alien introgression and 'foreign' chromatin transmission into cultivated species (Khrustaleva and Kik , 2000).

Meiosis in *Allium cepa* and other alliums

In alliums breeding systems are variable, some are reproduced as inbreeders or outbreeders i.e., reproduced sexually, some asexually with apomixis or vivipary and some are vegetatively propagated. But majority of genus *Allium* is sexually propagated which allows for gene exchange and more genetic variation consequently. *Allium cepa* L. belongs to the genus *Allium*, subgenus *Rhizirideum*, section *cepa* (Mill.). It is a diploid (2n=2x=16) (Bal *et al.*, 2021; Bal, 2022; Bal *et al.*, 2022) and is known to possess one of the largest genomes among cultivated plants. Onion's nuclear genome contains 35.8 pg per 2C reflecting very large chromosome. It has a complement of eight pairs of metacentric and sub-metacentric chromosomes including a set of terminal satellite chromosomes. Meiosis in onion is reported to be normal and regular having eight bivalents. During metaphase I in PMC in onion, chiasmata are distributed in each bivalent at random and they become terminal or sub-terminal by the mid-metaphase. The cross-over points are therefore located mainly in distal and interstitial regions of chromosome arms. But no such information are found till date on localized chiasmata in onion (Emsweller and Jones, 1945). Majority of species within genus *Allium* carry sub-metacentric or metacentric chromosomes. Levan (1935) observed that species with x=7 possesses quite larger chromosomes to that of ones having x=8 or x=9. Besides arm-length asymmetry was found more frequent in the "16" and "18"-chromosome types, whereas, "14"-chromosome types were reported to be most primitive. Reports estimated that 40% of the forms within the genus possessing eight chromosomes had actually varying number of asymmetrical chromosomes occasionally carrying nucleolar organizer regions (NOR), whereas, 60% of the remaining had symmetrical chromosomes. In addition, reports on differences in telomeric sequences and stabilization of chromosomal ends using highly repetitive rDNA and satellites in *Allium* exists (Jones and Mann, 1963).

Meiosis in alliums was reviewed by Levan (1931). The diploid forms of chiasmata in prophase I was visible at early diakinesis. Terminalization of chiasmata in each conjugating pair causes a decrease in their number from 10-15 to 2-3. In metaphase chromosomal shapes was commonly rings and rods. In case of *A. nutans* chromosomal pairing observed to be incomplete in meiosis. Nevertheless, chromosomal association/pairing was common during meiosis in polyploidy forms. In addition, trivalents were observed in different forms i.e., two rods on one arm or with V-shaped trivalents with one rod on each arm. Trivalents in anaphase I were randomly

distributed usually twelve to the pole, however, 8 to one pole and 16 to the other was reported. Besides, formation of unreduced gametes and occurrence of laggard chromosomes developing micronuclei was reported in triploids. In meiosis I quadrivalents were formed in the PMC of *Allium porrum* and *Allium schoenoprasum* and the most frequent quadrivalent types were chains and rings. Besides, in polyploid *A. oleraceum* ($2n=24, 32, 40$) chromosomal elimination during meiosis was noticed by Levan (1931, 1935).

Analysis of meiosis and pollen fertility on *A. cepa* × *fistulosum* and in subsequent generations

Japanese bunching onion or Welsh onion, botanically which is known as *Allium fistulosum* L. is a diploid species ($2n=2x=16$). Its nuclear DNA is 26.3 pg (2C) which is reported to be 20% less than that of onion (Levan, 1935). During meiosis I eight bivalents were formed in *A. fistulosum* which were held together by two chiasmata, one on each side of the centromere where they appear like a cross in metaphase plate. In the bivalent, chiasmata were usually proximally localized mostly adjacent to the centromere. However, occasional formation of more interstitial and randomized chiasmata were observed.

Among all interspecific crosses in the genus *Allium* progenies obtained from generative hybridization of onion with *A. fistulosum* have been studied most extensively. Both of these species can be easily hybridized in the greenhouse and the success rate can be increased by using tissue culture techniques such as embryo rescue. First hybrids between *A. cepa* and *A. fistulosum* was developed by Emsweller and Jones in 1935. Meiotic studies revealed that in early prophase I the hybrid chromosome appear as single threads following the patterns to that of parents. Abnormalities in cell division starts at late prophase was confirmed by Maeda (1937). In pachytene, chiasmata were doubled and single threads were doubled between some conjugating partners, wherein other chromosomes overlapped each other at entire length of arms. When the chromosomes form bivalents of different length, they were separated sometimes in association with long chromatid as well as long plus short chromatid. In addition, extension of long arm of conjugated chromosomes beyond the end of the other was also observed and such condition depends upon arrangement of chiasmata at the ends of a bivalent. In diakinesis, chromosomes get usually well-separated and their configuration could easily be determined. In *A. fistulosum* chiasmata were localized near the centromere region and in *A. cepa*, chiasmata were predominantly terminal. Hybrids had high frequency of complete bivalent pairing along with presence of unpaired chromosome. In anaphase I, occasional chromosome bridges were present where chromosomal separation occur (Emsweller and Jones, 1945). Levan (1931) reported regular bivalent formation in only 2% of the tested PMC in his *A. cepa* × *A. fistulosum*. In that plant material early stages of meiosis showed a number of unpaired threads, where the maximum pairing was found before diplotene. Few pachytene chromosomes had threads longer than their pairing partner in consequence of which it leads to the occurrence of loop shape formations. In addition loops were localized on one side of pairing complex, whereas in some cases longer threads get folded and paired with itself. Besides, inter-chromosomal differences, resulting in pairing of more than two chromosomes were observed. Levan (1931) observed frequent trivalents (14%) in metaphase forming rings. Quadrivalents, pentavalents and hexavalents were seldom reported.

However, Maeda (1937) observed absence of such associations to be higher in bivalents in his hybrids. Abnormalities were observed in anaphase I which includes laggard chromosomes in 10% and bridge formation in 28% of the cells. Besides, occurrence of inversions, translocations and presence of heteromorphic pairing in F_1 interspecific hybrids were also reported (Maeda, 1937). By using GISH (Genomic *In Situ* Hybridization) reciprocal crossover events was detected in F_1 interspecific hybrids by some researchers, where the frequencies of crossovers were detected as label exchanges in anaphase I and chromosomes were reported to be about 20% higher than metaphase I chiasma frequency. Albin and Jones (1990) reported irregularities in the synaptonimal complex (SC) and incomplete synapsis in the centromeric region in the *A. cepa* × *A. fistulosum* hybrids. Additionally, the differences in DNA between *A. cepa* and *A. fistulosum* chromosomes were localized in the centromeric region. Such condition prevented progression of synapsis and possible proximal chiasma formation. The F_1 hybrids between *A. cepa* and *A. fistulosum* can be produced easily and possesses low pollen fertility along with successful production of F_2 progenies. F_2 progenies were exclusively triploids and tetraploids. Meiotic events exhibited frequent disturbances such as micronuclei in dyads and tetrads as well as chromatin bridges and random distribution of chiasmata. On cytological analysis of the tetraploid forms revealed that amphidiploids were build

up from 2 *fistulosum* and 2 *cepa* genomes. In fact morphology of tetraploids reported to be intermediate between the parents with varying pollen fertility. One tetraploid was reported to be sterile while other had moderate (50%) pollen fertility, whereas, the frequency of trivalents were reported to be high (80-90%). Chromosomal bridges and micronuclei observed to be 33% of tested cells having low pollen fertility; not exceeding 10%.

Attempt to backcross (BC) *A. cepa* × *fistulosum* to *cepa* were unsuccessful by some researchers. The reasons behind such difficulty lies in pre-fertilization barriers, as growth of pollen tube of onion in the style of the hybrid was disturbed. Maeda (1937) took an attempt to backcross F₁ to *A. fistulosum* and obtained viable seeds. Several BC progenies were obtained by Emsweller and Jones (1945) only when the hybrid was used as pollen source. In BC₁, the most common meiotic configuration were eight univalent and eight bivalents along with the presence of localized and randomized chiasmata. In addition chromosomal bridges, laggard chromosomes, multivalents (includes both quadrivalents and pentavalents) were observed in 40% of the meiocytes. Emsweller and Jones (1945) backcrossed F₁ to both *A. cepa* and *A. fistulosum* using F₁ as pollen source where on an average 44% of PMC in population when backcrossed to *A. cepa* showed regular pairing of chromosome and eventually segregated with respect to male fertility. In addition, after self-pollination some individuals yielded few seeds and some were completely sterile. The first backcross to *A. fistulosum* possessed both localized and randomized chiasmata but the second backcross revealed a high frequency of localized chiasmata. In fact BC₂ fertile plants had localized chiasmata and their morphology was similar to *A. fistulosum*, while sterile plants were morphologically *A. cepa* types and had mostly randomized chiasmata. Some researchers while studying 16 BC₃ plants observed very high pollen fertility (90-92%) along with eight bivalents at metaphase. In addition, those plants exhibited low percentage of chromosomal aberrations such as micronuclei, bridges and laggards. Following similar GISH patterns Khurstaleva and Kik (2000) observed differences in distribution of heterochromatin at the telomeres between two parental species.

CONCLUSION

Hybrids between *A. cepa* and *A. fistulosum* including their backcross progenies were deeply studied in this article, using *A. cepa* either as female or pollen parent. Although pollen sterility of hybrids act as a barrier to introgression of genes, but occurrence of fertile F₁ hybrids can fetch further progenies and introduce them in the further breeding process. The idea of meiosis in F₁ hybrids and subsequent generations of onion and *A. fistulosum* has been discussed but meiotic studies of hybrids between *A. cepa* and *A. sativum* and *A. porrum* is limited. Thus change on such status is highly needed as understanding of meiosis is crucial for further research pertaining to fertility, reproduction, genetics and breeding of *Allium* species.

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