

# Cytomixis in tobacco microsporogenesis: are there any genome parts predisposed to migration?

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**Abstract** Cytomixis is a poorly studied process of nuclear migration between plant cells, discovered in microsporogenesis of several hundreds of plant species. The chromosomes that migrate between tobacco microsporocytes have been for the first time identified using fluorescence *in situ* hybridization (FISH), and the question whether cytomixis is a random or a targeted process is answered. The distribution of four repetitive sequences used for identifying the tobacco chromosomes—NTRS, 5S rDNA, GRS, and HSR60—has been examined in the migrating chromatin, and the micronuclei formed after cytomixis. The distribution of tobacco S and T genomes has been analyzed in the cytotoxic chromatin using genomic *in situ* hybridization (GISH). As has been shown ( $\chi^2$  test), the labeled DNA probes marking the listed sequences in tobacco genome are observed in the micronuclei formed after cytomixis with the probability not exceeding the theoretically expected value if cytomixis considered as a random process. Thus, it is shown that cytomixis is not a targeted process, and the chromosomes migrate between microsporocytes in a random manner.

**Keywords** *Nicotiana tabacum* · Fluorescence *in situ* hybridization (FISH) · Genome *in situ* hybridization (GISH) · Meiosis · NTRS

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## Introduction

Cytomixis is the migration of nuclei between plant cells, discovered in microsporogenesis of several hundreds of plant species (for review, see Lone and Lone 2013; Mursalimov et al. 2013). The interest to cytomixis is explainable not only because it is a specific type of communication between plant cells but also owing to its potential evolutionary significance as a putative mechanism involved in changes in the karyotype of produced pollen.

Currently, the cytological pattern in cytomixis is best studied for tobacco microsporogenesis (Yu et al. 2004; Wang et al. 2006; Sidorchuk et al. 2007; Mursalimov et al. 2013). Various methods of light and electron microscopies have shown that the cytomixis in tobacco microsporocytes is most frequently detectable during the first meiotic prophase and that the nuclei migrate between cells through specialized cytotoxic channels, which are considerably larger as compared with the plasmodesmata (Yu et al. 2004; Wang et al. 2006; Mursalimov and Deineko 2015). All nuclear components, including chromatin, nucleolus, and nuclear matrix, migrate between cells enclosed within the nuclear membrane, which shows no signs of damage. As a rule, only part of the migrating nucleus of a donor cell passes through the cytotoxic channel. Then, this part of the migrating nucleus buds off in the recipient cell to give one or several micronuclei. Migration of a whole nucleus to a recipient cell is a considerably rarer event; in this case, a binucleate microsporocyte and a cell completely lacking the chromatin are formed (Mursalimov and Deineko 2015). Recent studies have demonstrated that the cytotoxic chromatin does not differ from the chromatin of intact cells in the histone methylation, acetylation, and phosphorylation patterns (Mursalimov et al. 2015). As has been shown, the chromatin migrating between cells is not selectively heterochromatized

before its migration to another cell, during the migration, and after entering the recipient cell as micronuclei. The cytotoxic chromatin contains normal euchromatin markers, which do not disappear in the micronuclei formed after cytotoxicity. The histones in cytotoxic chromatin display a normal phosphorylation pattern matching a current stage of the meiotic division. The migrating chromatin contains the synaptonemal complex proteins and lacks the apoptosis markers (Mursalimov et al. 2015).

Although new data have been accumulated on the functional state of cytotoxic chromatin, it is still vague which particular chromosomes migrate between cells. It is unknown whether cytotoxicity is a random process or such migration of nuclear material is a directed transfer/elimination of part of the chromosomes. The tobacco is a convenient cytological object; however, a large genome and small chromosome size hinder its cytogenetic analysis and identification of individual chromosomes. Currently, only one method makes it possible to identify the tobacco chromosomes with the help of a combination of markers associated with repetitive sequence specific of individual chromosomes (Lim et al. 2000; Shibata et al. 2013).

In this work, the distributions of four repetitive sequences used for tobacco chromosome identification—NTRS, 5S rDNA, GRS, and HSR60—were studied in the migrating chromatin, and the micronuclei formed after cytotoxicity. The distribution of the tobacco S and T genomes in the cytotoxic chromatin was also analyzed.

## Materials and methods

### Plant material

Tobacco line SR1 (*Nicotiana tabacum* L. cv. Petit Havana SR1,  $2n = 4x = 48$ , genome constitution SSTT, S genome from *Nicotiana sylvestris*, T genome from *Nicotiana tomentosiformis*) was used in the work. SR1 line was obtained by Maliga et al. (1973). Anthers were collected from five individual plants. Plants were grown in a hydroponic greenhouse with a photoperiod of 16/8 h (day/night) at a temperature of 22/18 °C (day/night).

### Fluorescence *in situ* hybridization

Fluorescence *in situ* hybridization (FISH) and genome *in situ* hybridization (GISH) were performed according to the protocol described in Shibata et al. (2013) modified for meiotic nuclei. Anthers were fixed with freshly prepared 4 % paraformaldehyde in phosphate-buffered saline (PBS, pH 7.3) for 30–60 min on ice. Anthers were then washed with 0.01 M citrate buffer (pH 4.8) 3 × 15 min, squashed into suspension

in citrate buffer; then microsporocyte cell walls were digested at 37 °C for 30 min in the mixture of 2 % cellulase (Sigma) and 2 % pectinase (Fluka) dissolved in citrate buffer. Cells were centrifuged at 7000×g for 6 min at 4 °C, suspended in citrate buffer, and centrifuged again at 7000×g for 6 min at 4 °C. Then cells in a minimum volume of citrate buffer (10–20 µl) were transferred to poly-L-lysine-coated slides and squashed between a glass slide and a cover slip. After freezing in liquid nitrogen for 60 s, the cover slips were removed, and the slides were immediately transferred into 2× saline-sodium citrate (SSC).

Before hybridization, the cells were pretreated with RNase A (Sigma) (100 µg/ml in 2× SSC) for 60 min at 37 °C. The slides were then washed 3 × 5 min with 2× SSC and digested with a freshly made proteinase K (Sigma) solution (0.1 µg/ml in 20 mM TRIS–HCl pH 7.5, 2 mM CaCl<sub>2</sub>) at 37 °C for 60 min followed by a rinse with 2× SSC 3 × 10 min. Slides were fixed with 4 % paraformaldehyde in 2× SSC for 10 min, rinsed with 2× SSC 3 × 10 min, and dehydrated in 70, 96, and 100 % ethanol series.

Pretreated slides were denatured with 70 % formamide in 2× SSC at 68 °C for 6 min and dehydrated in the ice cold ethanol series. The DNA probes were denatured at 95 °C for 5 min; 100 ng of biotinylated DNA in 20 µl of hybridization mixture (2× SSC, 50 % formamide, 10 % dextran sulfate, and 100× unlabelled blocking salmon sperm DNA) was applied to each slide; after hybridization (at 37 °C overnight), the slides were washed with 2× SSC at 42 °C.

The repetitive sequences NTRS, 5S rDNA, GRS, and HSR60 were amplified from genomic DNA of *N. tabacum* using primers according to Shibata et al. (2013) and simultaneously labeled with biotin-11-dUTP. For GISH, the genomic DNAs of *N. sylvestris* and *N. tomentosiformis* extracted from leaves (the seeds were kindly provided by J. -L. Verrier, Tobacco Institute of Bergerac, France) were labeled with biotin-11-dUTP by nick translation using a nick translation DNA labeling system (Enzo Life Sciences).

The biotin-labeled probes were visualized using fluorescein isothiocyanate (FITC) conjugated avidin (Sigma, cat. no. A2901). The signal was amplified using FITC-conjugated antiavidin antibody (Sigma, cat. no. F1269). Chromosomes were counterstained with 4,6-diamino-2-phenylindole (DAPI). FISH/GISH signals and stained chromosomes were captured using an AxioImager M1 microscope (Carl Zeiss), AxioCam HRm camera (Carl Zeiss), and Isis software (MetaSystems).

Single DAPI images were presented in gray as the best way to discriminate chromosome structures. In the merged images, the DAPI staining is blue. In order to demonstrate the migration of chromatin between cytotoxic cells, the cytoplasm red autofluorescence signal, denoting cell boundaries, was added to merge images.

## Statistical data processing

The  $\chi^2$  test was used for statistical processing of the data.

## Results

DNA probes were hybridized to the tobacco chromosomes in pachytene (meiotic prophase I). All probes used for FISH (NTRS, 5S rDNA, GRS, and HSR60) and GISH (S and T genomes) hybridized to the chromosomes of intact cells, of the cells involved in nuclear migration, and cells carrying the micronuclei formed after cytomixis. The repetitive sequences NTRS, 5S rDNA, GRS, and HSR60 are present in one, two, five, and 11 chromosomes of the tobacco haploid karyotype, respectively. Both haploid S and T tobacco genomes have 12 chromosomes with translocations (Shibata et al. 2013).

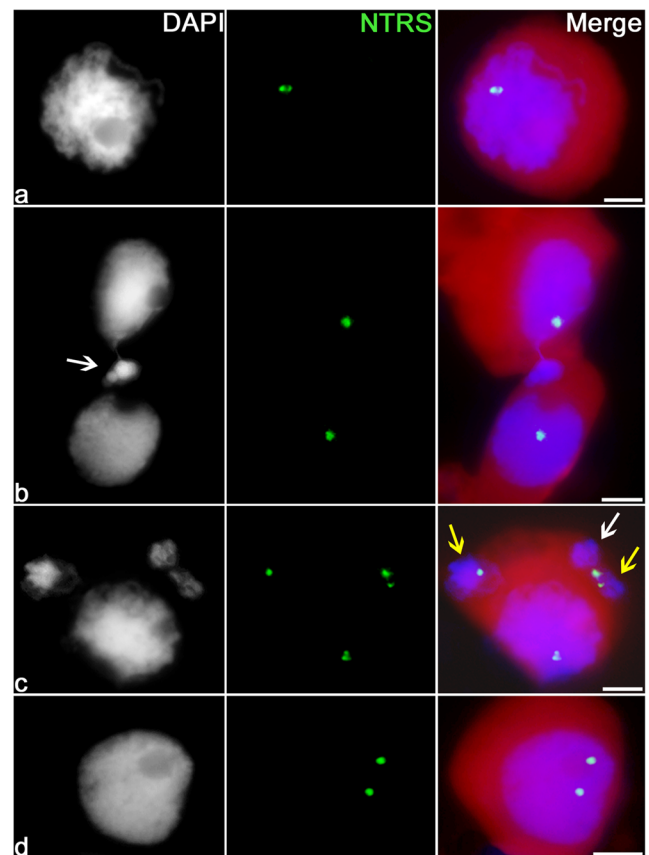
The NTRS repetitive sequence is located in intercalary site of the T3 tobacco chromosome long arm (Lim et al. 2000; Shibata et al. 2013). As was expected, analysis of the distribution of NTRS probe in tobacco microsporocytes in the pachytene after chromosome cohesion and bivalent formation detected a single signal in the intact cells (Fig. 1a). The cells involved in cytomixis also displayed a single signal (Fig. 1b), and the micronuclei containing this signal or lacking it were formed in recipient cells after cytomixis depending on whether the bivalent carrying this sequence was present in the migrating part of the nucleus (Fig. 1c).

In some cases, the intact cells simultaneously containing two NTRS signals were observed among the tobacco microsporocytes (Fig. 1d); the origin of these cells is discussed below.

The GRS and HSR60 repetitive sequences are located in five (T2–T4, T5/s, and S2/t) and 11 (S1, S2/t, S4–S9, T1/s, T5/s, and T8/s) tobacco chromosomes, respectively. The GRS is specific for T genome chromosomes and is localized mostly at intercalary sites of the long arms, while the HSR60 is specific for S genome and is localized mostly in subterminal chromosome regions (Lim et al. 2000; Shibata et al. 2013). The corresponding number of signals was detectable in the nuclei of intact tobacco microsporocytes (Fig. 2a, d). As for the cells involved in cytomixis, GRS and HSR60 signals are present in both the nuclei and migrating chromatin (Fig. 2b, e). Two types of micronuclei are evident after cytomixis, namely, containing these signals and lacking them (Fig. 2c, f).

An analogous pattern is observed for the 5S rDNA probe, which is contained in two tobacco chromosomes, S8 and T8/s in intercalary sites of the long arms (Lim et al. 2000; Shibata et al. 2013). The 5S rDNA signal is detected at an expected number in the nuclei of intact cells (Fig. 3a). Different numbers of additional signals are detectable in the micronuclei formed in cells after cytomixis (Fig. 3b).

GISH analysis was conducted separately for each of the tobacco genomes. In squash preparations, the probes for the



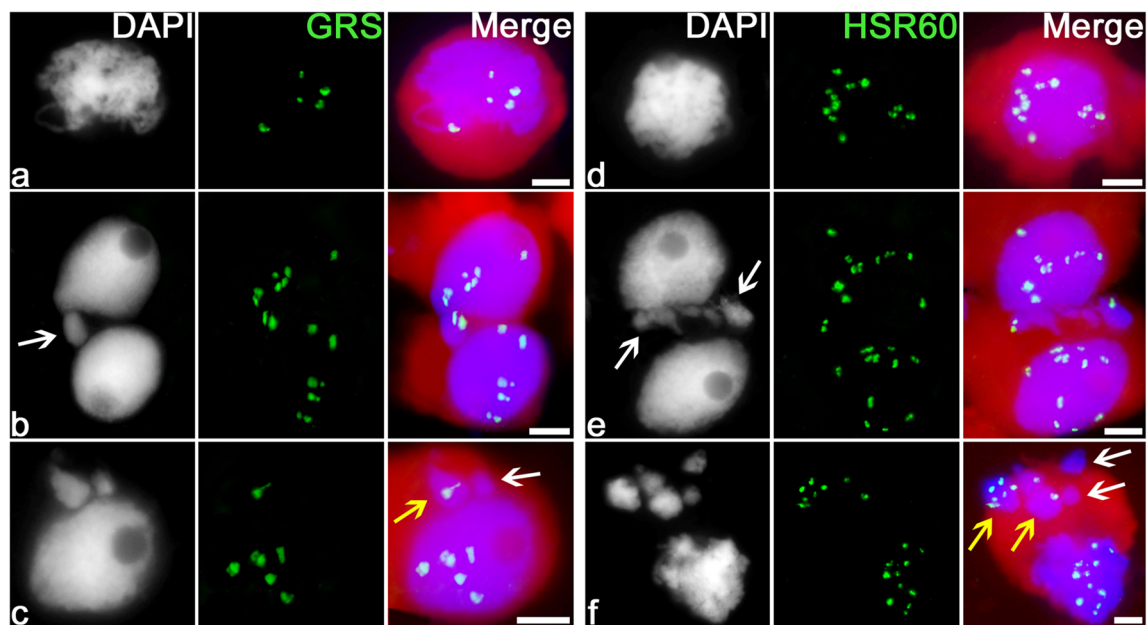
**Fig. 1** Distribution of NTRS signal in tobacco microsporocytes. **a** Intact microsporocyte. **b** Cytomictic microsporocytes; *arrow* denotes the migrating part of the nucleus. **c** Microsporocyte after cytomixis; *yellow arrows* denote the micronuclei containing NTRS, signal and *white arrow* denotes the micronucleus without the signal. **d** Intact microsporocyte with two NTRS signals. DAPI is *blue*, and cytoplasm is *red* in merged figures. *Bars*, 5  $\mu$ m

S and T tobacco genomes are rather evenly distributed in the microsporocyte nuclei in pachytene (Fig. 3c, e). As for the micronuclei formed after cytomixis, the presence or absence of the signal is rather distinct (Fig. 3d, f).

The numbers of FISH and GISH signals were statistically processed on completion of cytomixis and formation of micronuclei in recipient cells (Table 1).

The data of statistical processing suggest that the observed number of the micronuclei with the signals does not exceed the theoretically expected value, that is, the null hypothesis is retained and demonstrates that the chromosome migration in cytomixis is of a random nature without any predisposition to this process in a certain part of the tobacco genome. This result is the same for all types of repeats assayed with FISH.

GISH analysis has shown that the signals for the S or T genome are detectable at the same rate in approximately 80 % of the micronuclei. Since the signals were assayed separately, the probes for one of the genomes also marked the micronuclei containing both genomes. Correspondingly, the



**Fig. 2** Distribution of GRS (a–c) and HSR60 (d–f) signals in tobacco microsporocytes. **a, d** Intact microsporocytes. **b, e** Cytomictic microsporocytes; *arrows* denote the migrating part of the nuclei. **c, f** Microsporocytes after cytomixis; *yellow arrows* denote the micronuclei

containing the corresponding signal, and *white arrows* denote the micronuclei without the signal. DAPI is *blue*, and cytoplasm is *red* in merged figures. *Bars*, 5  $\mu$ m

micronuclei without any signal contained only the other genome. As is shown, the cytomictic micronuclei containing only one of the tobacco genomes are formed at a rate of about 20 % for S genome and about 20 % for T genome; thus, micronuclei simultaneously contain both genomes at a rate of 60 %.

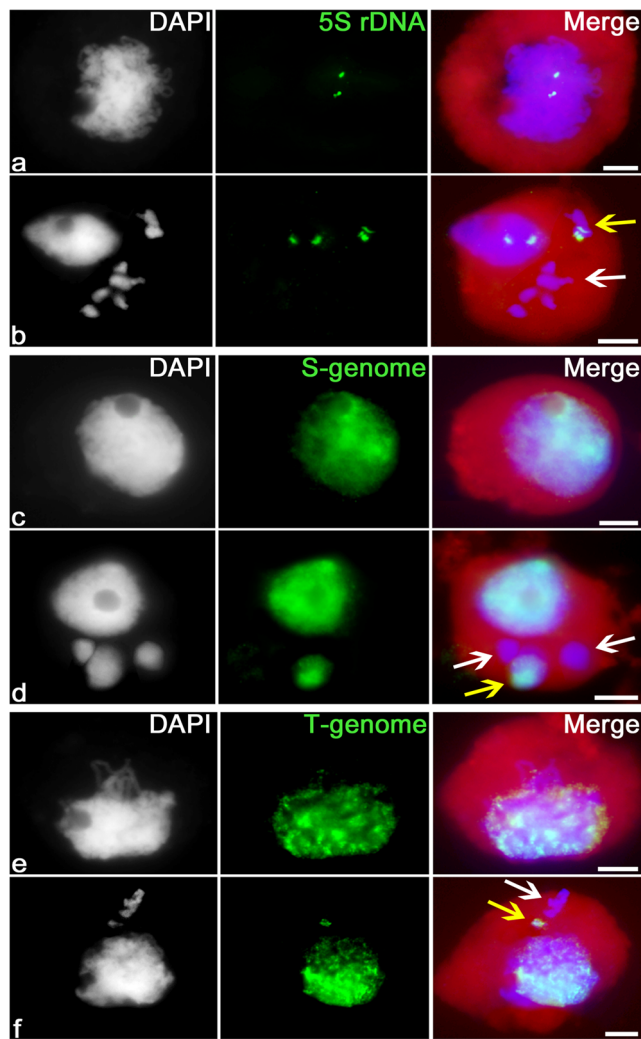
The data listed in Table 1 allow for assessing of the average number of micronuclei formed in the cell after cytomixis. In total, 736 cells and 1649 micronuclei were analyzed; thus, the tobacco microsporocytes form approximately two (2.24) micronuclei per cell after cytomixis.

## Discussion

The tobacco has a complex genome and is assumed to be an ancient allotetraploid derived from a natural hybrid between *N. sylvestris* and *N. tomentosiformis* (Lim et al. 2000; Shibata et al. 2013). Identification of individual tobacco chromosomes is a complex problem because of their small size, large number, and considerable amount of repetitive sequences. That is why combinations of markers are used for karyotyping the tobacco, since this allows a larger part of the chromosomes to be identified (Lim et al. 2000; Shibata et al. 2013). In our work, we used the markers for several groups of chromosomes to assess the probability of these chromosomes to appear in the migrating chromatin. In total, the distributions in the cytomictic chromatin of 15 chromosomes of the 24 ones constituting the tobacco haploid genome were analyzed using

NTRS, 5S rDNA, GRS, and HSR60 markers. The analyzed markers are located on chromosomes of both S and T tobacco genomes at intercalary and subterminal sites. This analysis demonstrates that the chromatin migration during cytomixis is not targeted and that the tobacco lacks any genome parts predisposed to migration to other cells. GISH analysis has shown that the cytomixis in tobacco microsporogenesis does not selectively eliminate one of the two constituent tobacco genomes. As has been demonstrated, approximately 60 % of the micronuclei simultaneously contain the chromosomes belonging to both tobacco genomes.

Our results allow both a fresh look at the phenomenon of cytomixis and a revision of the hypotheses on the role of chromatin migration in plant microsporogenesis. Researchers have been earlier inclined to regard cytomixis as a mechanism providing selective elimination of part of the chromatin that is “surplus” or damaged (Kravets 2011; Barton et al. 2014). The surplus chromatin may appear as a result of hybridization and polyploidization, which, as a rule, entail active and frequently targeted elimination of particular genome parts (Li et al. 2015). As has been assumed, cytomixis can contribute to this selective elimination by discharging it to another cell with subsequent degradation of the migrated DNA (Kalinka et al. 2010; Kravets 2011). However, we have shown in tobacco plants that there is no purposefulness in the chromatin migration. Moreover, we have earlier demonstrated that the cytomictic chromatin before, during, and after the migration to another cell displays no signs of being damaged (Mursalimov et al. 2015). These data allow the hypothesis



**Fig. 3** Distribution of 5S rDNA signals (**a, b**) and the signals for S (**c, d**) and T (**e, f**) genomes in tobacco microsporocytes. **a, c, e** Intact microsporocytes. **b, d, f** Microsporocytes after cytomixis; *yellow arrows* denote the micronuclei containing the corresponding signal, and *white arrows* denote the micronuclei without the signal. DAPI is blue, and cytoplasm is red in merged figures. Bars, 5  $\mu$ m

implying that cytomixis is the mechanism intended for elimination of “surplus” or damaged DNA to be discarded.

On the other hand, it has been repeatedly assumed that the chromatin migrating between cells during cytomixis is not degraded but rather included into the recipient cell nucleus, thereby changing the karyotype of the generated pollen and future gametes (Falistocco et al. 1995; Ghaffari 2006; Negron-Ortiz 2007; Lavia et al. 2011; Pécirix et al. 2011; Reis et al. 2016). In this case, the authors propose to regard cytomixis as a putative mechanism of genetic recombination and/or polyploidization in plants. Numerous data have been obtained for the tobacco and other plant species suggesting that cytomixis is a cause of unreduced pollen formation (Falistocco et al. 1995; Ghaffari 2006; Negron-Ortiz 2007; Lavia et al. 2011; Pécirix et al. 2011). However, migration of a whole nucleus between microsporocytes and the consequent formation of a binucleate cell are rather a rare event in the tobacco microsporogenesis. As a rule, only part of a migrating nucleus passes to recipient cells and is further detectable in its cytoplasm as micronuclei (Mursalimov and Deineko 2015). It has been shown that the chromatin in these micronuclei is surrounded by the nuclear membrane and lacks any markers that would suggest its damage or degradation (Mursalimov et al. 2013; Mursalimov et al. 2015). It has been also demonstrated that the nuclear membrane of cytomictic micronuclei can fuse with the nuclear membrane of recipient cell (Mursalimov and Deineko 2015). These data suggest that the migrated chromatin can be incorporated into the recipient cell nucleus and, thus, change the karyotype of future pollen and gametes.

In this perspective, the idea that the double NTRS signals appearing in tobacco microsporocytes, described here, is explainable by fusion of a cytomictic micronucleus carrying additional signal to the recipient cell nucleus looks most appealing. However, this statement is currently premature. The presence of a double signal is not a direct evidence for a putative fusion of the migrating chromatin and recipient cell nucleus after cytomixis. The double signal can result from the absent cohesion of homologs in meiotic prophase. Note also

**Table 1** Distribution of FISH and GISH signals in tobacco microsporocytes after cytomixis (null hypothesis states that the chromatin migration during cytomixis is random)

Repeat type	Number of analyzed cells	Number of analyzed micronuclei	Observed number of micronuclei with signal	Expected number of micronuclei with signal	$\chi^2$ (cutoff value at $p < 0.05 = 3.8$ )	$p$ value
NTRS	133	256	15	10.66 (1/24)	1.83	0.1753
5S rDNA	191	393	37	32.74 (2/24)	0.6	0.4379
GRS	117	316	67	65.83 (5/24)	0.02	0.8716
HSR60	120	283	124	129.7 (11/24)	0.46	0.4964
GISH S	106	212	178 (83.96 %)			
GISH T	69	189	153 (80.95 %)			

that additional chromosomes can appear not only owing to cytomixis but also as a result of certain disturbances during premeiotic divisions. Nonetheless, the hypothesis that cytomixis contributes to the changes in pollen karyotype attracts ever increasing interest.

In addition to the hypothesis that cytomixis can contribute to a change in the main set of chromosomes, it is assumed that the chromatin migration between cells is associated with emergence of B chromosomes in some plant species (Patra et al. 1988). In this case, it is possible that the chromatin in cytotoxic micronuclei is remodeled and transformed into additional chromosomes. However, any experimental data that would confirm this hypothesis are still absent; in particular, we have not observed any heterochromatinization of the cytotoxic chromosomes in our studies (Mursalimov et al. 2015), while this is a major specific feature of the B chromosomes. Unfortunately, the tobacco is a rather inconvenient object for studying B chromosomes because its chromosomes constituting the core set are numerous and small. Nonetheless, the hypothesis implying emergence of additional chromosomes as a result of cytomixis deserves attention, since additional chromosomes have been observed in the karyotype of tobacco SR1 (Shibata et al. 2013), the line used in this work, which may be associated with chromatin migration in developing microsporocytes.

An important result of this work is that both the FISH and GISH probes display similar good signal quality in the nuclei of intact cells, of the microsporocytes involved in cytomixis, and cytotoxic micronuclei. This once again confirms that the DNA in the microsporocytes involved in cytomixis is not subject to a massive degradation, which would rule out specific hybridization of the probes.

Note in conclusion that the specific features of cytomixis may well be species-specific. Although the tobacco has a complex hybrid genome, we cannot state that the patterns detected for it will be also observable for other hybrid and polyploid plants. By our work, we hope to inspire other researchers to conduct analogous studies into cytomixis in tobacco with new chromosome-specific markers and in other plant forms and species using FISH and GISH.

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#### Compliance with ethical standards

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

## References

- Barton DA, Cantrill LC, Law AMK et al (2014) Chilling to zero degrees disrupts pollen formation but not meiotic microtubule arrays in *Triticum aestivum* L. *Plant Cell Environ* 37:2781–2794
- Falstocco E, Tosti N, Falcinelli M (1995) Cytomixis in pollen mother cells of diploid *Dactylis*, one of the origins of 2n gametes. *J Hered* 86:448–453
- Ghaffari SM (2006) Occurrence of diploid and polyploid microspores in *Sorghum bicolor* (Poaceae) is the result of cytomixis. *Afr J Biotech* 5:1450–1453
- Kalinka A, Achrem M, Rogalska SM (2010) Cytomixis-like chromosomes/chromatin elimination from pollen mother cells (PMCs) in wheat-rye allopolyploids. *Nucleus* 53:69–83
- Kravets E (2011) The role of cell selection for pollen grain fertility after treatment of barley sprouts (*Hordeum distichum* L.) with UV-B irradiation. *Acta Biol Slov* 54:31–41
- Lavia GI, Ortiz AM, Robledo G et al (2011) Origin of triploid *Arachis pintoi* (Leguminosae) by autopolyploidy evidenced by FISH and meiotic behaviour. *Ann Bot* 108:103–111
- Li H, Guo X, Wang C, Ji W (2015) Spontaneous and divergent hexaploid triticales derived from common wheat × rye by complete elimination of D-genome chromosomes. *PLoS One* 10, e0120421
- Lim KY, Matyásek R, Lichtenstein CP, Leitch AR (2000) Molecular cytogenetic analyses and phylogenetic studies in the *Nicotiana* section *Tomentosae*. *Chromosoma* 109:245–258
- Lone A, Lone S (2013) Cytomixis—a well known but less understood phenomenon in plants. *Int J Recent Sci Res* 4:347–352
- Maliga P, Sz.-Breznovits A, Marton L (1973) Streptomycin-resistant plants from callus culture of haploid tobacco. *Nat New Biol* 244:29–30
- Mursalimov S, Permyakova N, Deineko E et al (2015) Cytomixis doesn't induce obvious changes in chromatin modifications and programmed cell death in tobacco male meiocytes. *Front Plant Sci* 6:1–13
- Mursalimov SR, Deineko EV (2015) How cytomixis can form unreduced gametes in tobacco. *Plant Syst Evol* 301:1293–1297
- Mursalimov SR, Sidorchuk YV, Deineko EV (2013) New insights into cytomixis: specific cellular features and prevalence in higher plants. *Planta* 238:415–423
- Negron-Ortiz V (2007) Chromosome numbers, nuclear dna content, and polyploidy in *Consolea* (Cactaceae), an endemic cactus of the Caribbean Islands. *Am J Bot* 94:1360–1370
- Patra NK, Srivastava HK, Chauhan SP (1988) B chromosomes in spontaneous and induced intercellular chromosome migration of *Papaver somniferum*. *Indian J Genet* 48:31–42
- Pécix Y, Rallo G, Folzer H et al (2011) Polyploidization mechanisms: temperature environment can induce diploid gamete formation in *Rosa* sp. *J Exp Bot* 62:3587–3597
- Reis A, Sousa S, Viccini L (2016) High frequency of cytomixis observed at zygote in tetraploid *Lippia alba*. *Plant Syst Evol* 302:121–127
- Shibata F, Nagaki K, Yokota E, Murata M (2013) Tobacco karyotyping by accurate centromere identification and novel repetitive DNA localization. *Chromosom Res* 21:375–381
- 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
- Wang CY, Li X, Wu QF, Wang X (2006) Cytoplasmic channels and their association with plastids in male meiocytes of tobacco, onion and lily. *Cell Biol Int* 30:406–411
- Yu CH, Guo GQ, Nie XW, Zheng GC (2004) Cytochemical localization of pectinase activity in pollen mother cells of tobacco during meiotic prophase I and its relation to the formation of secondary plasmodesmata and cytoplasmic channels. *Acta Bot Sin* 46:1443–1453