

# High frequency of cytomixis observed at zygotene in tetraploid *Lippia alba*

Aryane C. Reis<sup>1</sup> · Saulo M. Sousa<sup>1</sup> · Lyderson F. Viccini<sup>1</sup>

Received: 17 June 2015 / Accepted: 14 September 2015 / Published online: 28 September 2015  
© Springer-Verlag Wien 2015

**Abstract** *Lippia alba* (Verbenaceae) is an aromatic shrub recently described as a new tropical polyploid species with five distinct chromosome numbers ( $2n = 30, 38, 45, 60,$  and  $90$ ) with  $2n = 30, 45,$  and  $60$  being the most frequent ones. Cytomixis is a cellular process known as the migration of genetic material mainly between meiocytes through cytoplasmic connections or cytomictic channels. This phenomenon has been reported in various plant families such as Leguminosae, Brassicaceae, Poaceae, Apocynaceae, Liliaceae, Rutaceae, and others. The transference of genetic material between pollen mother cells (PMCs), by cytomictic channels, induces the formation of unbalanced and unreduced ( $2n$ ) gametes, and is considered a possible source of aneuploid and polyploid plants. Here, we describe for the first time, the occurrence of cytomixis in meiotic cells of *L. alba* (tetraploid cytotype) analyzing data obtained from meiotic behavior assays. In addition, the pollen size and viability were also evaluated. A high index of irregularities during meiosis was observed as well as unviable pollen with heterogeneous size. Approximately 80 % of zygotene cells showed genetic material exchange. Considering that *L. alba* shows different chromosome numbers, the contribution of cytomictic to cytotypes formation is also discussed.

**Keywords** Meiosis · Pollen viability · Polyploidy · Unreduced gametes

## Introduction

Cytomixis, a migration of cytoplasmic or nuclear materials between adjacent cells, is an intricate phenomenon well reported in plants (Lattoo et al. 2006; Singhal and Kumar 2008; Pierre and Sousa 2011; Guan et al. 2012; Mursalimov et al. 2013; Mursalimov and Deineko 2015). This natural process was reported for the first time around 100 years ago by Kornicke (1901) although the phenomenon was named by Gates (1911). Cytomixis was observed in different plant groups including Pteridophyta, Gymnosperms, Monocots, and Dicots. More specifically, the process was mainly reported in hybrids, aneuploids, mutants, polyploids, and apomictic individuals (de Nittancourt and Grant 1964; Gottschalk 1970; Mantu and Sharma 1983; Li et al. 2009; Pierre and Sousa 2011). Cytomixis was most frequently observed during meiosis I of microsporogenesis (Koul 1990; Lattoo et al. 2006; Kumar et al. 2010). Although cytomictic is not common in somatic cells, the phenomenon was also reported in root tips (Jacob 1941; Sarvella 1958; Tarkowska 1960; Kostritsyna and Soldatov 1991; Guzicka and Wozny 2005) and tapetal cells, among others (Cooper 1952).

The transference of DNA occurs by cytoplasmic connections called cytomictic channels. These channels derive from an atypical plasmodesmata structure, which persists throughout meiosis (Risueno et al. 1969; Pierre and Sousa 2011). The origin of cytomictic channels is still unknown and different explanations have been described: failure in cellular wall formation during cytokinesis (Sarvella 1958; Bisalpufra and Stein 1966); adjacent

---

Handling Editor: Martin Lysak.

✉ Lyderson F. Viccini  
lyderson.viccini@ufjf.edu.br

<sup>1</sup> Departamento de Biologia/Laboratório de Genética e Biotecnologia, Universidade Federal de Juiz de Fora, Juiz De Fora, MG 36036-900, Brazil

plasmodesmata fusion (Wang et al. 1998) and defects in callose synthesis (Sheidai and Fadaei 2005; Sidorchuk et al. 2007). In general, cytomictic channels connect two or more cells in the same division stage (Souza and Pagliarini 1997) but some reports also indicate the exchange of genetic materials between cells in different stages (Song and Li 2009; Lone and Lone 2013).

Cytomixis may be influenced by stress conditions such as physical and chemical factors (Bobak and Herich 1978; Narain 1979; De and Sharma 1983; Dwivedi et al. 1988; Souza and Pagliarini 1997; Bhat et al. 2006; Gulfishan et al. 2010). Independently of its origin, cytomixis results in serious genetic consequences. When it occurs in pollen mother cells (PMCs) it frequently affects gametogenesis and fertility (Mursalimov et al. 2013; Mursalimov and Deineko 2015). Whereas the normal product of meiosis division must possess half of the DNA of the mother cells, the transference of genetic material between cells may result in gametes with increased or decreased DNA content leading to unbalanced gametes (Mursalimov and Deineko 2015). Many reports indicate the presence of unbalanced or unreduced pollen originated from meiotic irregularities, mainly in individuals with cytomixis (Bell 1964; Lattoo et al. 2006; Mursalimov and Deineko 2015). In an evolutionary context, cytomixis has been studied over time (Kamra 1960) assuming an important role in new species formation, especially by polyploidization via unreduced gametes (Bell 1964; Falistocco et al. 1995; Lattoo et al. 2006).

*Lippia alba* (Verbenaceae), an important aromatic shrub used in Brazilian folk medicine, is characterized by various chemotypes as well as large phenotypic plasticity and genome variation (Pierre et al. 2011; Reis et al. 2014; Viccini et al. 2014). A recent study published by Reis et al. 2014 indicates the occurrence of a putative polyploid complex naturally formed in this species with diploids ( $2n = 30$ ; 64 individuals), aneuploid ( $2n = 38$ ; one individual), triploids ( $2n = 45$ ; 16 individuals), tetraploids ( $2n = 60$ ; 24 individuals), and one hexaploid individual ( $2n = 90$ ), collected in 14 states of the five major geographical regions of Brazil (North, Northeast, Center-West, Southeast, and South). The authors hypothesized an autopolyploid origin of each chromosome number by unilateral and bilateral crossing between cytotypes via unreduced gametes. Nevertheless, the mechanism involved in the formation of unreduced pollen is still unclear and presents an interesting point to be elucidated, which could help understand the origin of the cytotypes.

Here, we describe, for the first time, the cytomixis phenomenon in microsporogenesis of *L. alba*, one possible mechanism associated with the formation of unreduced gametes.

## Materials and methods

### Biological material

The tetraploid accession of *L. alba* (BGEN-92,  $2n = 4x = 60$ ) was cultivated in standard conditions at the Plant Experimental Station of the Universidade Federal de Juiz de Fora (UFJF), Brazil. Flower buds were collected in the morning and immediately fixed in cold ethanol: acetic acid solution 3:1 (v/v) for a minimum of 24 h.

### Meiotic preparation

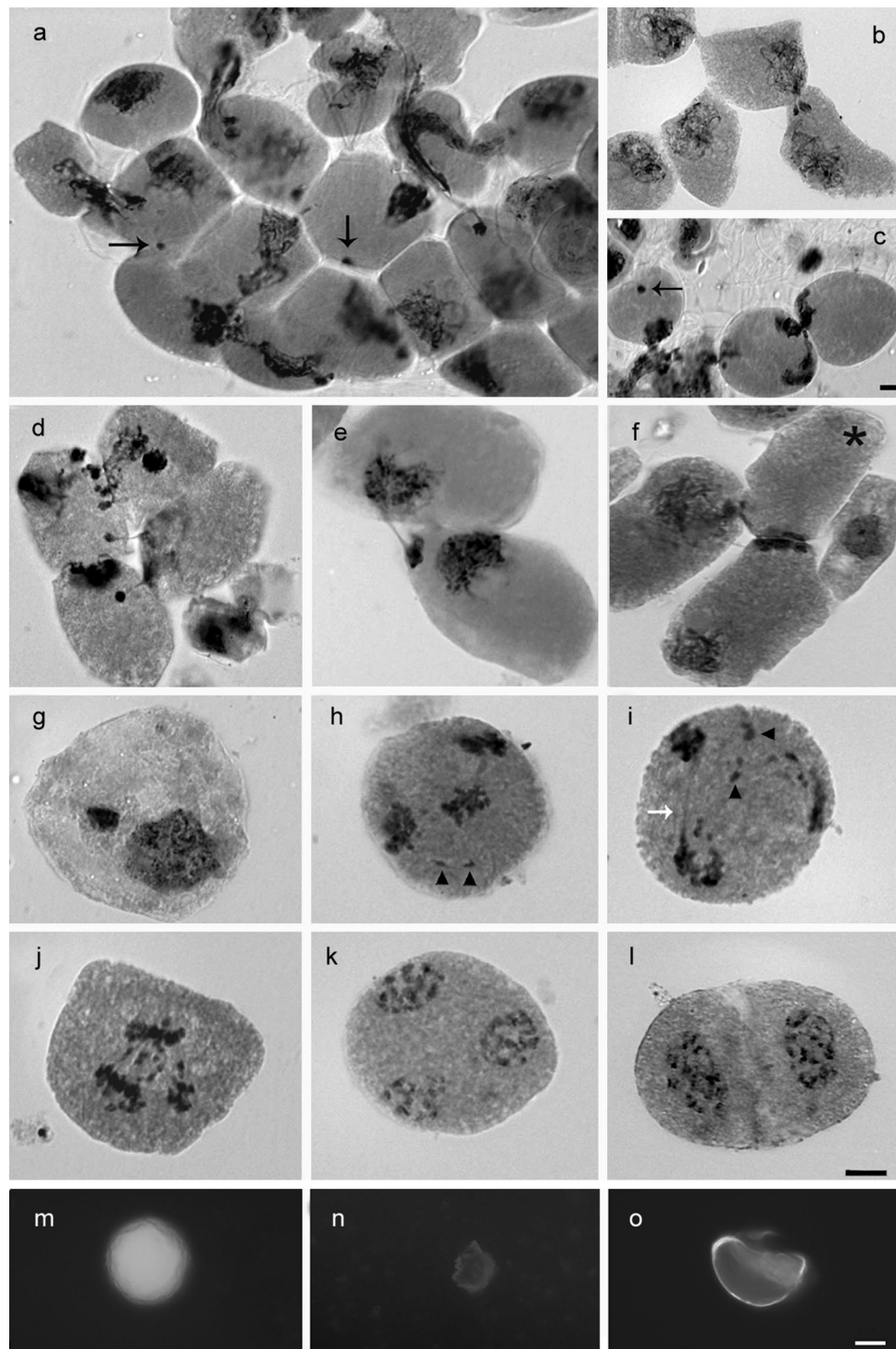
Slides were prepared by squash technique. The anthers were excised from the flower buds in a drop of 45 % acetic acid, squashed using a coverslip and stained with 5 % acetocarmine. Chromosomal abnormalities of different meiotic stage cells (zygotene, metaphase I, anaphase I, metaphase II, anaphase II, and telophase II) were quantified considering at least 100 cells per stage. The images were registered using the CellSens software (Olympus).

### Pollen viability

Pollen viability was investigated according to Heslop-Harrison and Heslop-Harrison (1970) with minor modifications. Mature and fresh anthers were excised and stained with fluorescein diacetate dissolved in acetone (2 mg/mL) combined with 10 % sucrose for 30 min. The fluorescent pollen were considered viable and the non-fluorescent ones as unviable. At least 1000 pollen grains were analyzed using a BX 51 microscope (Olympus) with appropriate filter. The diameter of ca. 100 grains was measured. The images were digitalized using the CellSens software (Olympus).

## Results and discussion

Here, we describe for the first time the cytomixis phenomenon in a tetraploid individual of *L. alba*. In this accession, the process occurs specifically in prophase I and among meiocytes only during the zygotene stage (Fig. 1a–f). Approximately 80 % of zygotene cells showed an exchange of genetic material (Table 1). Although previous reports revealed that this species showed a high index of irregularities during meiosis and unviable pollen (mainly in polyploid cytotypes), no event of cytomixis has been observed so far (Brandão et al. 2005; Pierre et al. 2011; Reis et al. 2014). In our study, in addition to cytomictic cells observed at zygotene stage, the analysis of meiotic behavior revealed a high percentage of irregularities at



**Fig. 1** Meiotic irregularities in a tetraploid accession of *Lippia alba*. **a–f** cytotoxicity among PMCs at zygotene stage cell; **g** PMC with micronucleus; **h** multipolar anaphase I with chromosome loss; **i** multipolar anaphase I with chromosome bridge and chromosome losses; **j** anaphase I with lagging chromosomes; **k** triad; **l** dyad;

**m** viable pollen; **n** unviable pollen with reduced size; **o** unviable pollen with irregular shape. *Black arrows* indicate micronuclei; *asterisk* indicates empty PMC after transference of chromatin; *white arrow* indicates chromosome bridge; *arrowheads* indicate chromosome losses. *Bar* 5  $\mu\text{m}$  (**a–l**); *bar* 10  $\mu\text{m}$  (**m–o**)

subsequent stages of prophase I: precocious migration and lost chromosomes (metaphase I); lagging chromosomes, chromosome bridge and multipolar anaphase (anaphase I);

chromosomal losses (metaphase II); late migration (anaphase II); dyads, triads, polyads and micronuclei (telophase II) (Table 1).

**Table 1** Meiotic irregularities observed at different stages in a tetraploid accession of *L. alba*

Abnormalities	Number of cells	Total	%	Stages
Cytomixis	135	167	81	Zygotene
Precocious migration, lost chromosomes	78	107	73	Metaphase I
Laggard chromosomes, bridge, multipolar anaphase	53	113	47	Anaphase I
Chromosomal losses	100	100	100	Metaphase II
Late migration, lost chromosomes	89	107	83	Anaphase II
Dyads, triads, polyads, micronuclei	96	109	88	Telophase II

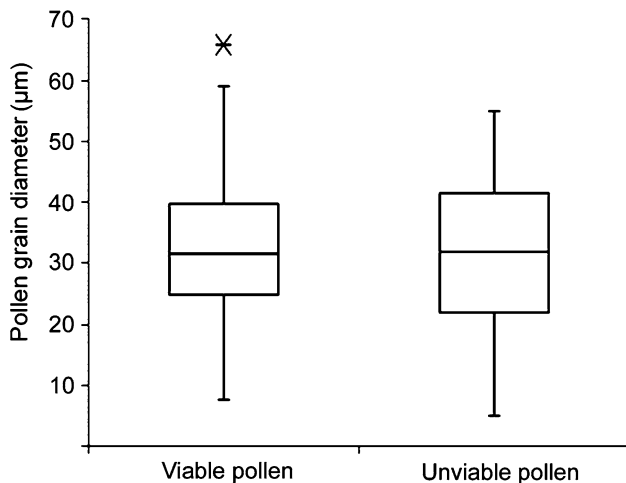
Many studies reported the formation of cytotoxic channels in PMCs, mainly in prophase I (Falistocco et al. 1995; Wang et al. 2002; Bellucci et al. 2003; Sidorchuk et al. 2004; Imeri-Buiza 2007; Negron-Ortiz 2007; Song and Li 2009). However, species such as *Nicotiana tabacum*, *Chlorophytum comosum*, *Medicago sativa*, and *Mecynopsis aculeate* showed communications between nuclei in interphase, in different stages of meiosis I and rarely in meiosis II (Bellucci et al. 2003; Lattoo et al. 2006; Singhal and Kumar 2008; Guan et al. 2012; Mursalimov et al. 2013). In the individual investigated here, cytomixis process involved from two to 16 adjacent cells and the cytotoxic channels varied in length and number (Fig. 1a–f). In some cases the nuclei migration resulted in almost empty cells and others apparently showed twice the amount of DNA content (Fig. 1f). After chromatin transfer, the receptor cell frequently shows condensed micronucleus and unorganized chromatin (Fig. 1a). The migration of chromatin from one cell to another directly interferes with its function and development. The increase or reduction of DNA content in PMCs may cause problems in microsporogenesis and consequently failure in the formation of balanced gametes (Bellucci et al. 2003; Singhal and Kumar 2008; Ferreira et al. 2009; Kumar et al. 2010). The most common abnormalities observed were condensed micronucleus, desynapsis, chromosome stickiness, incorrect pairing, unequal segregation, laggard chromosomes, precocious migration, and chromosome bridge (Lattoo et al. 2006) as was observed in a tetraploid accession of *L. alba* investigated here (Table 1; Fig. 1g). All of these abnormalities can result in unreduced or unbalanced gametes (Bretagnolle and Thompson 1995).

The frequency of cytomixis can vary within the same species with different ploidy levels (Lavia et al. 2011; Guan et al. 2012). Jeelani et al. (2011), for example, observed different cytomixis indices in diploid and tetraploid cytotypes of *Silene conoidea*, *S. edgeworthii*, and *S. vulgaris* (Caryophyllaceae). In *Ranunculus hirtellus* (Ranunculaceae) the tetraploid individuals showed transfers of chromatin and abnormal microsporogenesis, while diploids exhibited normal meiosis (Kumar and Singhal 2011). In *L. alba*, up to now, only the tetraploid cytotype showed chromatin migration in PMCs.

Cytomixis is usually observed in plants with some level of genetic, physiological, or biochemical instability such as haploids, triploids, hybrids, mutants, aneuploids, and apomicts individuals (De Nittancourt and Grant 1964; Gottschalk 1970; Mantu and Sharma 1983). In *L. alba*, meiotic instability, genome restructuring, and chromosome rearrangements were already reported in polyploids (Reis et al. 2014). Genomic rearrangements are common in neopolyploids and tend to stabilize after successive generations of karyotype “adjustment” (Soltis and Soltis 2009; Chester et al. 2010, 2013; Lipman et al. 2013). This nuclear restructuring is an adaptation to the new conditions imposed by the increase of DNA content (Chester et al. 2013). Therefore, for these individuals, considering that cytomixis allows a large scale of DNA losses and addition, this process can help genome adjustments, accelerating the adaptation/stability of new polyploids.

As outlined above, cytomixis is a potential mechanism to generate meiotic instability and to form abnormal gametes. Thus, the chromatin transfer between adjacent PMCs can result in unreduced or unbalanced gametes that could contribute to the formation of aneuploids and polyploid individuals (Bell 1964; Lattoo et al. 2006). It is well known that polyploidy is the major driver of speciation in plants (Masterson 1994; Soltis et al. 2003; Mittelbach et al. 2007; Soltis et al. 2009; Soltis and Soltis 2009; Jiao et al. 2011), and cytomixis might be one of mechanisms that contributes to the formation of new polyploids via unreduced gametes (Bretagnolle and Thompson 1995; Falistocco et al. 1995; Ramsey and Schemske 1998; Ghaffari 2006; Lattoo et al. 2006). Several papers reported the formation of viable  $2n$  pollen in cytotoxic plants and also that cytomixis was directly associated with the formation of new cytotypes (Falistocco et al. 1995; Zhou 2003; Ghaffari 2006; Lattoo et al. 2006; Negron-Ortiz 2007; Singhal and Kumar 2008; Song and Li 2009; Singhal et al. 2011; Mursalimov and Deineko 2015).

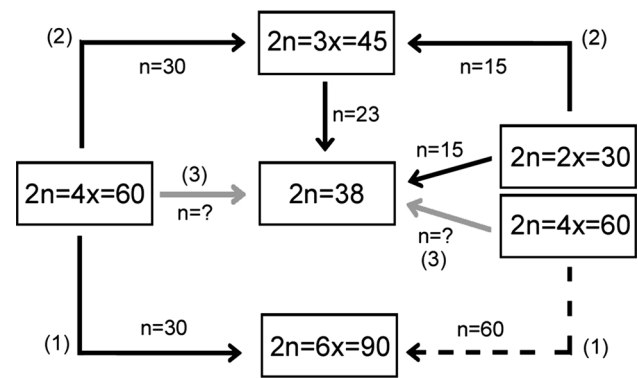
In general, unreduced pollen grains show larger volume and diameter than haploid ones. On the other hand, cytomixis can also produce pollen grains with reduced size (pollen with less DNA) (Ramsey and Schemske 2002; Ramanna and Jacobsen 2003; Soltis et al. 2004; Larrosa et al. 2012). In wild potatoes (*Solanum tuberosum*), for



**Fig. 2** Variation of diameter length for viable and unviable pollen grains in a tetraploid accession of *Lippia alba*. The box plots show the median (central horizontal line inside the boxes); the interquartile range (white box) and the outlier (X). The boxes comprise 50 % of the values (upper and lower; 25 % quartile around the median). Measured values larger than the upper quartile are plotted as outlier

example, the authors suggested that pollen grains larger and smaller than the average size formed diploids and aneuploids gametes contributing to the formation of new hybrids (Larrosa et al. 2012). In our results with *L. alba*, the fluorescein diacetate test revealed a high percentage of unviable pollen (ca. 87 % of 1069 grains analyzed). Moreover, most of the pollen grains showed irregular shape and heterogeneous size (Fig. 1m–o). A large variation was also observed in pollen diameters both for viable (from 7.65 to 65.44 µm) and unviable (from 4.87 to 54.84 µm) ones (Fig. 2), suggesting the existence of variation in the DNA content of pollen grains (Ramsey and Schemske 2002; Ramanna and Jacobsen 2003; Larrosa et al. 2012).

In *L. alba* complex, a hypothesis to explain the formation of different cytotypes involves unilateral and bilateral crossings via unreduced pollen (Reis et al. 2014). We suggest that the irregularities at telophase II (dyad, triad, and polyads) observed in our data constitute an evidence of unreduced and unbalanced pollen formation. According to the hypothesis of polyploid complex formation, the tetraploid individuals can originate hexaploids ( $6x = 90$ ) via union of unreduced and reduced gametes ( $n = 60 + n = 30$ ) (Fig. 3). Triploid individuals ( $3x = 45$ ) could be a result of crossing involving reduced gametes between diploids and tetraploids cytotypes ( $n = 15 + n = 30$ ) (Fig. 3). In addition, aneuploid individual might be formed by unbalanced gametes with aneuploid chromosome numbers, as observed in the individual with 38 chromosomes (Fig. 3; Reis et al. 2014). In *L. alba*, giant pollen (possibly with somatic DNA amount) were previously quantified and the authors suggested that they



**Fig. 3** Representative scheme for the origin of *Lippia alba* cytotypes from tetraploid individuals. Continuous arrows represent reduced gametes; discontinuous arrows represent unreduced gametes; gray arrows represent unbalanced gametes. Chromosome numbers in each box represent the somatic number observed for each individual with different ploidy levels. (1) tetraploid ( $2n = 60$ ) individuals can originate the hexaploid via crossing involving reduced and unreduced gametes; (2) tetraploid can form triploid cytotype by crossing with diploid; and (3) tetraploid can originate the aneuploid individual ( $2n = 38$ ) from unbalanced gametes which are resulted from cytomixis process

were a consequence of dyads and triads observed in meiosis (Reis et al. 2014). Here, we also observed pollen grains that were bigger and smaller than those previously reported for *L. alba* (Pierre et al. 2011), giving an additional evidence to aneuploid gametes formation.

Although additional studies should be conducted in a higher number of cytotypes to evaluate the occurrence of cytomixis in the species, the present data open a new perspective to better understand the reproductive biology and the origin of the polyploid complex in *L. alba*.

**Acknowledgments** The authors wish to thank, CNPq, CAPES, and FAPEMIG for financial support.

#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

#### References

- Bell CR (1964) Cytomixis in *Tauschia nudicaulis* Schlecht (Apiaceae). *Cytologia* 29:369–398
- Bellucci M, Roscini C, Mariani A (2003) Cytomixis in pollen mother cells of *Medicago sativa* L. *J Heredity* 94:512–516
- Bhat TA, Parveen S, Khan AH (2006) MMS-induced cytomixis in pollen mother cells of broad bean (*Vicia faba* L.). *Turkish J Bot* 30:273–279
- Bisalpufra T, Stein JR (1966) The development of cytoplasmic bridges in *Volvox aureus*. *Canad J Bot* 44:1697–1702
- Bobak M, Herich R (1978) Cytomixis as a manifestation of pathological changes after application of trifluralin. *Nucleus* 21:22–26

- Brandão AD, Viccini LF, Reccopimentel SM (2005) Meiotic analysis of two putative polyploid species of Verbenaceae from Brazil. *Cytologia* 58:315–319
- Bretagnolle F, Thompson JD (1995) Gametes with the somatic chromosome number: mechanisms of their formation and role in the evolution of polyploid plants. *New Phytol* 129:1–22
- Chester M, Leitch AR, Soltis PS, Soltis DE (2010) Review of the application of modern cytogenetic methods (FISH/GISH) to the study of reticulation (polyploidy/hybridization). *Genes* 1:166–192
- Chester M, Gallagher JP, Symonds VV, Silva AVC, Mavrodiev EV, Leitch AR, Soltis PS, Soltis DE (2013) Extensive chromosomal variation in a recently formed natural allopolyploid species, *Tragopogon miscellus* (Asteraceae). *Proc Natl Acad Sci USA* 9:1176–1181
- Cooper DD (1952) The transfer of deoxyribose nucleic acid from the tapetum to the microsporocytes at the onset of meiosis. *Cytologia* 86:271–274
- de Nittancourt D, Grant WF (1964) La cytogenetique de *Lotus* (Leguminosae) III. Un cas de cytomixie dans un hybride interspecific. *Cytologia* 29:191–195
- De M, Sharma AK (1983) Cyto-mixis in pollen mother cells of an apomictic ornamental *Ervatamia divaricata* (Linn.) Alston. *Cytologia* 48:201–207
- Dwivedi NK, Sikdar AK, Jolly MS, Susheelama BN, Suryanarayana N (1988) Induction of tetraploidy in colchicine- induced mutant of mulberry I: morphological and cytological studies in cultivar Kanva-2. *Indian J Genet* 48:305–311
- Falisticco E, Tosti N, Falcinelli M (1995) Cyto-mixis in pollen mother cells of diploid *Dactylis*, one of the origins of 2n gametes. *J Heredity* 86:448–453
- Ferreira K, Torres GA, Carvalho IV, Davide LC (2009) Abnormal meiotic behavior in three species of *Crotalaria*. *Pesq Agropecu Brasil* 44:1641–1646
- Gates RR (1911) Pollen formation in *Oenothera gigas*. *Ann Bot (Oxford)* 25:909–940
- Ghaffari SM (2006) Occurrence of diploid and polyploidy microspores in *Sorghum bicolor* (Poaceae) is the result of cyto-mixis. *African J Biotechnol* 5:1450–1453
- Gottschalk W (1970) Chromosome and nucleus migration during microsporogenesis of *Pisum sativum*. *Nucleus* 13:1–9
- Guan JZ, Wang JJ, Cheng ZH, Liu Y, Li ZY (2012) Cyto-mixis and meiotic abnormalities during microsporogenesis are responsible for male sterility and chromosome variations in *Houttuynia cordata*. *Genet Molec Res* 11:121–130
- Gulfishan M, Khan AK, Bhat TA (2010) Studies on cytotoxicity induced by DES and SA in *Vicia faba* var. major. *Turkish J Bot* 34:31–37
- Guzicka M, Wozny A (2005) Cyto-mixis in shoot apex of Norway spruce [*Picea abies* (L.) Karst.]. *Trees* 18:722–724
- Heslop-Harrison J, Heslop-Harrison Y (1970) Evaluation of pollen viability by enzymatically induced fluorescence: intracellular hydrolysis of fluorescein diacetate. *Stain Technol* 45:115–120
- Imeri-Buiza J (2007) Inestabilidad cariológica durante la formación de células madres del polen em *Aloe vera* (Aloaceae). *Int J Trop Biol* 55:805–813
- Jacob KT (1941) Certain abnormalities in the root tips of cotton. *Curr Sci* 10:174–175
- Jeelani SM, Rani S, Kumar S, Kumar S, Gupta RC (2011) Meiotic studies in some members of Caryophyllaceae Juss. from the Western Himalayas. *Acta Biol Cracov Ser Bot* 53:86–95
- Jiao Y, Wickett NJ, Ayyampalayam S, Chanderbali AS, Landherr L, Ralph PE, Tomsho LP, Hu Y, Liang H, Soltis PS, Soltis DE, Clifton SW, Schlarbaum SE, Schuster SC, Ma H, Leebens-Mack J, dePamphilis CW (2011) Ancestral polyploidy in seed plants and angiosperms. *Nature* 473:97–100
- Kamra OP (1960) Chromatin extrusion and cyto-mixis in pollen mother cells of *Hordeum*. *Hereditas* 46:592–600
- Kornicke M (1901) Über ortsveränderung von Zellkernern S B Niederhein, *Ges Natur-U Heilkunde Bonn A*:14–25
- Kostritsyna TV, Soldatov IV (1991) Cyto-mixis in the shoot apical meristem of hybrids of *Prunus domestica* L. *Persica vulgaris* Mill. *Genetika* 27:1790–1794
- Koul KK (1990) Cyto-mixis in pollen mother cells of *Alopecurus arundinaceus* Poir. *Cytologia* 55:169–173
- Kumar P, Singhal VK (2011) Male meiosis, morphometric analysis and distribution pattern of 2× and 4× cytotypes of *Ranunculus hirtellus* Royle, 1834 (Ranunculaceae) from the cold regions of Northwest Himalayas (India). *Comp Cytogen* 5:143–161
- Kumar P, Singhal VK, Kaur D, Kaur S (2010) Cyto-mixis and associated meiotic abnormalities affecting pollen fertility in *Clematis orientalis*. *Biol Pl* 54:181–184
- Larrosa FH, Maune JF, Erazu LE, Camadro EL (2012) Meiotic abnormalities underlying pollen sterility in wild potato hybrids and spontaneous populations. *Pl Biol* 14:223–233
- Lattoo SK, Khan S, Bamotra S, Dhar AK (2006) Cyto-mixis impairs meiosis and influences reproductive success in *Chlorophytum comosum* (Thunb) Jacq.—an additional strategy and possible implications. *J Biosci* 31:629–637
- Lavia GI, Ortiz AM, Robledo G, Fernández A, Seijo G (2011) Origin of triploid *Arachis pintoi* (Leguminosae) by autopolyploidy evidenced by FISH and meiotic behaviour. *Ann Bot (Oxford)* 108:103–111
- Li XF, Song ZQ, Feng DS, Wang HG (2009) Cyto-mixis in *Thinopyrum intermedium*, *Thinopyrum ponticum* and its hybrids with wheat. *Cereal Res Commun* 37:353–361
- Lipman MJ, Chester M, Soltis PS, Soltis DE (2013) Natural hybrids between *Tragopogon mirus* and *T. miscellus* (Asteraceae): a new perspective on karyotypic changes following hybridization at the polyploid level. *Amer J Bot* 100:2016–2022
- Lone FA, Lone S (2013) Cyto-mixis—a well-known but less understood phenomenon in plants. *Int J Recent Sci Res* 4:347–352
- Masterson J (1994) Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. *Science* 264:421–423
- Mittelbach GG, Schemske DH, Cornell HV, Allen AP, Brown JM, Bush MB, Harrison SP, Hurlbert AH, Knowlton N, Lessios HA, McCain CM, McCune AR, McDade LA, McPeck MA, Near TJ, Price TD, Ricklefs RE, Roy K, Sax DF, Schluter D, Sobel JM, Turelli M (2007) Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecol Letters* 10:315–331
- Mursalimov SR, Deineko LV (2015) How cyto-mixis can form unreduced gametes in tobacco. *Pl Syst Evol* 301:1293–1297
- Mursalimov SR, Sidorchuk YV, Deineko EV (2013) New insights into cyto-mixis: specific cellular features and prevalence in higher plants. *Planta* 238:415–423
- Narain P (1979) Cyto-mixis in the pollen mother cells of *Hemerocallis* Linn. *Curr Sci* 48:996–998
- Negron-Ortiz V (2007) Chromosome numbers, nuclear dna content, and polyploidy in *Consolea* (Cactaceae), an endemic cactus of the Caribbean Islands. *Amer J Bot* 94:1360–1370
- Pierre PMO, Sousa SM (2011) Citomixia em plantas: causas, mecanismos e consequências. *Braz J Biosci* 9:231–240
- Pierre PMC, Sousa SM, Davide LC, Machado MA, Viccini LF (2011) Karyotype analysis, DNA content and molecular screening in *Lippia alba* (Verbenaceae). *Anais Acad Brasil Ci* 83:993–1005
- Ramanna MS, Jacobsen E (2003) Relevance of sexual polyploidization for crop improvement—a review. *Euphytica* 133:3–18
- Ramsey J, Schemske DW (1998) Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Rev Ecol Syst* 29:467–501

- Ramsey J, Schemske DW (2002) Neopolyploidy in flowering plants. *Annual Rev Ecol Syst* 33:589–639
- Reis AC, Sousa SM, Vale AA, Pierre PMO, Franco AL, Campos JMS, Viera RF, Viccini LF (2014) *Lippia alba* (Verbenaceae): a new tropical autopolyploid complex? *Amer J Bot* 101:1002–1012
- Risueno MC, Gimenez-Martin G, Lopez-Saez JF, R-Garcia MI (1969) Connexions between meiocytes in plants. *Cytologia* 34:262–272
- Sarvella P (1958) Cytomixis and loss of chromosomes in meiotic and somatic cells of *Gossypium*. *Cytologia* 23:14–24
- Sheidai M, Fadaei F (2005) Cytogenetic studies in some species of *Bromus* L., section *Genea* Dun. *J Genet* 84:189–194
- Sidorchuk YV, Deineko EV, Shumny VK (2004) Cytomixis in pollen mother cells of transgenic tobacco (*Nicotiana tabacum* L.) plants. *Dokl Biol Sci* 394:47–50
- Sidorchuk YV, Deineko EV, Shumny VK (2007) Peculiarities of cytomixis in pollen mother cells of transgenic tobacco plants (*Nicotiana tabacum* L.) with mutant phenotype. *Cell Tissue Biol* 1:570–576
- Singhal VK, Kumar P (2008) Impact of cytomixis on meiosis, pollen viability and pollen size in wild populations of *Himalayan poppy* (*Meconopsis aculeata* Royle). *J Biosci* 33:371–380
- Singhal VK, Rana PK, Kumar P, Kaur D (2011) Persistent occurrence of meiotic abnormalities in a new hexaploid cytotype of *Thalictrum foetidum* from Indian cold deserts. *Biologia (Bratislava)* 66:458–464
- Soltis PS, Soltis DE (2009) The role of hybridization in plant speciation. *Pl Biol* 60:561–588
- Soltis DE, Soltis PS, Tate JA (2003) Advances in the study of polyploidy since plant speciation. *New Phytol* 161:173–191
- Soltis DE, Pires JC, Kovarik A, Tate J, Mavrodiev E (2004) Recent and recurrent polyploidy in *Tragopogon* (Asteraceae): cytogenetic, genomic and genetic comparisons. *Biol J Linn Soc* 82:485–501
- Soltis DE, Albert VA, Leebens-Mack J, Bell CD, Paterson AH, Zheng C, Sankoff D, dePamphilis CW, Wall PK, Soltis PS (2009) Polyploidy and angiosperm diversification. *Amer J Bot* 96:336–348
- Song ZQ, Li XF (2009) Cytomixis in pollen mother cells of *Salvia miltiorrhiza*. *Caryologia* 62:213–219
- Souza AM, Pagliarini MS (1997) Cytomixis in *Brassica napus* var. *oleifera* and *Brassica campestris* var. *oleifera* (Brassicaceae). *Cytologia* 62:25–29
- Tarkowska J (1960) Cytomixis in the epidermis of scales and leaves and in meristems of root apex of *Allium cepa* L. *Acta Soc Bot Poloniae* 29:149–168
- Viccini LF, Silveira RS, do Vale AA, de Campos JMS, Reis AC, de Oliveira Santos M, Campos VR, Carpane AG, Grazul RM (2014) Citral and linalool content has been correlated to DNA content in *Lippia alba* (Mill.) N.E. Brown (Verbenaceae). *Industr Crops Prod* 59:14–19
- Wang XY, Guo GQ, Nie XW, Zheng GC (1998) Cytochemical localization of cellulose activity in pollen mother cells of david lily during meiotic prophase I and its relation to secondary formation of plasmodesmata. *Protoplasma* 204:128–138
- Wang XY, Nie XW, Guo GQ, Pan YF, Zheng GC (2002) Ultrastructural characterization of the cytoplasmic channel formation between pollen mother cells of david lily. *Caryologia* 55:161–169
- Zhou SQ (2003) Viewing the difference between the diploid and the polyploid in the light of the upland cotton aneuploid. *Hereditas* 138:65–72