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Cytomixis with associated chromosomal anomalies and the reproduction of *Chlorophytum borivilianum* Santapau & R. R. Fern.

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ABSTRACT: Cytomixis, a phenomenon of mixing up of cell contents of adjacent cells, though unusual in nature, have been witnessed in plenty of angiospermous plants. In addition, coexistence of some other irregularities in chromosomal behavior has also been noted in many a case to violate the normal process of meiosis. In this paper an account of the occurrence of cytomixis in the pollen mother as well as tapetal cells of *Chlorophytum borivilianum* Santapau & R. R. Fern. has been presented for the first time. A variety of chromosomal abnormalities has been recorded too. The species, mainly known for aphrodisiac properties, also provides biochemicals of curative properties against many ailments. The herb reproduces mostly by vegetative means and seed germination is meager. The nature and extent of cytomixis associated anomalies seem to provide a plausible cue in understanding the occurrence of only vegetative means of reproduction. Cytomixis of an accrued amount of 4.21 % occurs with greater preponderance only in meiosis I; while, chromosomal abnormalities, altogether of 21.16 %, are recorded in different stages of both of meiosis I and II. In PMCs the varieties of unusual features recorded are chromatin transfer to adjacent cell, chromosome stickiness, loss of chromosomes, improper orientation of chromatin/chromosomes, chromosome bridges and formation of micronuclei at different stages. A considerable extent (27.20 %) of pollens has been noted to be sterile.

KEY WORDS: *Chlorophytum borivilianum*, Chromosome anomalies, Cytomixis, Meiosis, Reproduction, Seed germination.

INTRODUCTION

Chlorophytum borivilianum Santapau & R. R. Fern. is an important medicinal plant belonging to the family Asparagaceae. Altogether 216 species of the genus *Chlorophytum* have been recorded worldwide and among them only 18 species are reported from India (Adsul *et al*., 2014; Mandal, 2015). *Chlorophytum borivilianum* is commercially important for its aphrodisiac saponin content (Mandal and Nandi, 2012, 2013 a, b). This species is now often claimed as endangered in India (Nayar and Sastry, 1988). The occurrences of cytomixis in both pollen mother cells and tapetal cells as well as various chromosomal anomalies in different phases of meiotic divisions of the species have been witnessed in the present study. The occurrence of such anomalies in generative cells is believed to interrupt the reproductive success, seed production and germination of seeds.

Cytomixis has been reported to occur in a large number of angiospermous plants (Lattoo *et al*., 2006; Mandal *et al*., 2013c; Mursalimov and Deineko, 2015), infrequently in tapetal cells (Cooper, 1952) and ovary cells (Koul, 1990) and also found in some somatic cells of plants (Wang *et al*., 2004; Guzicka and Wozny, 2005). Its prospective evolutionary significance often being apprehended has still remained controversial (De Souza and Pagliarini, 1997; Malallah and Talaat, 2003). Meiosis is an event of high evolutionary stability, any defiance of which culminates in a reduction or enhancement of chromosome number. The normal and harmonious course of meiosis ensures gamete viability. The cytological events of gametogenesis are known to be controlled by a large number of genes that act from premeiotic to postmeiotic stages. Mutations in those genes or any structural change in chromosome impair fertility by causing male sterility in plants (Baker *et al*., 1976; Wijnker and Schnittger, 2013; Kravets, 2013).

MATERIAL and METHODS

The plant *Chlorophytum borivilianum* Santapau & R. R. Fern. was collected from Jeevan Herbs, Madhya Pradesh and Medicinal Plants Garden under the Forest Department of West Midnapore in West Bengal, India. Accessions numbers of two populations are Cbvu 100 and Cbvu 151 respectively. Specimens of the accessions have been kept in the collection of the Department of Botany & Forestry of Vidyasagar University. All the germplasms are maintained in the Garden of Vidyasagar University.

Cytological Study

Fresh juvenile flower buds were taken from 15 plants of each of two populations. They were preserved in 70% alcohol after keeping in modified Carnoy's solution (6 : 3: 1 of ethyl alcohol, glacial acetic acid and chloroform) for 2 hours. Both fresh and preserved

buds were stained in 1% Aceto-Carmine and observed under the microscope (Leica DM100).

Reproductive Study

30 plants of each of two populations from Medicinal plants garden of West Midnapore and Madhya Pradesh were planted and randomly 15 plants were selected from each population. The study of PMC, total number of flowers per inflorescence, total number of fruits per inflorescence, total number of ovules per fruit and total number of seeds per fruit were carried out with this selected 30 plants. Fertility of pollen was studied by staining them with aceto-carmine. Sterile pollens took either no stain or less stain with respect to the fertile ones. For the purpose of estimation of seeding efficiency, totally 30 inflorescences, by randomly selecting single inflorescence from each of 30 selected plants, were selected and tagged randomly for scoring the total number of flowers and ovules, as well as the number of seeds in a capsule (Table 2). Reproductive success (Number of fruit / Number of flower × Number of seed / Number of ovule) was calculated according to Weins *et al.*, (1987). Seed germination percentage was calculated from randomly selected 100 seeds.

Statistical analysis

The numerical data were statistically analyzed with SPSS 21.1 version software. The level of significance used in test was P = 0.05.

RESULTS

Cytoplasmic fusion was found to occur among the cells of different divisional stages of pollen mother cells. Cytoplasmic connections were observed in 2 to 9 cells at a time (Fig. 1a). Chromatin migration was also noticed in some tapetum cells during the mitotic division (Fig. 2a).

During meiosis I and II various types of chromosome clamping and stickiness were observed at Prophase II, Metaphase I & II and Telophase I & II (Fig. 1b, n-q). Excessive stickiness of chromosomes was often noted to disorganize the chromosome structure (Fig. 1p). The intensity of stickiness was 10.73 % in both meiosis I & II.

Chromosome bridges at anaphase stage of PMCs and also in mitosis of tapetal cells were noted in C. borivilianum (Fig. 2b). Percentage of chromosome bridge was high in meiosis than in mitosis of tapetal cells. Unequal segregation of chromosomes and cells with laggard chromosome were detected with a frequency of 4.52% and 2.86 % respectively, in different divisional stages (Table 1, Fig. 1i, j).

Separation of a lump of chromatin from the main part was noted in both of prophase I & II with a frequency of occurrence of 6.56 % in pollen mother cell (Table 1, Fig. 1b, c). It was noted that the amount of scattered chromatin lump was more during leptotene to pachytene in prophase I.

Table 1. Cytological features in ***Chlorophytum borivilianum***

Feathures	Mean (%) ± SD
Chromosome Number	2n = 28; X = 7
Cyto-mixis (%)	4.21 ± 0.60
Chromosome abnormality (%)	21.16 ± 12.62
Laggard Chromosome (%)	2.86 ± 1.24
Chromosome bridges (%)	01.23 ± 1.12
Chromosome stickiness (%)	10.73 ± 5.53
Chromatin colony (%)	6.56 ± 4.92
Micro-Nuclei (%)	4.17 ± 3.64
Loss of chromosome (%)	3.51 ± 2.87
Unequal segregation of chromosome (%)	4.52 ± 2.59
Pollen sterility (%)	27.20 ± 11.18

SD = Standard deviation.

Presences of micronuclei or migrated chromosomes were noted in 4.17% cells (Table 1). Micronuclei and migrated chromosomes were recorded in different stages of meiotic division (Fig. 1f). Existences of migrated chromosomes were witnessed in the contiguous cells at metaphase-I (Fig. 1g).

Some pollen mother cells were noted to have no chromosome or with a very low number of 3 or 4 only (Fig. 1k). In such a condition the divisional stage of the affected cell becomes confounding.

The average numbers of pollen grains found to be present in an anther were 31260.02 ± 11.42, the pollen ovule ratio per flower was 74166.68 ± 4.46 (Table 2). Although every plant had more than 30 flowers, only less than 60% of them could get matured into fruits (Table 2). The number of flowers that matured into capsules per plant were 13.89 ± 7.28 and the number of ovules that got matured into seeds per capsules were 3.49 ± 1.56 (Table 2). The seed germination percentage in the species was noted to be about 10.20%. Reproductive success (Fr/Fl × S/O) was extremely low (0.425) (Table 2).

DISCUSSION

Cytomixis in different angiospermous plants has been reported since the beginning of nineteenth century by several workers (Heslop-Harrison, 1966; Lattoo *et al*., 2006; Mandal *et al*., 2013c). However, the origin and significance of cytomixis have still remained controversial. According to previous reports it is a normal and genetically controlled phenomenon influenced by physiological and environmental factors (Baker *et al*., 1976; Lattoo *et al*., 2006; Wijnker and Schnittger, 2013). Notwithstanding the unknown origin and nature of cytomixis, most workers agreed with its significant role in plant evolution (Malallah and Talaat, 2003; Guan *et al*., 2012).

Two species of *Chlorophytum* have shown cytomixis

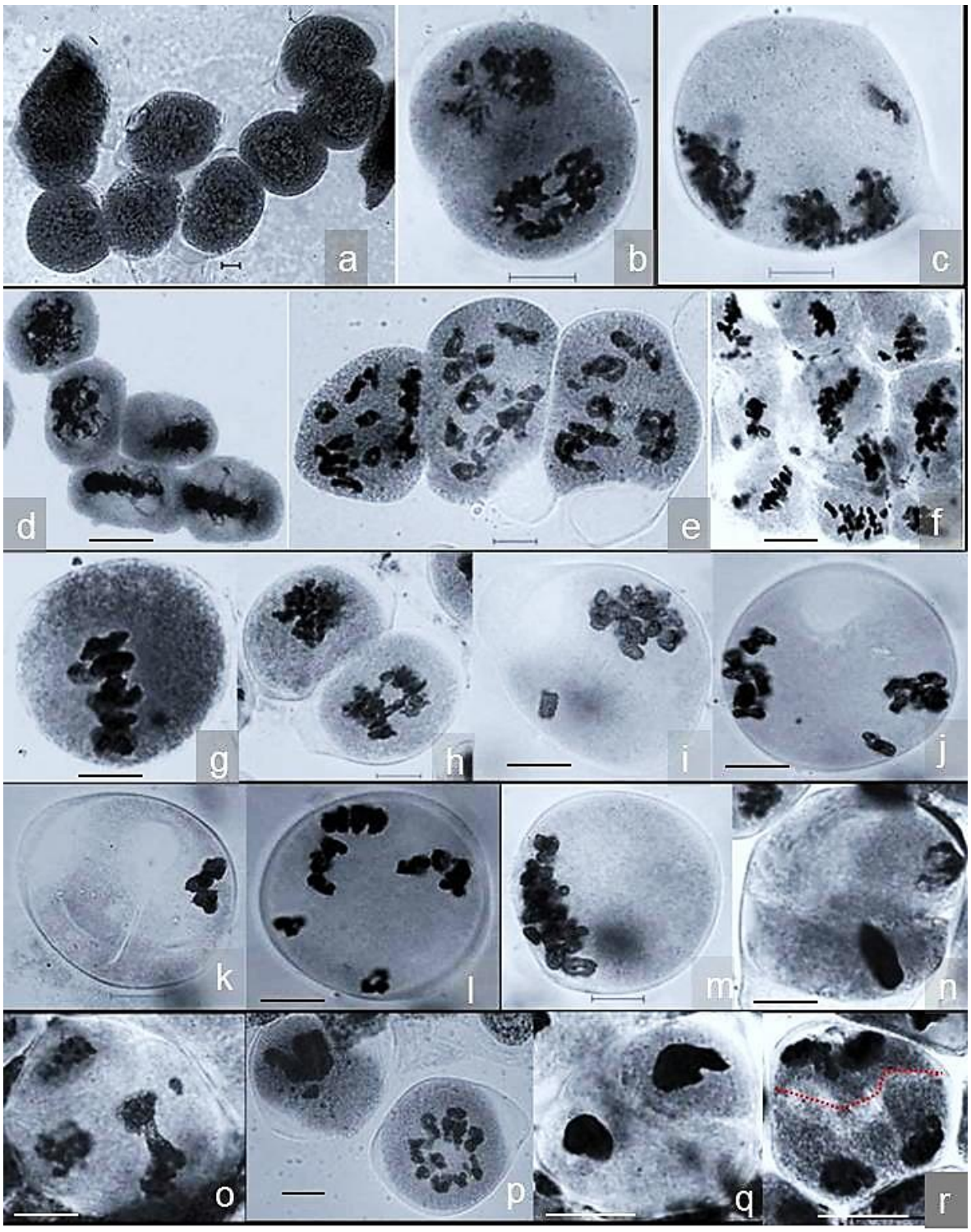

Fig. 1. Meiotic chromosome abnormalities at different stages. **a)** fused pollen mother cells at early stage of Zygotene, **b)** clumped chromosomes in groups at Prophase II, **c)** eccentric placement of chromosomes at Prophase I, **d)** abnormal configuration of chromatin at Leptotene of Prophase I, **e)** cytoplasmic fusion at Diakinesis of Prophase I, **f & g)** migrated or fragmented chromosomes, **h)** chromosome bridges at Anaphase I and highly clamped chromosome at Anaphase I, **i)** unequal chromosome separations at Anaphase I, **j)** laggard and sticky chromosome at Anaphase I, **k)** massive loss of chromatin at Meiosis I, **l)** spindle disturb chromosomes at Metaphase I, **m)** eccentric placement of chromosomes at Anaphase I/II, **n)** loss of chromosome at Telophase II, **o)** highly clumped chromatin and micronucleus at Telophase II, **p)** clamped chromosomes & normal Diakinesis, **q)** clamped and sticky chromosomes loss their morphological appearance, **r)** unequal separation of cytoplasm and unusual orientation of chromosome lump at Telophase II. (Bar scale 10 µm).

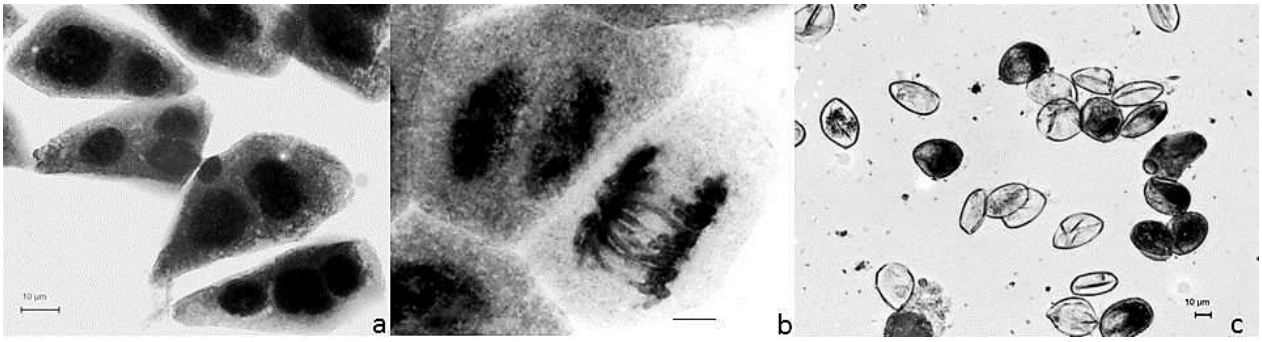

Fig. 2. Chromosome abnormalities in tapetal cells and pollens of ***C. borivilianum***. **a)** Chromatin transfer through cytomitic channel in tapetum cells, **b)** chromosome bridges at Anaphase of tapetum cell, **c)** sterile pollens along with fertile pollens. (Bar scale 10 µm).

Table 2. Parameters showing reproductive efficacy of ***Chlorophytum borivilianum***.

Characters	Mean (%) ± SD
Number of flowers/plant (Fl)	21.37 ± 7.41
Number of anther/flower	6
Number of ovules/flower (O)	5.34 ± 0.67
Number of pollen grains/anther	31260 ± 11.42
Number of pollen grains/flower	170226 ± 1.85
Number of capsules/plant (Fr)	13.89 ± 7.28
Number of seed/capsule (S)	3.49 ± 1.56
Seed germination (%)	10.20 ± 2.30
Pollen/ovules ratio	74166.68 ± 4.46
Seed efficiency (%)	58.11 ± 16.07
Reproductive success(Fr/Fl × S/O)	0.425

SD = Standard deviation.

with chromosomal abnormalities in pollen mother cells (Lattoo *et al*., 2006; Gudadhe *et al*., 2012; Mandal *et al*., 2013c). But cytomixis in tapetum cells as well as cytoplasmic fusion among the PMCs have been registered here in *Chlorophytum borivilianum* for the first time. Authors have also witnessed several chromosomal anomalies in both PMCs and tapetum cells of this species (Figs. 1 & 2). Several chromosomal disorders have been documented here and it has also been observed that meiosis I is more affected than meiosis II (Table 1). These anomalies may have different implications. 1) The loss of chromatin and unequal separation of chromosome can play a vital role in the evolution of the species (Table 1, Fig. 1 i, k & n). 2) Irregular orientation of chromosomes arises due to impediment of spindle fiber (Table 1, Fig. 1 i, m, o & r). Highly clumped or extremely sticky chromosomes are not separated from each other and as a result they produce eccentric chromosomes at anaphase (Fig. 1m). Earlier reports also support this observation (Liu *et al*., 2004; He *et al*., 2011). 3) Unequal separation of cytoplasm may produce squeezed and sterile pollens (Table 1, Fig.2 c). 4) Migrated chromosome or acquittal part of chromosome i.e. excess DNA in the PMCs may produce pollen with greater amount of DNA or greater ploidy (Table 1, Fig. 1 c, f & g). With the progress of meiosis the pycnotic masses were either eliminated or resulted into the formation of micronuclei (Table 1, Fig. 1o). Earlier works agree with such findings (Guan *et al*., 2012; Mursalimov *et al*., 2013). As a result, cytomixis could be considered as a possible reason for the development of polyploid or aneuploid plant (Morikawa and Leggett, 1996; de Souza and Pagliarini, 1997). Incidences under cytomixis may culminate into the development of B chromosome(s) (Cheng *et al*., 1975) or different basic numbers of chromosomes as well. In the light of these considerations the occurrence of two basic chromosome numbers, i.e. X = 7 and X = 8, in this genus appears to be significant, though the species *C. borivilianum* consistently has X =7.

Previous works directed that cytomixis and irregular behavior of chromosomes have an impact on the development of sterile pollens in plants (Lattoo *et al*., 2006; Kravets, 2013; Mursalimov and Deineko, 2015). The phenomenon is also quite apparent in case of *Chlorophytum borivilianum*, as adduced by the occurrence of plenty of sterile pollens in it (Table 1, Fig. 2c).

Pollen fertility has a direct impact on the production of vigorous seeds, which increases the rate of reproduction success in plants. Cytomixis having a bearing on pollen fertility can also play an important role in the reproductive performance of plants (Lattoo *et al*., 2006; Kravets, 2012), as also revealed by the occurrence of low seed germination 10.20 % in *C. borivilianum* leading to a concomitant decline in the reproductive success of the species (Table 2).

Thus, the present investigation visualizes the preponderance of cytomixis as well as other irregular chromosomal behaviors in the first meiotic cycle of this species. Breakage of spindle occurs at metaphase I and unequal separation of chromosomes at anaphase obstructs the proper segregation of chromosomes at meiosis I. The fate of such PMCs with extra or less number of chromosomes may produce either sterile pollen or pollen with different chromosome numbers. All these chromosomal and cytological changes purport their possible link with the regular vegetative reproduction of the species and paucity of seed production in *Chlorophytum borivilianum*.

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