# STUDIES ON MEIOTIC CONFIGURATIONS AND POLLEN FERTILITY IN *CHLOROPHYTUM LAXUM* R.BR.

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***Abstract***
Meiosis is a key feature of eukaryotic sexual reproduction that properly reduces the chromosome number of gametes in anticipation of fertilization and the reconstitution of the diploid state. It also increases the genetic variability in the population of organisms from one generation to the next and variations are very important for the process of evolution. During present study the meiotic course in *Chlorophytum laxum* R. Br. and pollen fertility have been detected. For this, the plant materials fixed in Carnoy's fixative and observed the detailed meiotic behavior in *Chlorophytum laxum* by standard squash method in 1% acetocarmine. Pollen viability was tested with 1 % acetocarmine and 0.5 % TTC stain. During the present study it is observed that along with the normal meiotic behavior of PMCs showed varied range of abnormalities in *Chlorophytum laxum* like chromosome stickiness, unequal distribution of chromatin material, change in orientation and unreduced gametes, etc. without any treatment. Pollen fertilityy was found to be maximum in acetocarmine: glycerine i.e. 93.57% compared to TTC (2, 3, 5- triphenyl tetrazolium chloride) i.e. 59.76%.

**Index Terms:** *Chlorophytum laxum, Meiotic configurations, Pollen fertility*

## I. INTRODUCTION

Meiosis is the process of chromosome division which play important role in the evolution of cellular organisms where it exists. The developmental stages in meiosis due to the chromosomal behavior especially in plants leads to the evolutionary changes in their life cycle generations to generations. As the literature concern many species evolved due to the continuous changing behavior of chromosomes due to the effects of genetical as well as environmental factors.

The genus *Chlorophytum* Ker-Gawl belongs to family liliaceae, one of the largest plant families. The genus is represented worldwide by about 215 species distributed mainly in the old world tropics especially in Africa and India. (Goaverts R, Zona SA (2006) In India the genus is represented by about 17 species of which 15 are found in peninsular India and 9 are endemic to the country. *Chlorophytum* is a complex and taxonomically difficult genus as a result many species are often misidentified by taxonomists (Sardesai *et al*, 2006). Like many other Liliaceous genera, *Chlorophytum* is good cytological material and despite this cytological information on the genus is very fragmentary. Cytological studies in different species of the genus *Chlorophytum* Ker-Gawl have been confined so far mainly to chromosome number and their morphology (Baldwin and Speese, 1951; Kumar and Rao, 1958; Boraiah, 1966; Datta and Mitra, 1968;

Pahuja and Virendrakumar, 1969; Sheriff and Chennaviraiah, 1972; Naik, 1974; Naik, 1976). There are many species in the Indian Subcontinent. Some of the species exhibit tremendous morphological, chromosomal and cytotytpic diversity (Patil *et al*, 1987). The comprehensive survey of literature reveals that the genus exhibits a wide range of somatic chromosome counts (2n=14, 16, 28, 40, 42 and 56) and is represented by two basic numbers (x=7 and 8). However, to the best of our knowledge, there are few published records on meiotic chromosome abnormalities and behavior in *Chlorophytum laxum*. In the present study, detail analyses of the meiotic configurations with meiotic abnormalities which are rarely reported earlier are mentioned here and attempt is made to interpret them.

## II. MATERIALS AND METHODS

Plant Material: *Chlorophytum laxum* R. Br. collected from Melghat forest of Amravati district and grown under suitable condition in pots containing garden soil and maintained in the departmental garden. Voucher specimens are deposited in the Herbarium of Botanical Survey of India, Western Circle, Pune. (*Chlorophytum laxum* voucher no. SMIGCHL1) and in the Herbarium of Department of Botany, S.G.B. Amravati University, Amravati.

**Cytological Preparation and Meiotic Analysis** : Freshly collected young inflorescence were fixed in a Cornoy's solution I for 24hrs Anthers were dissected from the flowers for chromosome count from PMC and the smears were made in 2% Aceto-orcein as well as 2% Acetocarmine. All meiotic phases were analyzed. Occurrence of chiasma was also observed at diplotene and diakinesis. Abnormalities that might impair the meiotic product were taken into account. All observations were made both from temporary and permanent preparations. Photomicrographs were taken mostly from freshly prepared slides using Trinocular Fluorescence Microscope (AXIOSTAR PLUS, M/S Carl Zeiss, Germany).

**Pollen fertility**: Pollen viability was evaluated using two different stains acetocarmine glycerine (1:1) and 0.5 % TTC. At least 200 to 300 pollen grains were evaluated (Alexander, 1969).

$$\textbf{Pollen fertility \%} = \frac{\textbf{No. of stained pollen}}{\textbf{Total no. of pollen}} \textbf{ X 100}$$

## III. OBSERVATIONS AND RESULT

**Table-1: - Meiotic configurations with abnormalities in *Chlorophytum laxum* R. Br.**

| Sr. No. | Phases | No. of PMCs analyzed | Percentage of abnormal PMCs | Abnormalities |
|---|---|---|---|---|
| **1** | Diakinesis | **163/273** | **59.70** | Multivalent chromosome configurations |
| **2** | Metaphase-I | **98/181** | **54.14** | stickiness/micronuclei/ equatorial arrangement of chromosome |
| **3** | Anaphase-I | **54/93** | **58.06** | Dicentric bridge formation with laggards/Snapping of bridge/ Unequal distribution of chromatin material |

| | | | | |
|---|---|---|---|---|
| **4** | Telophase-I | **115/222** | **51.80** | Laggard formation/precocious chromosome movement |
| **5** | Metaphase-II | **56/145** | **38.62** | Change in orientations/ unoriented univalents |
| **6** | Anaphase-II | **68/167** | **40.71** | Undistributed chromatin material/change in orientation/ formation of laggards |
| **7** | Telophase-II | **101/254** | **39.76** | Laggard formation |
| **8** | Tetrads | **54/197** | **27.41** | Unreduced gametes/Change in orientation/disturbed tetrads |

**Table-2: - Pollen viability in *Chlorophytum laxum* R.Br. by using acetocarmine and glycerine (1:1)**

| Sr. No. | Total no. of pollen | No. of viable pollen | No. of nonviable pollen | Percentage of viable pollen grain | Mean of pollen viability |
|---|---|---|---|---|---|
| 1. | 95 | 90 | 05 | 94.73 | **93.57%** |
| 2. | 234 | 198 | 36 | 84.61 | |
| 3. | 286 | 273 | 13 | 95.45 | |
| 4. | 290 | 256 | 34 | 88.27 | |
| 5. | 173 | 169 | 4 | 97.68 | |
| 6. | 115 | 110 | 5 | 95.65 | |
| 7. | 93 | 85 | 8 | 91.39 | |
| 8. | 278 | 267 | 11 | 96.04 | |
| 9. | 213 | 206 | 7 | 96.71 | |
| 10. | 166 | 158 | 8 | 95.18 | |

**Table-3:- Pollen viability in *Chlorophytum laxum* by using 0.5% TTC (2,3,5- triphenyl terazolium chloride)**

| Sr. No. | Total no. of pollen | No. of viable pollen | No. of nonviable pollen | Percentage of viable pollen grain | Mean of pollen viability |
|---|---|---|---|---|---|
| 1. | 116 | 72 | 44 | 62.06 | **59.76%** |
| 2. | 233 | 106 | 127 | 45.49 | |
| 3. | 281 | 112 | 169 | 39.85 | |
| 4. | 102` | 56 | 46 | 54.90 | |
| 5. | 99 | 76 | 23 | 76.76 | |
| 6. | 157 | 83 | 74 | 52.86 | |
| 7. | 242 | 196 | 46 | 80.99 | |
| 8. | 200 | 168 | 32 | 84.00 | |
| 9. | 286 | 201 | 85 | 70.27 | |
| 10. | 138 | 42 | 96 | 30.43 | |

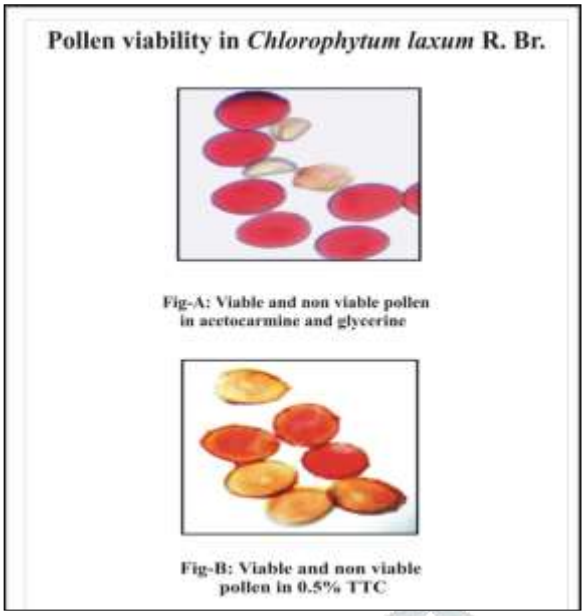


**Figure: Pollen viability in *Chlorophytum laxum* R. Br. A)** Viability and Non viability of pollen grains in acetocarmine and glycerine (1:1). **B)** Viability and Non viability of pollen grains in 0.5% TTC

**Figure: Meiotic configurations and abnormalities in *Chlorophtum laxum* R. Br.**

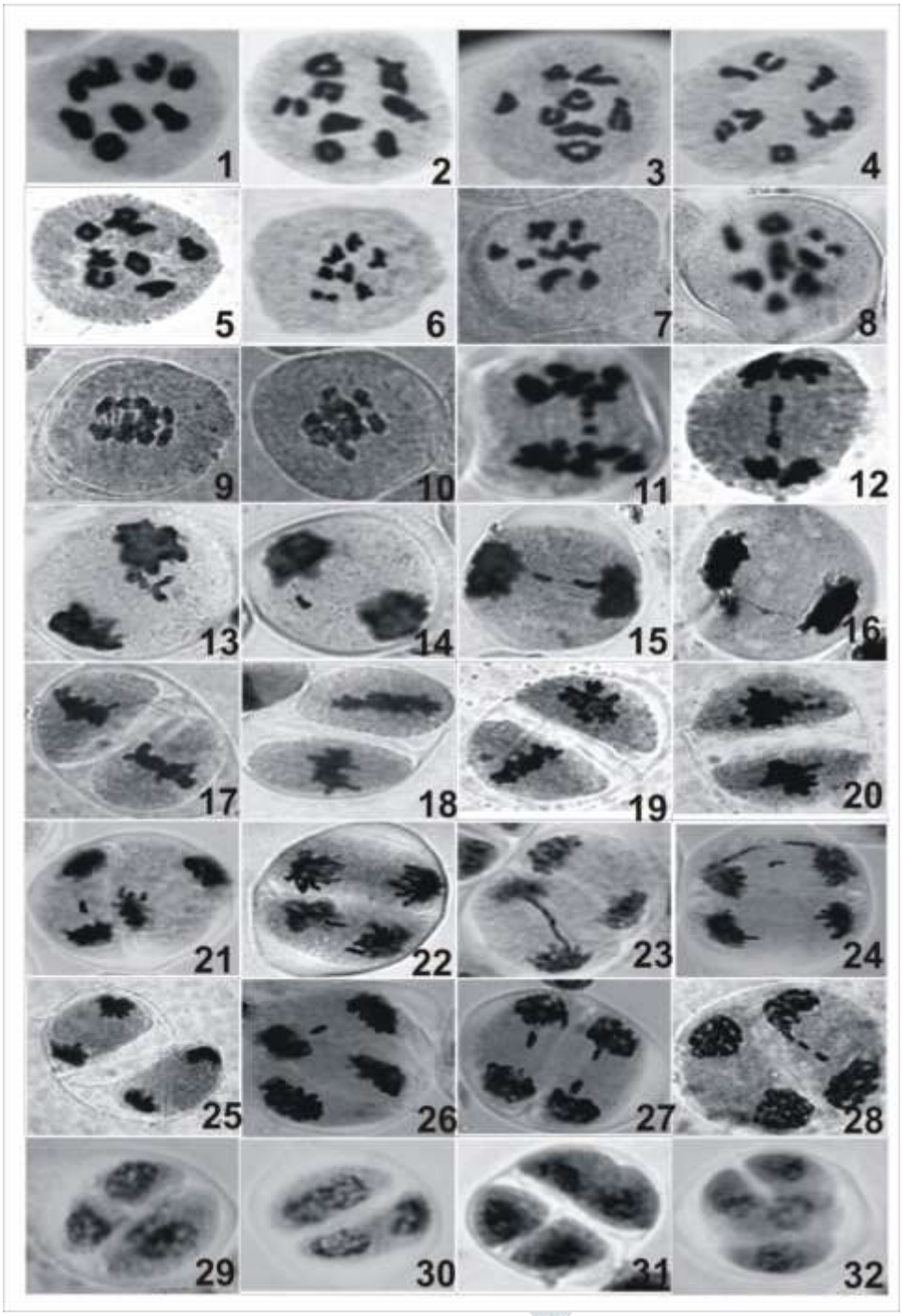


**Figure:-Chromosome configurations and meiotic abnormalities in *C. laxum* R. Br.** 1) Diakinesis with 8 II 2) Diakinesis showing 1III+5II+3I, 3-4) Diakinesis showing 8II with different shapes (ring, rod, c&v-shape, etc), 5) Metaphase I with 8II with different shapes 6) Metaphase I with 2III+4II+2I 7) Metaphase I showing 1III+6II+1I 8) Metaphase I showing 2III+3II+4I 9-10) Sticky Metaphase I with micronuclei 11) Anaphase I showing laggards 12) Anaphase I with chromatid bridge 13-14) Telophase I with precocious chromosome/laggard 15-16) Telophase I showing formation of chromatid bridge 17) Metaphase II showing equal distribution of chromatin material 18) Metaphase II showing change in orientation 19) Metaphase II showing change in orientation with laggard 20) Sticky metaphase II with laggards 21) Anaphase II showing change in orientation with laggard 22) Anaphase II showing laggard 23) Anaphase II with chromatin bridge 24) Anaphase II showing formation of chromatid bridge with laggard 25) Telophase II showing equal distribution of chromatin material 26-27) Telophase II with laggard/multiple laggards 28) Telophase II showing formation of chromatin bridge 29) Triad (tetrad showing unreduced gametes) 30) disturbed tetrad 31) Tetrad showing unequal distribution of chromatin material 32) Tetrad with change in orientation.

**Meiotic configurations:-** In the present investigation *Chlorophytum laxum* shows regular meiotic configuration with some meiotic abnormalities (Table-1) which include chromosomal multiple associations, chromosome stickiness, micronuclei, equatorial arrangement of chromosome, dicentric bridge formation with laggards, Snapping of bridge, Unequal distribution of chromatin material, cytomixis, abnormal spindle, laggards and chromatin bridges, and united pollen grains. The meiotic analysis in *Chlorophytum laxum* was

studied by observing several number of microsporocytes and near about 200 microspore tetrad. The different meiotic abnormalities from diplotene to tetrads are represented in Table-1. Most of the PMC's i.e. 85-90% shows 8 biavalents at diakinesis (Fig: 1) in *C. laxum*. Very rarely diakinesis with 1III+ 5II+ 3I was seen (Fig: 2). Few chromosomes showed chiasmata in diakinesis showing 8 II with different shapes (Ring, rod, c &v shape, etc) (Fig: 3-4). Metaphase I shows 8 bivalents with different shapes (Fig: 5) along with few multivalent associations like 2III + 4II + 2I (Fig: 6), 1III + 6II + 1I (Fig: 7), 2III + 3II + 4I (Fig: 8) were found. The diakinesis with normal 8 bivalents showed the perfect pairing of chromosomes during the division with multivalent state at Metaphase I (Fig-5,7,8) may be due to the early disjunction of bivalents at diakinesis or failure of their pairing during Prophase-I. Sticky metaphase-I (Fig: 9, 10) and M-II with with micronuclei (Fig: 20) shown in several PMcs it seems that seven bivalents congregate on the equatorial plate and one remain unoriented and lags behind in Anaphase-I (Fig: 11, 12) and A-II (Fig: 21, 22, 24) with irregular meiotic spindle (Fig: 21) formation of chromatid bridge (Fig: 23, 24) along with equal distribution of chromatin material (Fig: 25). Also Metaphase II showing equal distribution of chromatin material (Fig: 17) and change in orientation (Fig: 18) along with laggard formation (Fig: 19). During Telophase-I and T-II the chromosomes were uncoiled to chromatin showing single to multiple laggards (Fig: 13, 14, 26-28) and showed the formation of chromatin bridge (Fig: 15, 16). The tetrads also showed some variations in their behavioral pattern. In *C. laxum* triads (tetrads with unreduced gametes) (Fig: 29) disturbed tetrad (Fig: 30) some tetrads with unequal distribution of chromatin material (Fig: 31) along with change in orientation (Fig: 32) were also observed.

**Pollen fertility: -** In the present investigation after analyzing the pollen slides about 10 times in available media it is observed that the pollen viability in acetocarmine and glycerine (1:1) was found to be 93.57% (Table-2) while in 0.5 % TTC (2,3,5- triphenyl terazolium chloride) was 59.76% (Table-3). In acetocarmine and glycerine viable pollen appeared red/pink whereas non viable pollen was colourless (Fig: A) and in 0.5 % TTC the viable pollen appeared red in colour due to accumulation of formazen (Fig: B). It was observed that pollen viability was greater than 90% in *C. laxum* where as average in 0.5 % TTC i.e. 50-60%.

## IV. DISCUSSION

The meiotic cell division is a critical reproductive process, and is tightly controlled to guarantee reductional homologous chromosome segregation and subsequent formation of haploid male and female gametes. In some instances, however, alterations in the meiotic program or cellular defects in meiosis I (MI) or meiosis II (MII) may switch the meiotic cell division into a mitotic-likeone, generating diploid spores out of a diploid mother cell. This mechanism is generally termed meiotic restitution or meiotic non-reduction, and the resulting gametes are referred to as unreduced or 2n gametes (Veilleux, 1985; Bretagnolle *et al*, 1995; Brownfield and Kohler, 2011; Storme and Geelen, 2013). In *Chlorophytum laxum* meiotic configuration in most of the PMCs is highly regular with 8 bivalents in diakinesis representing the basic number x=8 which confirmed that this species is diploid in nature with 2n=16 chromosome number which supports the findings of regular meiosis with 8II in this genus was reported by earlier workers (Datta and Mitra, 1958; Patil, 1988; Kumar and Rao 1958, Basu and Jha, 2011). Naik (1976) studied the meiotic behavior in *C. laxum* and concluded that this species is a segmental allopolyploids from two closely related

but still unknown species with 2n=8 chromosomes. This is evident from the occasional non-disjunction in the entire complement, which suggests the segregation of ancestral genomes. Further evolution proceeded in two different directions. In one diploidization by chromosomal rearrangements resulted in to species with 2n=16 chromosomes, while in other, elimination of a non-homologous segment gave rise to species with 2n=14 chromosomes and several autopolyploids.

As the literature concerned there are various ploidy level in *Chlorophytum.* The diploid chromosome number of some species of *Chlorophytum* is 2n=16 with 8II in *Chlorophytum tuberosum* (Gudadhe *et al*, 2019) and while other species *Chlorophytum comosum* shows 2n=28 with 14 bivalents (Gudadhe *et al*, 2012) ploidy up to 2n=56 in *Chlorophytum nepalensis* (Basu and Jha, 2008) and this support the investigation that in this genus polyploids from tetraploid to octaploid level are identified in the population with two basic chromosome number, X=7 and X=8 (Baldwin and Speese, 1951). In *C. laxum*, diploids (2n=16) and a rare tetraploid (2n=32) have been recorded by Sheriff and Nagraj (1976) and (Patil and Gandhi, 1988). The meiotic analysis of three species of *Chlorophytum* namely *C. elatum*, *C. heynei* and *C. laxum* and showed regular type of meiosis with the presence of 14, 7 and 8 bivalents. Thus the cytological studies of different populations have revealed that Indian populations of *C. laxum* are characterized by having 16 chromosomes (Fig: 2). The diploidy being found predominantly, in most of the populations while the tetraploids are of rare occurrence. The cytological studies clearly show 7 and 8 basic euploid series in the genus *Chlorophytum.* Such a phenomenon where in different ploidy exist in different species of the same genus is not very uncommon in the Liliaceae (Kameshwary and Muniyamma, 2001).

In *C. laxum* the presence of rearrangements very common but along with that first time such chromosome abnormalities were observed in the particular range (Table-1). In the present investigation most of the PMc's shows normal meiotic behavior but 50-60% Pmcs shows multivalent chromosome configurations (Fig: 2,7-8) and its too hard to understand the behaviour of the paired chromosomes and their segregation during the first division of meiosis. The multivalent association in C. *laxum* shows trivalents, bivalents and univalents and during the analysis most of the meiotic configurations were observed but from them only few are shown here. The occurrence of two univalents may be due to the failure in their pairing during prophase-I. Early disjunction of a bivalent at diakinesis would also lead to a similar situation. (Gudadhe *et al*, 2019). Some knowledge of the structure of the kinetochore region is also important for an understanding of certain features of chromosome behavior (Ostergren, 1995). Genetic variation in chromosome rigidity and in the capacity of the centromeres to reorientate must be the cause of the observed genetically conditioned variations in preference for a certain orientation type (Sybenga, 1968).

The reduction of interstitial chiasma in *C. laxum* suggest the random distribution, free terminalization and least linear differentiation. On contrary *C. laxum* with x=8 have restricted their occurrence under arid and semiarid conditions (Patil and Gandhi, 1988). Chiasmata which is observed in various meiotic stages is important because it is responsible for maintenance and segregation of chromosomes. Generally one to two chiasma/bivalent was seen in *C. laxum* (Fig: 2,3,5). There is good reason to suppose that adjustments in chiasma frequency at meiosis may be adaptive, especially insofar as they regulate the amount of gene recombination in outbreeding populations. One should expect, in consequence, to find the chiasma

frequencies of populations within a species to vary according to their habit or habitat (Rees and Ahmad, 1963).

Chromosome stickiness was the other anomaly found in *Chlorophytum laxum* (Fig: 9, 10, 11, 12, 5, 6, 23, 24). Although the first reports of chromosome stickiness were published at the beginning of the century (Kiiernicke 1905), the term "stickiness" was first employed by Beadle (1932) when he described the sticky aspect of chromosomes in maize cells that had suffered a mutation. The recessive gene was denoted sticky (st). Chromosome stickiness is caused due to genetic and environmental factors and several agents have been reported to cause chromosome stickiness (Pagliarini, 2000). Stickiness has been reported to be a result of partial dissociation of nucleoproteins and alteration in the pattern of organization of chromosomes (Evans, 1962) or due to disturbances in cytochemically balanced reactions (Jayabalan and Rao, 1987). Gaulden (1987) postulated that stickiness may result from the defective functioning of one or two types of specific non histone proteins involved in chromosome organization which are needed for chromosome separation and segregation. The altered functioning of these proteins is caused by mutation in the structural genes coding for them (hereditary stickiness) or by the direct action of mutagens (induced stickiness). Sticky metaphases with clumped chromatin material and the precocious accessions were observed in this species. Chromosome bridges resulting from stickiness were observed in anaphase-I and II as well as in telophase-I and II stages (fig:-15, 16, 23, 24, 27).

Among the most common type of meiotic abnormalities was PMCs with laggards. These laggards when failing to get included in telophase nuclei resulted in the formation of micronuclei at late telophase and sporad stage. (Puneet Kumar and Singhal, 2011).This type of abnormality was observed in both first and second meiotic divisions in *Chlorophytum laxum*. The induction of laggards at metaphase I in diploids may be extending in all subsequent meiotic stages. In this respect many phases such as Metaphase I and II, Anaphase I and II, Telophase I and II with laggards were observed (Fig:9, 11, 14, 19, 20, 21, 24, 26. 27 ). The induction of laggards could be attributed to irregular orientation of chromosomes (Dimitrave and Gadeva, 1997). Lagging chromosomes did not reach the poles as a result of the absence of spindle fibers or irregular formation. This has a role in the formation of micronuclei. In another case, PMCs were mitotically divided without reducing the chromosome number, chromosomes move towards the poles by seperating longitudinally and form diads in which chromosome number has not been reduced. Chromosome distribution is generally irregular between these dyads. Size of the nuclei is also different in dyads with unequal chromosome number (Silva-Stort, 1984).

Other most prominent meiotic anomalies noticed were disorientation of spindles during the second meiotic divisions owing to spindle irregularities. In *Chlorophytum laxum* disturbed spindle apparatus orientation at metaphase II (Fig: 19) anaphase II (Fig. 21) and tetrads (Fig: 32) was observed resulted in unoriented chromatin material at meiotic stages. Disturbances in chromosomal movements occur due to suppression of spindle movement which in turn may be due to changes in cytoplasmic viscosity (Kostoff, 1930; Sax, 1937; Sheidai and Inamdar, 1991). Randomly scattered chromosome fragments or micronuclei are often associated with irregularly shaped spindles. The normal functioning of spindle apparatus is crucial

for chromosome alignment during metaphase and correct segregation of chromosomes to poles (Shabrangi *et al.*, 2010).

Unequal distribution of chromosomes may result in a larger number of chromosomes in one of the daughter nuclei than in the other and consequently the former nucleus would be larger. During anaphase II the separations in such cell would result in equal distribution of the chromosomes, the larger nucleus forming two larger daughter nuclei and the two chromosomes of the smaller nucleus forming two smaller nuclei. Such irregularities would result in the production of a variety of tetrad formation with unequal sized nuclei, micronuclei and polysporous condition (Sheriff and Rao, 1974). All the described meiotic irregularities lead either to meiotic arrest or gametic loss and cell restitution, with the eventual formation of unbalanced gametes. As a consequence of these disturbances during meiosis, the plants have greatly reduced their fertility (Sanso and Wulff, 2007). Presently unreduced gametes were found in *Chlorophytum C. laxum* (Fig: 29) along with unequal distribution of chromatin material (Fig: 31) and change in the orientation (Fig: 32). Triad was observed in case of C. *laxum* (Fig: 29) but in *C. comosum* the meiocytes showed single microspore (monad), dyad, and variations in orientation of triad (Gudadhe *et al*, 2012). The occurance of unreduced gametes along with hybridization may have played important role in the production of a higher polyploidy level and the evolution of the genus *Chlorophytum*. In general, these abnormalities affect the pollen fertility and the seed production as consequence. The data reinforce that seed production depends upon regularity of meiotic process among other factors.

The meiotic abnormalities obstruct the normal cell division and partially affect pollen fertility. High meiotic stability ensures high pollen fertility. The amount and quality of pollen produced by a flower is an important component of fitness (Nathar, *et al*, 2013). The amount and quality of pollen produced by a flower is an important component of fitness. Pollen quality is often equated to pollen viability, i.e., the proportion of pollen grains that are viable. While viability can be measured in number of ways (Stanley and Linkens, 1974). The production of viable pollen involves a complex series of developmentally regulated processes, involving the differentiation and degeneration of specialized tissues, and the coordinated expression of thousands of sporophytic and gametophytic genes (Peirson *et al*, 1996). Pollen viability is always greater than germinability. The highest pollen viability in case of *C. laxum* was obsereved in acetocarmine and Glycerine (Table: 3/Fig: A) media than in 0.5% TTC (Table: 3/Fig: B). Many genetic studies demonstrate that poor quality pollen can also reduce seed production which is interpreted as a quality limitation (Aizen and Harder, 2007).

## V. CONCLUSION

From the present study it is concluded that the variations in meiotic configuration during diakinesis and other further processes along with meiotic abnormalities in *Chlorophytum laxum* may lead to the change in the final product and can be affect ploidy level as well as pollen fertility in the genotype and this may be the reason of having different ploidy level in *Chlorophytum laxum* as mention in earlier reports. There are various meiotic configurations were obsereved in *Chlorophytum laxum* which supports the earlier published reports of various authors that C. *laxum* shows 2n=16 with 8 bivalents with regular meiosis. But the detailed analysis of meiotic behavior of chromosomes in C. *laxum* showed the various meiotic

abnormalities which are not reported earlier and for this anomaly may be the genetic, environmental or any other factors are responsible.

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