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ORIGINAL ARTICLE

Meiotic aberrations during 2n pollen formation in *Begonia*

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Unreduced gametes are the driving force for the polyploidization of plants in nature, and are also an important tool for ploidy breeding. The final heterozygosity of a 2n pollen grain depends on the cytological mechanism behind 2n pollen formation. In this study, chromosome pairing and chromosome segregation during the microsporogenesis of seven *Begonia* genotypes were analysed using fluorescent chromosome staining on (squashed) pollen mother cells. Among the seven genotypes, five genotypes produce 2n pollen (*B.* 'Bubbles', *B.* 'Florence Rita', *B.* 'Orococo', *B.* 'Tamo' and B276) and two genotypes produce only normal n pollen (*B. fischeri* and B243). All 2n pollen producers showed a mechanism equivalent to first division restitution (FDR), in which chromosomes did not segregate during meiosis I but only during meiosis II. This FDR was the result of (a) an irregular chromosome pairing in *B.* 'Tamo', (b) stickiness of chromosomes associated with numerous chromosome bridges in *B.* 'Florence Rita' and B276, and (c) a combination of irregular chromosome pairing and stickiness of chromosomes in *B.* 'Bubbles'. The exact mechanism of the nuclear restitution in *B.* 'Orococo' could not be determined. Other mechanisms, such as early asymmetric cytokinesis, omission of meiosis II, parallel or tripolar spindle formation, were rather uncommon. Unpaired chromosomes (univalents) were observed in all genotypes, but they had moved to one of the poles by the end of anaphase I or II. Only *B.* 'Tamo' formed a high number of micronuclei. Consequently, this genotype formed a large number of malformed pollen. Obviously, chromosome behaviour during meiosis in *Begonia* is very dynamic, which may have important consequences for chromosome evolution and biodiversity within the genus.
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Introduction

Unreduced gametes, or gametes with the somatic chromosome number, are considered to be the driving force for polyploidization of plants in nature. Polyploidization is an important mechanism for speciation and adaptation in plants. Some of today's most important crops, such as wheat and potato, are polyploid (Ramanna and Jacobsen, 2003). Although the existence of 2n gametes has been known for a long time, it was assumed that the production of these gametes was rather sporadic and not useful for plant breeding (Ramanna and Jacobsen, 2003). New methods for synthetic polyploidization of plants through the use of spindle inhibitors, such as colchicine or dinitroanilines (for example, oryzalin and trifluralin), have been developed and successfully used in several plants (Vaughn and Lehnen, 1991; Hancock, 1997; Zlesak *et al*., 2005; Dhooghe *et al*., 2009). However, synthetic polyploidization has contributed little to crop improvement, likely owing to fixed heterozygosity (Soltis and Soltis, 2000). Contrary to the assumption of Stebbins (1950), Harlan and De Wet (1975) showed that almost all plant species produce 2n gametes with some frequency. Sexual polyploids, resulting from the fusion between a 2n gamete and a normal (or another 2n) gamete, have been shown to be useful for crop improvement (Ramanna and Jacobsen, 2003). In sexual polyploids, heterozygosity is not fixed because of recombination between parental chromosomes. Owing to this recombination, introgression can be achieved (Lim *et al*., 2001).

The heterozygosity within a 2n pollen grain depends on the cytological mechanism operating in 2n pollen formation. These mechanisms are currently subdivided as first division restitution (FDR), second division restitution (SDR), indeterminate meiotic restitution (IMR) or post-meiotic restitution (Ramanna and Jacobsen, 2003). In FDR, the pairing and/or the separation of the homologous chromosomes at meiosis I does not occur (univalent formation), whereas the second division occurs normally with the two sister chromatids of each chromosome moving to opposite poles. FDR is typical in synaptic mutants or distant hybrids, in which homologous chromosome pairing (bivalent formation) is completely absent, although other mechanisms, such as cytokinesis failure or spindle abnormalities during metaphase II, can also lead to an equivalent of FDR (Ramanna and Jacobsen, 2003). With the exception of cross-over segments, FDR pollen retain all homologous

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parental chromosomes. FDR pollen are very important in producing heterozygous hybrids, because of the highly heterozygous 2n gametes formed (Bretagnolle and Thompson, 1995). In SDR, the pairing and the separation of the homologous chromosomes during meiosis I occurs normally (bivalent formation). In meiosis II, the centromeres of the half-bivalents divide, but the chromatids do not migrate to the poles. As SDR gametes contain random combinations of sister chromatids, the heterozygosity within one pollen grain is lower than pollen produced by FDR. SDR rather occurs in hybrids in which the genomes are closely related (Bretagnolle and Thompson, 1995; Ramanna and Jacobsen, 2003). Less frequently reported are IMR and post-meiotic restitution. IMR was detected in lily (Lim *et al*., 2001), and showed characteristics similar to both SDR and FDR. In this intermediate type, both univalents and bivalents are formed during metaphase I. Although the univalents divide equationally as happens in FDR, bivalents disjoin reductionally as seen in SDR. Post-meiotic restitution, where chromosomes duplicate after meiosis, was observed by Bastiaanssen *et al*. (1994) in potato.

Traditional cytological methods based on fixed anthers or ovaries are frequently used to identify mechanisms operating in 2n pollen formation. The identification is based on abnormalities during chromosome orientation, chromosome division, spindle formation (parallel or tripolar spindles) and cytokinesis (Bretagnolle and Thompson, 1995; Jauhar, 2003; Taschetto and Pagliarini, 2003). However, identifying the mechanisms of origins of unreduced gametes is a complex process because different cytological mechanisms can be active within one genotype (Ramanna and Jacobsen, 2003). Other methods focus on the use of molecular cytological techniques, such as GISH (Lim *et al*., 2001), or molecular genetic markers as AFLP (Crespel *et al*., 2002) or RFLP (Barone *et al*., 1995; Chen *et al*., 1997).

The pantropical genus *Begonia* is one of the largest *Angiosperm* genera in the world, with more than 1500 species. Cultivation of the genus has produced several economically important groups, for example, tuberous begonias (*B. x cheimanta*, *B. x hiemalis*, *B. x tuberhybrida*), begonias grown for their ornamental foliage (*B. rex* cultorum group) and the *B. semperflorens* cultorum group (Haegeman, 1979; Anderson, 2007). Begonias are easily grown from seed and cross-hybridize well (Anderson, 2007). A wide variety of chromosome numbers have been reported for *Begonia* species and cultivars, ranging from $2n = 16$ for *B. Rex* to 156 for *B. acutifolia* (Legro and Haegeman, 1971; Sarkar, 1989; Doorenbos *et al*., 1998; Oginuma and Peng, 2002). This suggests frequent polyploidy and aneuploidy in the genus. Owing to this diversity, there is no general basic chromosome number for the genus, although $x = 11$ (several Asiatic begonias; Oginuma and Peng, 2002), 13 and 14 (tuberous begonias; Legro and Haegeman, 1971) are mostly suggested. Several tuberous cultivars are tetraploid, and also tetraploid cultivars of the *B. semperflorens* cultorum group surpassed the diploid cultivars during their breeding history (Horn, 2004). Although several polyploidization events must have occurred during past *Begonia* breeding, the potential role of unreduced gametes to create genetic variability through sexual polyploidization is unexploited in begonias. Such breeding would require knowledge of the mechanisms behind 2n gamete production, because the genetic information (or heterozygosity) stored in a 2n gamete and subsequently passed on to the progeny depends on the exact mechanism of nuclear restitution.

In *Begonia*, 10 genotypes that produce unreduced pollen have recently been identified (Dewitte *et al*., 2009). In all these genotypes, meiotic aberrations resulted mainly in the formation of dyads. Five 2n pollen-producing genotypes were selected and their chromosome behaviour during meiosis was observed microscopically using fluorescent chromosome staining on (squashed) pollen mother cells (PMCs). Our objective was to analyse the meiosis in order to determine the exact mechanism behind 2n pollen formation within the five selected genotypes. Two other genotypes, characterized by normal tetrad formation and high pollen fertility, were used as controls.

Materials and methods

Plant material

On the basis of previously published results (Dewitte *et al*., 2009), five 2n pollen-producing *Begonia* genotypes were selected: *B.* 'Orococo' ($2n = 40 + 1$ or 2 fragments), *B.* 'Tamo' ($2n = 23 + 0$ to 2 fragments), *B.* 'Bubbles' ($2n = 78$), *B.* 'Florence Rita' ($2n = 54$) and B276 ($2n = 50$). As a control, two genotypes that produce only normal pollen were used: *B. fischeri* ($2n = 92$) and B243 ($2n = 22$). *Begonia* plants were grown in glasshouses, under standard conditions ($20 \pm 2\,^{\circ}C$, with an extended natural photosynthetic period of $16\,h\,day^{-1}$ (photosynthetic photon flux density of $30\,\mu mol\,m^{-2}\,s^{-1}$)).

Microsporogenesis and pollen characteristics of these genotypes are described in Tables 1 and 2 (adapted from Dewitte *et al*., 2009).

Study of the meiosis

Immature anthers were removed from young floral buds and one single anther was immediately excised onto a slide in a drop of saline sodium citrate (SSC) buffer (0.3 M NaCl, 0.03 M sodium citrate; pH 7) containing 1 μM 4′,6-diamidino-2-phenylindole (DAPI). A coverslip was added and the preparation was examined using light or fluorescence microscopy. When the anther contained PMCs in the process of meiosis, the other anthers of the same flower were treated one by one in the same way to evaluate the different stages of meiosis (prophase I to telophase II). At least 100 cells were scored at each stage.

Table 1 Microsporogenesis characteristics of the investigated *Begonia* genotypes

	Tetrad	*Triad*	*Dyads*	*Monads*	*Others*[a]
B. fischeri	99	0	0	0	1
B243	100	0	0	0	0
B. 'Orococo'	42	4	52	0	2
B. 'Tamo'	55	1	4	1	39
B. 'Bubbles'	81	4	12	2	1
B. 'Florence Rita'	5	1	82	12	0
B276	4	2	89	6	0

Percentage (%) of observed tetrads, triads, dyads or monads.
[a]Pentads, hexads or dyads with micronuclei.

For observation of chromosome pairing at metaphase I, anthers were fixed in ethanol/acetic acid (3:1). After overnight incubation at 4 °C, the anthers were excised on a slide in 45% acetic acid and the anther debris was removed. After squashing under a coverslip, the slides were frozen in liquid nitrogen. The coverslip was removed using a sharp razor blade and the preparation was rinsed in 98% ethanol, then air-dried for at least 1 h. For staining, slides were washed in SSC buffer. A drop of 100 µl DAPI counterstain (1 µM DAPI in SSC) was dropped onto the slide and then covered with a 25 × 50 coverslip. After 5 min incubation, the coverslip was removed and the slides were washed in SSC buffer. A drop of Vectashield (Vector Laboratories, Burlingame, CA, USA) Mounting Medium was added, covered with a 25 × 50 coverslip and examined under the microscope (slides may be stored at 4 °C). The evaluation of chromosome pairing was based on at least 50 PMCs.

Table 2 Frequency (%) of malformed pollen and 2n pollen, as well as pollen germination capacity for the different investigated *Begonia* genotypes

	% Malformed pollen	*% Germination*	*% 2n pollen*[a]
B. fisheri	7	78	0
B243	13	74	0
B. 'Orococo'	71	9	88
B. 'Tamo'	98	1	—
B. 'Bubbles'	58	5	14
B. 'Florence Rita'	38	0	100
B276	29	52	100

Malformed pollen are observed microscopically as flat, shrunken pollen.
[a]On the basis of pollen size and shape. Only good-shaped pollen are considered for the calculations. For *B.* 'Tamo', the accuracy of using pollen size or shape to estimate the frequency of 2n pollen is rather poor as discussed in Dewitte *et al*. (2009).

Results

Formation of restitution nuclei during meiosis

A simultaneous type of meiosis was observed in all genotypes: cytokinesis started only after the formation of the four nuclei. Within one single anther of the same flower in the controls B243 and *B. fischeri*, PMCs developed simultaneously through meiosis (Figure 1). In contrast, 49% of the PMCs of *B.* 'Orococo' did not progress with the other PMCs to anaphase I in order to form two equal nuclei. Instead, a single restitution nucleus was formed (Figures 2a–d). The same phenomenon was observed in *B.* 'Tamo' in 27% and in *B.* 'Bubbles' in 16% of the PMCs. In *B.* 'Florence Rita', only 16% of the PMCs formed two distinctive nuclei at the end of anaphase I. The other PMCs of *B.* 'Florence Rita' formed spherical or 8-shaped restitution nuclei. This was the result of an incomplete separation of the two nuclei during anaphase I (Figure 2f). A similar event was observed in B276: only 11% of the PMCs reached the binuclear stage at the end of anaphase I, whereas the other PMCs formed restitution nuclei. A small micronucleus was sometimes formed during restitution. In all genotypes, the restitution nuclei divided at anaphase II

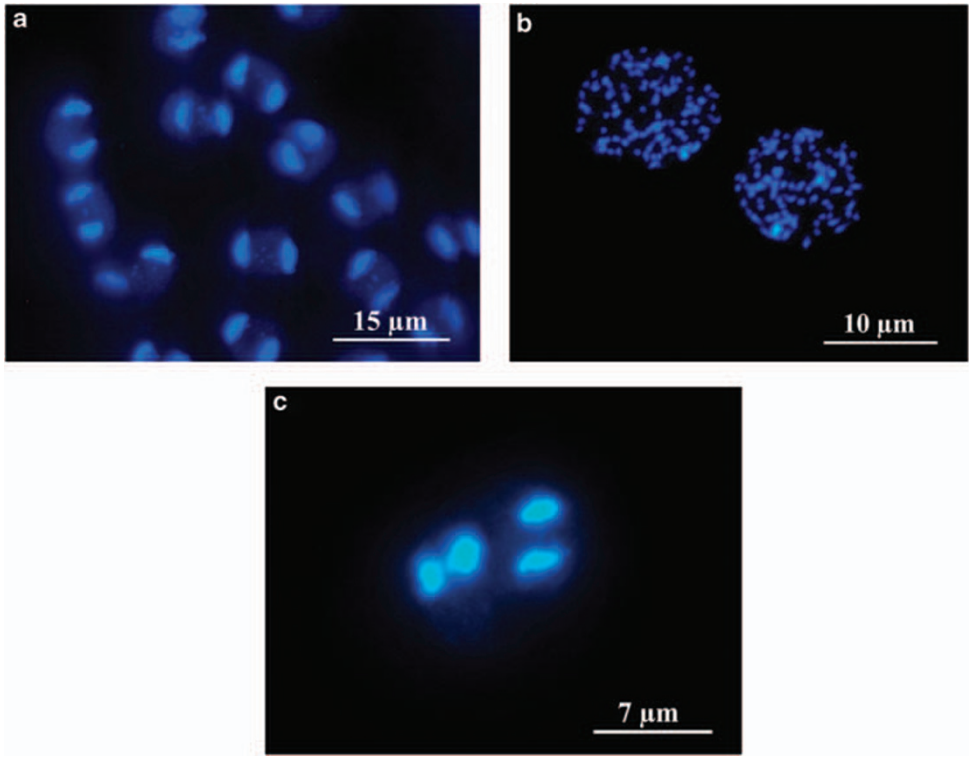

Figure 1 Different stages in the meiosis of the control *Begonia fischeri*. (**a**) All PMCs progress simultaneously through anaphase I, although in several PMCs lagging chromosomes were observed. (**b**) At telophase I, two equal nuclei were formed. (**c**) At anaphase II, the two nuclei formed after meiosis I divided with a different spindle orientation.

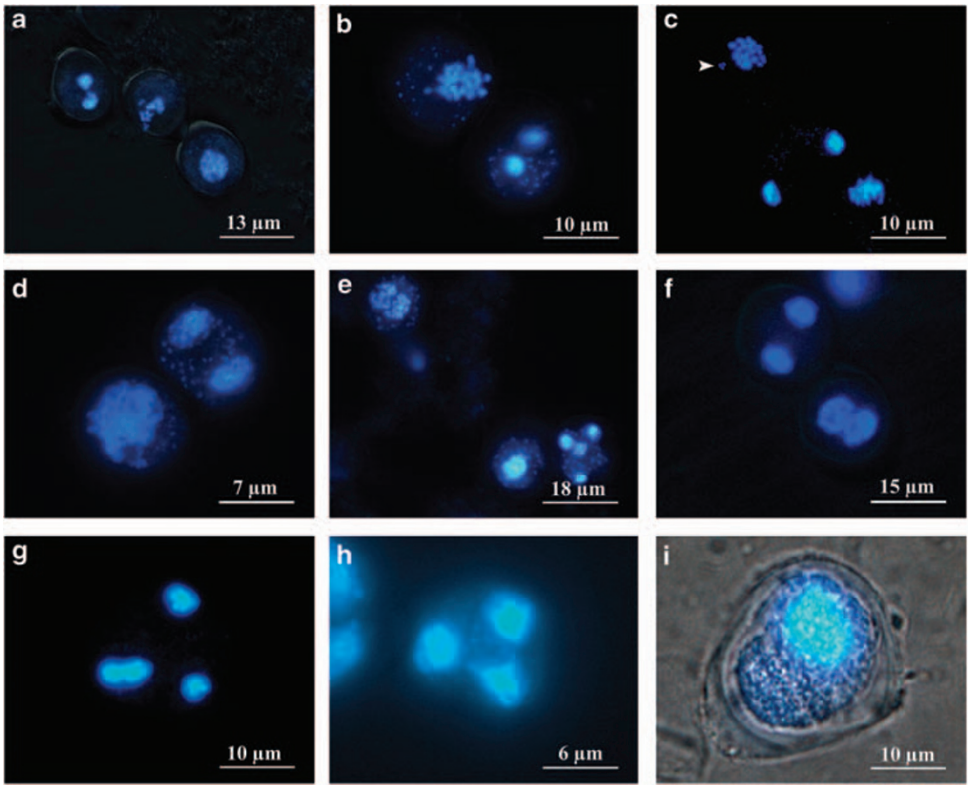

Figure 2 Nuclear restitution events observed within the investigated genotypes. (**a**) Two restitution nuclei and one dividing nucleus during anaphase I of *B.* 'Orococo'. (**b**) Restitution nucleus next to a dividing nucleus at anaphase I in *B.* 'Orococo'. The restitution nucleus of *B.* 'Orococo' consists of an unoriented chromatin mass. (**c**) One dividing nucleus (middle) next to two restitution nuclei in *B.* 'Orococo' at anaphase I. One of the restitution nuclei forms a small micronucleus of univalents (arrowhead). (**d**) Telophase I of a restitution nucleus and a normally dividing nucleus in *B.* 'Orococo'. (**e**) Although normal pollen mother cells of *B.* 'Orococo' reach the four-nuclear stage after anaphase II (right below), restitution nuclei divide to reach the binuclear stage. (**f**) At the end of anaphase I in B276 and *B.* 'Florence Rita', frequently two nuclei could be observed, which did not completely separate and form an 8-shaped restitution nucleus. (**g**) Furthermore in anaphase II, incomplete division of one of the two nuclei could be observed (*B.* 'Orococo'). (**h**) A tripolar spindle at anaphase II in *B.* 'Orococo' will form a triad. (**i**) Early asymmetric cytokinesis, leading to an empty microspore and a 4n microspore in *B.* 'Orococo'.

to form a FDR dyad, whereas the normal PMCs developed to a 4-nuclear stage and formed a tetrad (Figure 2e). Restitution nuclei did not develop into dyads but resulted in monads in 8% (*B.* 'Florence Rita') and 5% (B276) of the PMCs.

In *B.* 'Tamo', 32% of the PMCs contained three nuclei after meiosis II, mostly accompanied by micronuclei. In these cases, one of the two nuclei present after a normal meiosis I did not completely divide during anaphase II (SDR), and finally resulted in the formation of triads (or polyads when micronuclei were formed) (Figure 2g). This type of restitution also occasionally occurred in *B.* 'Orococo' (4% of the PMCs) and *B.* 'Bubbles' (3% of the PMCs). Triads also originated from tripolar spindles (Figure 2h) at meiosis II, but this was rather uncommon. Parallel spindles were highest in the control B243 with a frequency of 6%, but did not result in restitution nuclei. In some PMCs of *B.* 'Orococo', *B.* 'Bubbles', *B.* 'Florence Rita' and B276, early (asymmetric) cytokinesis was also observed (Figure 2i).

An overview of the nuclear restitution types can be found in Table 3.

Chromosome pairing at metaphase I

Sufficient chromosome spreading at metaphase I could only be obtained in *B. fisheri*, B243, *B.* 'Orococo' and *B.* 'Tamo'. In *B.* 'Bubbles', B276 and *B.* 'Florence Rita', large sticky chromosome clusters were formed, in addition to paired (bivalents or multivalents) or unpaired (univalents) chromosomes. The exact chromosome pairing in B276 and *B.* 'Florence Rita' was not evaluated because the large chromosome clusters made it impossible to evaluate the exact chromosome pairing.

In the controls B243 and *B. fisheri*, and in the 2n pollen producer *B.* 'Orococo', the number of univalents was low compared with the numbers of bivalents (Table 4; Figures 3a–c). In B243, *B.* 'Orococo' and *B.* 'Tamo', no chromosome clusters were observed; all chromosomes could be identified as bivalents, univalents or—in case of *B.* 'Tamo'—multivalents. In *B. fisheri*, one or two very small chromosome clusters were occasionally observed. Very few chromosome bridges in *B. fisheri* were present at this stage. *B.* 'Tamo' formed a high number of univalents, whereas the other chromosomes formed bivalents or multivalents of 3–7 chromosomes (Figure 3d). In *B.* 'Bubbles', most chromosomes clumped together in one or two large chromosome clusters (Figure 3e). Next to these chromosome clusters, several univalents and occasionally also bivalents or multivalents were present. *B.* 'Florence Rita' and B276 created various chromosome clusters of paired chromosomes and also several chromosome bridges (Figure 3f).

Table 3 Importance of several restitution mechanisms within the different investigated *Begonia* genotypes

Genotype	*% Omission of MI*[a]	*% Early cytokinesis*	*% Omission of MII*[a]	*% Parallel spindles at MII*	*% Tripolar spindles at MII*
B. fischeri	0	0	0	3	0
B243	0	0	0	6	0
B. 'Orococo'	49	3	4	6	1
B. 'Tamo'	27	0	32	5	1
B. 'Bubbles'	16	2	3	1	0
B. 'Florence Rita'	84	1	0	2	0
B276	89	1	0	2	0

Abbreviations: MI, metaphase I; MII, metaphase II.
Values represent the percentage (%) of PMCs in which the specific restitution type was observed.
[a]Absence or incomplete division of the nuclei.

Table 4 Pairing behaviour of chromosomes during metaphase I (%) as observed during pollen meiosis in the different *Begonia* genotypes

	Univalents	*Bivalents*	*Multivalents*	*Chromosome clusters*
B243	7	93	0	0
B. fisheri	10	88	0	2
B. 'Orococo'	16	84	0	0
B. 'Tamo'	58	30	12	0
B. 'Bubbles'	79	22	2	10

In *B.* 'Florence Rita' and B276, chromosome pairing was not evaluated due to the large number of chromosome clusters formed.

Behaviour of univalents during meiosis

During metaphase I, several univalents did not accumulate at the equatorial plane. This happened in only 4–6% of the PMCs in the controls, but occurred in 93% of the PMCs in *B.* 'Bubbles' (Figure 4a). In the other genotypes, this percentage varied between 16 and 29% (Table 5). The further behaviour of these univalents depended on the genotype and resulted in retarded movement to the pole, formation of micronuclei and precocious separation in sister chromatids as described below (Figures 4b–f).

In *B. fischeri*, B243, *B.* 'Orococo', *B.* 'Bubbles', *B.* 'Florence Rita' and B276, the univalents joined the large chromosome sets at the poles, although they were retarded in their movement (lagging chromosomes). During metaphase and anaphase II, lagging chromosomes were also observed, but were finally incorporated in the same microspore as the other chromosomes after cytokinesis. This finally resulted in a tetrad; formation of micronuclei was rather uncommon.

In *B.* 'Tamo', lagging chromosomes rather formed new micronuclei. Micronuclei formed after meiosis I resulted in two daughter micronuclei after anaphase II (Figure 4e). New micronuclei were also formed during meiosis II. This resulted in the formation of pentads and hexads.

Precocious separation of univalents into two sister chromatids during anaphase I was observed in about 1% of the PMCs of both *B.* 'Florence Rita' and B276. These univalents stayed at the equatorial plane and divided later than the half-bivalents (Figure 4f).

Remarkably, the control *B. fisheri* showed a very high number of cells with lagging chromosomes during anaphase I. However, in contrast to the other genotypes, the lagging chromosomes consisted mostly of bivalents that divided shortly after the other bivalents. The half-bivalents joined the other bivalents at the poles.

Occurrence of chromosome bridges during meiosis

In *B.* 'Florence Rita', B276 and *B.* 'Bubbles', about half of the PMCs that progressed to the binuclear stage during anaphase I contained chromosome bridges (Figure 5). In *B.* 'Florence Rita' and B276, numerous bridges were formed, frequently accompanied by fragments or univalents. In *B.* 'Bubbles', up to three bridges without fragments were observed.

Chromosome bridges were completely absent in B243 and *B.* 'Tamo', and appeared only rarely (<1% of the anaphase I cells) in *B.* 'Orococo' and *B. fischeri*.

Two types of chromosome bridges were observed: single and double arm bridges. The latter type was only observed exceptionally in *B.* 'Florence Rita' and B276.

Discussion

Our results show that restitution occurred mainly during the first division of the meiosis, resulting in FDR dyads and 2n pollen grains. This concurs with previous observations where 2n pollen producers formed mostly dyads (Dewitte *et al*., 2009). In all genotypes, a large number of the PMCs did not progress to anaphase I to form two equal nuclei; they formed restitution nuclei instead. Only during meiosis II did restitution nuclei divide normally. This is equivalent to a meiosis, where meiosis I is omitted and FDR gametes are formed. In addition, some less important mechanisms may result in an equivalent of FDR (abnormal spindle geometry during meiosis II, resulting in triads). Other mechanisms were also observed that result in SDR (incomplete separation of nuclei during meiosis II, resulting in triads) or IMR pollen (precocious separation of univalents, resulting in dyads), but they occurred only sporadically. Only in *B.* 'Tamo', triads were frequently observed in addition to dyads, but they were mostly accompanied by micronuclei owing to unbalanced chromosome segregation. Several of these cytological abnormalities responsible for 2n gamete production have been observed in other crops and discussed more extensively by Bretagnolle and Thompson (1995) and Ramanna and Jacobsen (2003).

FDR is typical in synaptic mutants or distant hybrids, where homologous chromosome pairing (bivalent formation) is low or absent (Ramanna and Jacobsen, 2003). A low frequency of chromosome pairing results only in

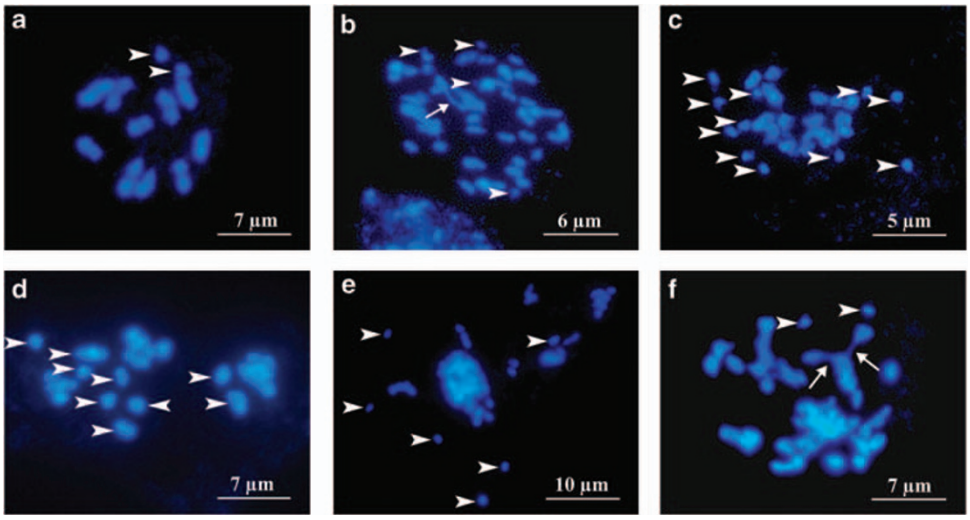

Figure 3 Representative meiotic chromosome spreads of the different investigated genotypes. (**a**) B243 with 10 bivalents and 2 univalents. (**b**) *B. fisheri* with four univalents and two small chromosome clusters, from which one possessed chromosome bridges. The other chromosomes formed bivalents. (**c**) *B.* 'Orococo' with 15 bivalents and 11 univalents. (**d**) Chromosomes of *B.* 'Tamo' frequently showed abnormal pairing or did not pair at all. During this abnormal pairing, several multivalents of non-homologous chromosomes were formed. (**e**) In *B.* 'Bubbles', typically one or two large chromosome clusters were formed in addition to several univalents, although also bivalents and multivalents were observed. (**f**) In B276 and *B.* 'Florence Rita', several small and large chromosome clusters were observed, frequently linked to each other with chromosome bridges. Arrows indicate bridges and arrowheads indicate univalents.

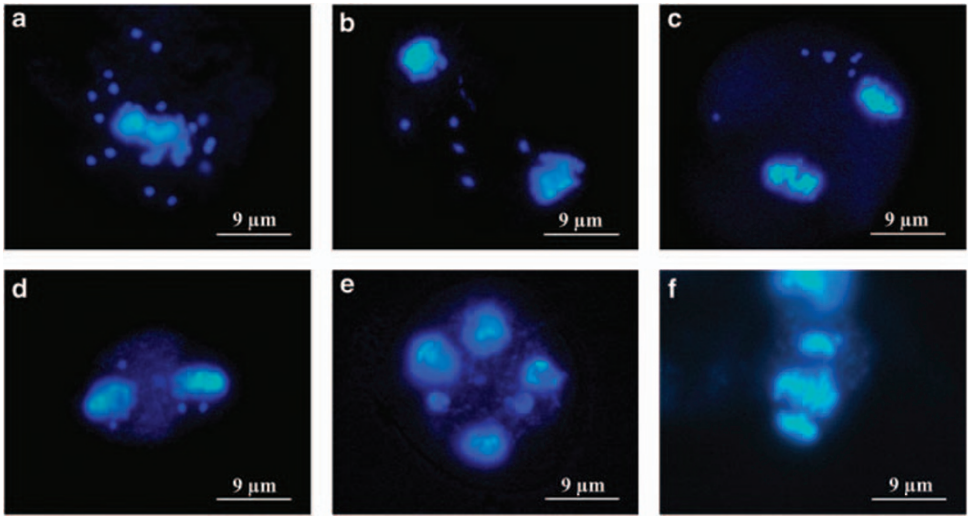

Figure 4 Univalent segregation behaviour during metaphase and anaphase I and II. (**a**) Univalents do not accumulate at the equatorial plate at metaphase I in *B.* 'Bubbles'. Exceptionally, also bivalents were involved. (**b**) Lagging chromosomes at anaphase I, retarded in their movement to a pole (*B.* 'Bubbles'). (**c**) Lagging chromosomes in *B.* 'Tamo' do not join the other chromosomes at a pole but will form micronuclei. (**d**) Similar to metaphase I, lagging chromosomes at metaphase II do not join the other chromosomes at the equatorial plate (*B.* 'Orococo'). (**e**) Micronuclei formed after meiosis I also divide during meiosis II (B. *'Tamo'*). (**f**) Precociously separation of univalents located at the equatorial plane in *B.* 'Florence Rita'.

an equational division of all sister chromatids, whereas no half-bivalents are formed. This is equal to a meiosis, where meiosis I is omitted and only meiosis II (a mitotic division) is completed, resulting in an FDR dyad. In wheat–barley hybrids for instance, it is postulated that restitution depends on a high frequency of univalent accumulation at the equatorial plate at metaphase I and the subsequent failure of the chromosomes to undergo anaphase I separation (Islam and Shepherd, 1980). As *B.* 'Orococo', *B.* 'Tamo' and *B.* 'Florence Rita' are described as interspecific hybrids (Tebbitt, 2005; no exact information is available on *B.* 'Bubbles' and B276), the presence of FDR was expected. However, only in *B.* 'Tamo' and *B.* 'Bubbles' meiosis pairing was very irregular and a high number of univalents were formed. The behaviour of these univalents was very unpredictable and resulted in retarded movement to the poles, micronucleus formation or precocious separation of the univalents (equational division). It has been reported in all cases that univalents divide after bivalents (Dawe, 1998). The above-mentioned aberrations have been reported in several genera as in durum wheat haploids (Jauhar, 2003), leek (Kazanehdari and Jones, 1997) and hybrids of *Nicotiana* (Trojak-goluch and Berbeć, 2003),

Table 5 Percentage PMCs with lagging chromosomes at each stage of the meiosis as observed in the different *Begonia* genotypes

	Metaphase I	*Anaphase I*	*Metaphase II*	*Anaphase II*
B. fisheri	4	41	6	3
B243	6	19	26	16
B. 'Orococo'	16	14	13	5
B. 'Tamo'	27	49	63	58
B. 'Bubbles'	93	27	31	24
B. 'Florence Rita'	26	28	10	15
B276	29	22	8	8

Abbreviation: PMC, pollen mother cell.
In metaphase, lagging chromosomes did not join the other chromosomes at the equatorial plate, whereas in anaphase, lagging chromosomes did not migrate simultaneously to the poles with the half-bivalents. At the end of meiosis, lagging chromosomes were incorporated within the microspores of the tetrad or formed new micronuclei.

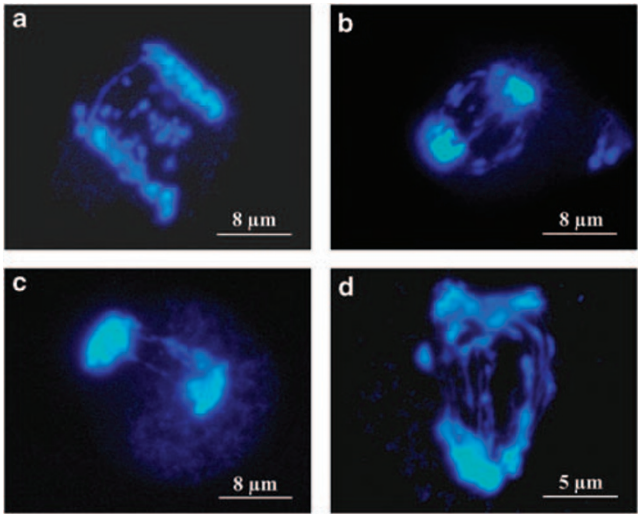

Figure 5 Chromosome bridge formation in *B.* 'Bubbles', *B.* 'Florence Rita' and B276. (**a**) Chromosome bridges were frequently observed simultaneously with fragments or univalents (*B.* 'Florence Rita'). (**b**) Three double chromosome bridges in *B.* 'Florence Rita'. (**c**) Up to three very thin chromosome bridges were observed in *B.* 'Bubbles'. (**d**) In *B.* 'Florence Rita' and B276, numerous chromosome bridges were formed. Both single and double arm chromosome bridges can appear simultaneously.

wheat–barley (Islam and Shepherd, 1980) and *Citrus* (Del Bosco *et al*., 1999), but are not necessarily associated with 2n pollen formation. With the exception of *B.* 'Tamo', which frequently formed micronuclei, the univalents mostly migrated to the pole and were incorporated into one of the microspores. This explains why Dewitte *et al*. (2009) observed a low frequency of micronuclei formation in all genotypes, with the exception of *B.* 'Tamo'. The numerous micronuclei in *B.* 'Tamo' resulted in a very high number of malformed pollen grains; the result of unbalanced chromosome segregation. On the basis of pollen malformation, it can also be concluded that chromosome segregation was more balanced in the controls than in the 2n pollen producers, although it does not always result in micronucleus formation. Jauhar (2003) showed in durum wheat (*Triticum turgidum*) that the distribution of univalents to a pole is indeed very irregular.

Although the high frequency of univalents in *B.* 'Tamo' may explain nuclear restitution, our results do not support an association between 2n pollen formation and a high number of univalents within *B.* 'Orococo', *B.* 'Florence Rita' and B276. Other causes must be present within these genotypes. One of these causes could be problems with orientation of the chromosomes at metaphase I. In *B.* 'Orococo', restitution nuclei consisted mostly of an unorientated chromatin mass, which finally restitutes. For normal chromosome segregation, chromosomes must align at the equatorial plane and sister kinetochores must orient together to the same spindle pole. This is known as co-orientation of the sister chromatids (Dawe, 1998). When the sister kinetochores fail to orient together, chromatids may disjoin.

B. 'Bubbles', *B.* 'Florence Rita' and B276 formed a large number of chromosome bridges. Chromosome bridges have often been studied by irradiating and damaging chromosomes within the cell or pollen grain (Viccini and de Carvalho, 2002; Carballo *et al*., 2006), or by observing cancer cells containing chromosomes damaged by spontaneous telomere loss (Fouladi *et al*., 2000; Lo *et al*., 2002; Acilan *et al*., 2007). Damaged chromosomes enter the breakage–fusion–bridge cycle, which is well investigated in maize (Zheng *et al*., 1999). In general, chromosome breakage results in chromosome stickiness, congregation in groups of bivalents and/or univalents and lagging chromosomes (Rao and Rao, 1977). This was similar to our observations of *B.* 'Bubbles', *B.* 'Florence Rita' and B276. The sticky chromosome ends can fuse together to yield unusual chromosome structures such as rings or dicentrics (Franklin and Cande, 1999). Sticky chromosomes, bridges and chromatin clustering have also been reported in natural systems. Examples include gametes of *Nicotiana tabacum* × *N. glauca* (Trojak-goluch and Berbeć, 2003), soybean (Palmer *et al*., 2000), *Solanum tuberosum* (Ünal and Alp, 2002), *Carica papaya* (Bajpai and Singh, 2006) and a meiotic mutant of pearl millet (Rao *et al*., 1990). The occurrence of bridges has frequently been used as indirect evidence for the presence of paracentric inversions during crossing-over (Newman, 1966), which result in both chromosome bridges and fragments. The stickiness of the chromosomes and the frequent occurrence of chromosome bridges in *B.* 'Florence Rita', B276 and *B.* 'Bubbles', together with the occurrence of fragments, indicates the existence of damaged chromosomes during meiosis. As there was no proof of unusual chromosome structures in chromosome spreads of somatic root tip cells of these *Begonia* genotypes (data not shown), chromosome damage must occur during crossing-over.

Chromosome bridges are one possible source of nucleus restitution. Bajer (1964) already remarked that kinetochore movement can be retarded by the presence of chromatin bridges, or, when numerous bridges are formed, it may result in nuclear restitution. The large number of bridges, mainly present in *B.* 'Florence Rita' and B276, but also in *B.* 'Bubbles', hinders normal spindle activity due to the tight associations between the chromosomes. This leads to a typical 8-shaped nucleus because of incomplete chromosome separation, which then results into restitution. The presence of chromosome bridges may also be the cause of the chromosome clustering at metaphase in these genotypes.

These results shed new light on chromosome evolvement in the genus *Begonia*. *Begonia* chromosomes

typically contain a high number of large secondary constriction (Oginuma and Peng, 2002). It is presumed that chromosome translocations occurred after polyploidization, followed by a decrease in chromosome number and genome stabilization (Oginuma and Peng, 2002). Our results indicate that large chromosomal rearrangements may also occur during restitution, when (damaged) chromosomes reassociate.

To conclude, FDR is the basic mechanism behind the 2n pollen formation in the investigated begonias. In *B*. 'Tamo', nuclear restitution originates from a highly irregular chromosome pairing, whereas in *B*. 'Florence Rita' and B276, chromosome stickiness and chromosome bridge formation have an important role in restitution. In *B*. 'Bubbles', a combination of both irregular meiosis pairing and chromosome bridges was observed. Only *B*. 'Orococo' had no obvious reason for nuclear restitution, although problems with chromosome orientation and segregation at metaphase I are suspected. The final genetic variability transmitted by the FDR pollen is dependent on the heterozygosity present within these pollen grains, which, in turn, depends on the frequency of meiotic recombination. FDR pollen generally transmit a higher level of heterozygosity than SDR pollen. When no recombination occurs, the heterozygosity transmitted by a pollen grain is theoretically 100%, whereas all pollen grains are genetically identical. When recombination occurs, the heterozygosity transmitted by a pollen grain is lower, and the population of 2n pollen becomes much more heterogeneous. It can be expected that chromosome rearrangements due to crossing-over are limited after irregular chromosome pairing as in *B*. 'Tamo'. In contrast, chromosome stickiness and chromosome pairing (*B*. 'Florence Rita', B276 and *B*. 'Bubbles') probably results from very abnormal chromosome rearrangements and may result in a higher variability in the progeny. Owing to this potential for genetic recombination, the production of polyploids by means of unreduced gametes presents significant advantages when compared with the that of synthetic polyploids by using cell cycle inhibitors.

We are now investigating meiotic activity in seedlings of B276 and *B*. 'Orococo', in order to gain more information about the inheritance of FDR pollen formation. When 2n pollen formation is genetically controlled, all genes involved in 2n pollen formation will be transmitted to a seedling by the FDR without crossing-over mechanism. Inheritance of 2n pollen is very important to study, as triploids obtained by polyploidization events are often sterile. In these cases, 2n pollen grains are often the only viable pollen produced by the triploids, as has been observed in lilies (Barba-Gonzalez *et al*., 2004). These pollen grains may be used to produce tetraploids. As such, 2n gametes have an important role in the establishment of new polyploid genotypes. The variability in chromosome dynamics during 2n gamete formation shows that 2n gametes may transmit new chromosome combinations, which may have important consequences for chromosome evolution and biodiversity within the genus.

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