Abstract

Till date, several classical and modern methodological tools had been employed to investigate the phenomenon of cytomixis for developing a clear understanding about its occurrence, causes, mechanism, and role in plant evolution. These attempts have succeeded to some extent in modifying general perception about it ever since its discovery. Based on the published literature as well as our own work, the authors opined that cytomixis has no correlation with ploidy level/genomic status. Rather, it is the genetic makeup and environmental conditions which are responsible for cytomixis. We also assume that recipient meiocytes adjust to extra chromatin either through exclusion or condensation of chromatin. Otherwise, meiocytes depicted spindle abnormalities leading to gametes with the variable genetic constitution. Such gametes led to the origin of aneuploids/polyploids. High frequency of cytomixis during early stages has a direct correlation with the easier passage of genetic material. In some cases, whole nucleus migration resulted into syncytes which yielded unreduced pollen, leading to the origin of polyploids. Another evolutionary aspect of cytomixis is that it induces high pollen sterility in plants of cold deserts acquiring perennial habits and vegetative modes of propagation. We are of the opinion that like other cytological processes, cytomixis is a natural meiotic aberration of potential evolutionary significance.

Keywords: Cytomixis; evolution; plants; polyploidy; pollen mother cell

Introduction

Inter-cellular chromatin migration (cytomixis) among proximate cells through cytomictic channels was observed for the first time by Arnoldy (1900) in gymnosperms and a year later by Körnicke (1901) in pollen mother cells of Crocus sativus and by Miehe (1901) in the leaf epidermis of Allium cepa. However, the term “cytomixis” was coined by Gates (1911) when he observed the phenomenon during microsporogenesis in Oenothera gigas. Such cytoplasmic connections originate from the pre-existing system of plasmodesmata which develop in anther tissue and then, in general, obstructed/plugged off by the progressive deposition of callose (Heslop-Harrison 1966). However, malfunctioning of certain genes during cell division failed to plug these pores which persist till the later stages to form intercellular channels through which transfer of nuclear material and other cell contents may take place (Shabrangi et al. 2010, Mursalimov and Deineko 2015).

Materials and Methods

Occurrence, causes, impact on meiosis, microsporogenesis and pollen grains

Cytomixis has been witnessed more often in meiocytes during microsporogenesis in flowering plants (Mandal et al. 2013, Mursalimov et al. 2013, Kumar et al. 2015, Kumar and Chaudhary 2016, Kumar and Singhal 2016, Mursalimov and Deineko 2018, Mursalimov et al. 2018). However, it has also been noticed in animals during spermatogenesis (Ventela et al. 2003), mammalian cells (Gerdes et al. 2007, Zani and Edelman 2010), lower plants like Chara (Kwiatkowska et al. 2003), transgenic plants of Nicotiana tabaccum (Sidorchuk et al. 2007), inter-generic hybrids (Sun et al. 1994) and in plants of Brassica napus exposed to electromagnetic fields (Shabrangi et al. 2010). In addition, cytomixis has been observed in other tissues such as root tip cells (Bobak and Herich 1978), tapetal cells (Wang et al. 2004), phloem parenchyma (Murmanis and Evert 1967), ovary cells (Koul 1990) and pro-embryos of cereals (Klyuchareva 1983). Cytomixis is the most characteristic of plants with imbalanced genomes such as haploids, triploids, hybrids, apomicts, and aneuploids (de Nettancourt and Grant 1964, Yen et al. 1993, Haroun 1995, Nirmala and Rao 1996, Peng et al. 2003, Li et al. 2009, Singhal et al. 2011a).

Ever since its first report, which appeared 117 years ago, the precise mechanism underlying the phenomenon is now vague and has acquired varied notions ranging from
Cytomixis causes, mechanisms and role

Cytomixis is an artifact of cytological preparation (Heslop-Harrison 1966), action of chemical agents (Dwivedi et al. 1988), effect of herbicides (Bobak and Herich 1978), changes in biochemical processes that involve microsporogenesis (Koul 1990), physiological and environmental factors (Lattoo et al. 2006), pressure difference (Tarkowska 1965) and stress factors and genetic control (Malallah and Attia 2003). Recently, researchers have assumed that cytomixis is a mechanism which provides selective removal of the damaged part of chromatin (Barton et al. 2014). On the other hand, some molecular cytogeneticists employed state-of-the-art technology (electron and confocal laser microscopy, immuno-staining, and fluorescence in situ hybridization) to inspect the migrating nuclei during cytomixis in pollen mother cells and concluded that migrated chromatin is not degraded but gets included in the recipient cell nucleus and helps in changing the karyotype of resulting pollen and future gametes (Pécrix et al. 2011, Mursalimov and Deineko 2015, 2018). Additionally, considering the above interpretation, Mursalimov and Deineko (2017) proposed that cytomixis may be regarded as a putative mechanism of genetic recombination and/or polyploidization in plants. It has also been presumed that cytomixis is a special kind of cell selection leading to apoptosis (Kravets 2011). Some cytologists also argued that chromatin transfer among cells is known to produce new phenotypic characters (Sheidai et al. 2001, 2003) and origin of B-chromosomes (Cheng et al. 1975). However, the authors are of the opinion that as per the nuclear/cytoplasmic ratio and volume of recipient meiocytes, cells possibly adjust to extra chromatin either by excluding extra chromatin through pycnosis or by acquiring the modified gene/s related mechanism instigating condensation and stickiness of chromatin. Beyond this, the microtubules of the spindle apparatus get disrupted. The space constrains created due to extra chromatin in hyperploid meiocytes after cytomixis resulted into various meiotic irregularities which included chromatin stickiness, spindle anomalies leading to non-synchronous separation of bivalent, laggards and chromatin bridges, micronuclei, multiple poles, restitution nuclei and so abnormal sporads. The products of such sporads yielded heterogeneous sized fertile and sterile pollen grains and micro-pollen (Kumar et al. 2010, 2012, 2014, Singhal and Kumar 2008, Singhal et al. 2008, Guan et al. 2012, Rana et al. 2013, Lovleen and Saggoo 2015, Reis et al. 2016). So far, the authors have not come across any aneuploid plants in the species, depicting the phenomenon of cytomixis. However, Malallah and Attia (2003) detected aneuploid plants of Diploptaxis harra and considered cyto-
mixis to be one of the probability for their possible origins.

Some researchers have opined that cytomixis is more prevalent in species with higher ploidy levels (Sheidai and Attaei 2005). While exploring the cytomorphic diversity in more than 1500 flowering plants (over 7000 accessions) from diverse phyto-geographical regions of Himalayas, Pachmarhi Hills, South Indian Hills, Shiwaliks and Punjab plains, have detected the phenomenon during male meiosis in c 250 species (Bedi 1981, Saggoo 1982, Singhal 1982, Kumar 2010, Bala 2012, Jeelani 2012, Kaur 2012, Kumar 2012, Malik 2012, Rani 2012, Kaur 2013, Farroq 2013, Himshihka 2013, Srivastava 2013, Kumar 2015, Lovleen 2015, Kaur 2017, Kumar 2018, Kumari 2018). It has been noticed that the occurrence and frequency of meiocytes involved in cytomixis have no correlation with ploidy level/genomic status of the plant. Rather, it is the genetic makeup and prevailing environmental conditions which are responsible for the presence or absence of cytomixis. We are of the opinion that plants inhabiting freezing stress conditions of Indian cold deserts are more prone to cytomixis. Another observation by the authors is that certain species/genera, especially those possessing large sized chromosomes viz. Aconitum, Anemone, Caltha, Clematis, Ranunculus, and Silene are more liable to chromatin transfer related meiotic anomalies (Kumar 2010). It has also been noticed that all the analyzed accessions of Anemone rivularis (Singhal et al. 2009, Kumar 2010, Kumar et al. 2015), Clematis grata (Kumar 2010), Caltha palustris (Kumar and Singhal 2008, Kumar, 2010) Ranunculus laetus and R. hirtellus (Kumar 2010, Kumar and Singhal 2011) showed the phenomenon of cytomixis. Another significant observation by the authors is that frequency of cytomixis is much higher during the early stages of meiosis-I which might be due to the fact that migration of chromatin through cytoplasmic channels is much easier during the early stages compared to the later stage/s when chromatin gets condensed into chromosomes.

Role of cytomixis in plants

On the basis of data gathered to date on the cytologically analyzed species, the authors are of the view that cytomixis is a phenomenon of evolutionary significance caused by migration of genetic material. Generally, the chromatin material transfer is of two types, one in which whole of the nuclei of donor meiocyte get transferred to the recipient cell, and in another type, the chromatin material transferred partly to the recipient from donor meiocyte. Owing to partial chromatin transfer some associated meiotic irregularities have often been observed in cytomictic plants. These associated meiotic irregularities have a much effect on the genetic constitution and viability of male gametes. Such viable gametes with different chromosome numbers/ploidy levels led to the origin of aneuploids and polyploids (Shamina et al. 2000, Lattoo et al. 2006, Guan et al. 2012). During our studies, in most of the cases, we have also observed that during cytomixis whole nuclei migrate resulting into syncytes/polyploid meiocytes (Singhal and Kumar 2008, Kumar and Singhal 2008, Kumar 2010, Kumar et al. 2010, Singhal et al. 2011b). Such modified cells contribute towards the formation of giant-sized unreduced pollen with "2n" chromosome constitution, which has doubtless significance in the origin of polyploids (Sheidai and Bagheri-Shabestarei 2007, Kim et al. 2009, Singhal et al. 2011b, 2016, Guan et al. 2012, Mursalimov et al. 2013, Reis et al. 2016). Bretagnolle and Thompson (1995) opined that for the origin of polyploids (whole genome duplication), the most important mechanism for polyploidization is through unreduced gametes which can be produced in several ways, but the authors are of the view that cytomixis is one of the most significant mechanisms for the production of such gametes. Mursalimov et al. (2016) analyzed the effect of plant ploidy level on the rate of cytomixis in microsporogenesis of tobacco plants of different ploidy level (2x, 4x, 6x, 8x) and found that the rate of cytomixis proportionally increases in 6x and 8x cytopotypes and was similar in 2x and 4x plants. The rate of cytomixis is highly variable, differing even in the genetically identical plants grown under the same conditions. Mursalimov et al. (2016) concluded that unreduced gametes are formed in the tobacco plants owing to cytomixis.

Another evolutionary aspect of cytomixis is that it induces high pollen sterility in the majority of plants, especially of cold deserts which ultimately reduces the reproductive success through seeds. Consequently, plants of such habitats have acquired the perennial habit and vegetative modes of propagation. We assume that like other cytological processes of evolution, cytomixis needs to be viewed as a natural meiotic aberration of potential evolutionary significance regulated by an array of genes.

Figure 2 (a-f). a & b) Meiocytes remained connected through pores (cytomictic channels, arrowed) depicting cytomixis; c) recipient meiocytes with a double chromosomes constitution (encircled); d) meiocytes with extra-chromatin (encircled); e) pycnotic chromatin material (encircled); f) meiocytes showing sticky chromatin material. Scale bar = 10 μm.
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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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Figure 3 (a-f). a & b) Unoriented daughter chromatids at late anaphase-II; c) abnormal sporad; d) unstained sterile and stained fertile pollen grains; e) fertile pollen grains with variable of sizes and genetic constitution; f) large (unreduced) and small pollen grains. Scale bar = 10 μm

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