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Gamma-induced infraspecific polyploidy via cytomixis-mediated syncyte formation in *Papaver somniferum* (Papaveraceae)

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Abstract: This is the first report on the phenomenon of syncyte manifestation through cytomictic transmigration in *Papaver somniferum* L. The occurrence characteristics and cytological depictions of cytomixis in the meiotic course of gamma-irradiated population reveal the mechanism leading to the origin of the cell fusions/syncytes. The event was more frequent in meiosis I and rare in meiosis II, indicating that the process atrophies at the end of the first meiotic division. The degree of occurrence of the phenomenon virtually concurred with the increase in the treatment dose of gamma irradiation. The phenomenon also affected the qualitative composition of postmeiotic products, i.e. tetrads and pollen grains. Heterosized pollen grains ranging from large to small size are also reported. In general, the occurrence of large-sized pollens is associated with unreduced gametes/syncytes and is an indication of the production of 2n pollens. Although occurring in low frequency, syncytes could play a decisive role in plant evolution as they can lead to the production of plants with higher ploidy levels. They may have potential applications in germplasm improvement in the sense of diversity and thus should be given more attention in future studies.

Key words: Gamma irradiation, cytomixis, syncyte, heterosized pollen grains, poppy

1. Introduction

Cytomixis is the phenomenon of migration of nuclear material, as well as other organelles and cytoplasm, between cells through the cytomictic channels. Gates (1911) assigned the term cytomixis to this cytological event in meiocytes of *Oenothera gigas* L. This phenomenon of inter-pollen mother cell (PMC) transfer of chromatin material through cytomictic channels was first described by Arnoldy (1900) in reproductive organs of gymnosperms; after that, Koernicke (1901) reported it during the microsporogenesis of *Crocus vernus* (L.) Hill and Miehe (1901) reported it in the leaf epidermis of *Allium cepa* L. Since then, the phenomenon has been frequently described for the PMCs of a large array of flowering plants (Falistocco et al., 1995; Caetano-Pereira & Pagliarini, 1997; Kumar & Sharma, 2002; Haroun et al., 2004; Singhal & Kumar, 2008). Conversely, cytomictic transmigration has also been reported in other tissues, for instance in the shoot apex of arboreal plants (Kostritsyna & Soldatov, 1991; Guzicka & Wozny, 2004), in the proembryos of cereals (Klyuchareva, 1983), and in anther vegetative tissues (Wang et al., 2004). The phenomenon has been seen in PMCs of transgenic tobacco plants, as well (Sidorchuk et al., 2007).

Diverse and conflicting opinions about the significance of cytomixis exist, but most researchers agree that it must have evolutionary significance (Ghanima & Talaat, 2003; Boldrini et al., 2006). Because the phenomenon involves the migration of chromatins or chromosomes from one cell to another in a donor-recipient manner, the recipient cell is destined to produce 2n gametes after meiosis (Falistocco et al., 1995; Tyagi, 2003; Ghaffari, 2006). Thus, 2n gametes may be formed by cytomixis or syncyte formation (Kim et al., 2009). Levan (1941) defined syntype formation as fusion of 2 or more PMCs or nuclei, usually in early prophase of the first meiotic division, such that the syncyte produces 2n gametes after meiosis (Levan, 1941; Sarbhoy, 1980). They are proposed to play a very significant role in the production of infraspecific polyploids (Kim et al., 2009).

The present study aimed to elucidate the consequences of cytomixis at the cellular level in relation to genetic behaviour and reproductive success of the plant. The study documents and reports the appearance of syncytes, which is the first report of its kind in *Papaver somniferum*. Efforts were also made to conduct a comparative analysis of characteristic cytomixis and syncyte frequency during

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a cytological investigation of gamma-irradiated diploid plants of poppy (2n = 22). An investigation has been done to determine whether cytomictic transmigration constitutes an efficient mechanism for significant modifications of the chromosome number and formation of 2n pollen or if it is just an abnormal phenomenon leading to disproportionate chromosomes at various meiotic stages due to induced mutagenesis. The evolutionary implication in the background of the existing literature has also been proposed.

2. Materials and methods

Seeds of a locally adapted inbred line of *Papaver somniferum* L. 'Vivek' were obtained from the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India. Dry and healthy seeds of *P. somniferum* were standardised for approximately 12% moisture content and were subjected to gamma irradiation at room temperature with a dose rate of 21 s/kR. The doses administered were 5 kR, 15 kR, and 25 kR. A seed sample consisting of 200 seeds was used for the irradiation for each dose of gamma. The source of gamma radiation was Co^{60} from gamma chamber 9000 at the National Botanical Research Institute, Lucknow. The irradiated seeds were sown immediately in the field at the experimental research farm of CIMAP using standard agronomic practices. The M1 seed samples were divided equally in 3 parts and were laid out in 3 replicates for each dose along with the control, adopting a randomised complete block design.

For meiotic studies, young floral buds about to emerge from the sheath were fixed in a freshly prepared 1:3 acetic alcohol solution (Carnoy's fixative) in which the acetic acid component was saturated with ferric acetate. The buds were kept for 24 h in the fixative and were preserved in 70% alcohol at 4 °C. Out of the 24 plants tested cytologically from each dose of gamma treatment, 4 plants in the case of 5 kR and 5 plants in both the 15 kR and 25 kR gamma treatments showed cytomixis and syncyte formation. Slides were prepared using anther squash technique with 2% acetocarmine. Pollen grains were also stained with 2% acetocarmine to study pollen fertility. Approximately 150 pollen particles were observed from each plant showing the phenomenon cytomixis. For comparative analysis of pollen size, diameter (polar view) of the pollen grains was measured by ocular micrometer. For the relative frequency of heterosized pollen grains, the observed number of pollens (small or large) / total number of fertile pollen grains was calculated. Slides were analysed and suitable cells were photographed under a Nikon research photomicroscope.

3. Results

For meiotic analysis of *Papaver somniferum*, studies on PMCs at various stages of division, from early prophase I to telophase II, were performed. In the control population, PMCs had species-standard chromosome sets (n = 11), and they performed meiosis independently, exhibiting regular meiotic divisions producing n pollen grains. They formed 11 bivalents at metaphase I (Figure 1) that segregated into 2 sets of 11 univalent chromosomes at anaphase I (Figure 1). In the case of gamma-treated sets, PMCs showed occasional inter-PMC transfer of chromatin material resulting in various meiotic abnormalities and malformed postmeiotic products.

Cytomixis/chromatin migration occurred in different directions from early prophase to anaphase I in the gamma-treated populations studied (Figure 1). Cytomixis between the PMCs was detected rarely at the late stages, i.e. meiosis II. Migration of chromatin material or chromosomes among the adjacent PMCs occurred through cytomictic channels, as well as by direct fusion of cell walls, but the former was more frequent than the latter. Occasionally, 2 proximate PMCs fused together and transferred the complete chromatin material to one cell that led to the formation of a syncyte, having double the normal chromosome number. Although they were found in low frequency, it was easy to distinguish them from the normal PMCs because they were fairly larger than typical cells. Syncyte manifestation primarily occurred at pachytene to diplotene and early diakinesis of meiosis I, and, subsequently, the chromosomes from the 2 PMCs in the syncytes acted as if they were of a single PMC. A syncyte with 44 bivalents at metaphase I could also be observed in the PMC (Figure 2).

The other consequence of cytomixis was the formation of cells with missing chromatin material, i.e. hypoploid/aneuploid cells, which leads to the production of abnormal postmeiotic products, i.e. abnormal tetrads and infertile and heterosized pollen grains. Thus, among the meiotic products in the cytomictic population, along with externally normal tetrads, polyads, monads, fertile and infertile pollens, and heterosized pollen grains could also be observed (Figure 2).

Data on the frequency of cytomixis along with syncytes in the gamma-treated population and in the control group of poppy at various meiotic stages are presented in Table 1. Cytomixis was revealed in all 3 of the doses of gamma rays administered and was found to be altogether absent in control population. The degree of variability concurred with the increase in gamma dose. The percentage mean and standard deviation (SD) was 12.09 ± 1.24%, 32.44 ± 2.55%, and 36.79 ± 2.30% for 5, 15, and 25 kR, respectively (Table 1). The percentage mean and SD for the syncytes was 2.15 ± 0.82% at 5 kR, 3.77 ± 1.20% at 15 kR, and 4.16

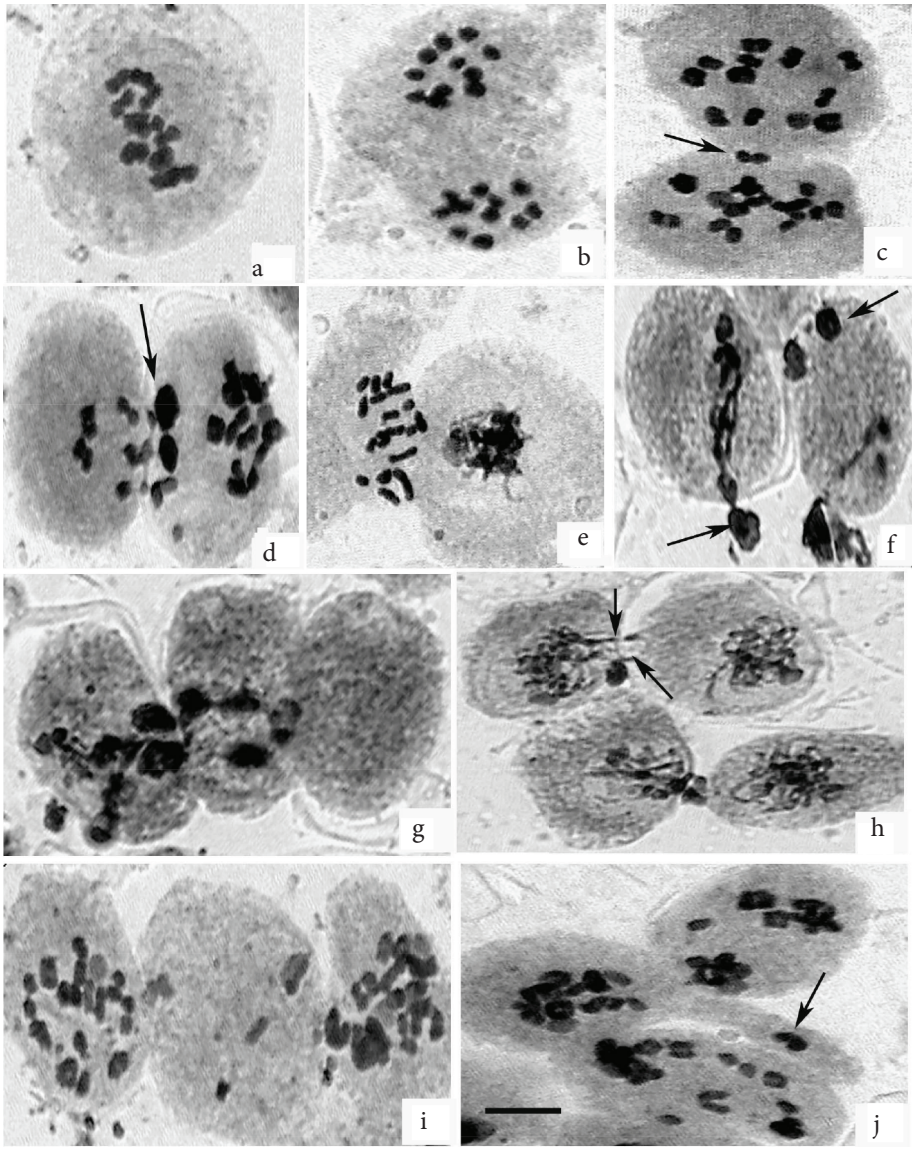

Figure 1. a- PMC with 11 bivalents at metaphase I plate; b- PMC with 11:11 chromosome distribution at anaphase I; c- PMCs showing single bivalent transmigration through a cytoplasmic channel; d- chromatin transfer through multiple cytoplasmic channels (arrow shows chromatin balls); e- chromatin transfer between 2 PMCs at different meiotic stage through broad cytoplasmic channel; f- PMCs showing pyknotic chromatin (arrows); g- group of PMCs involved in unidirectional transmigration; h- group of PMCs involved in chromatin transfer through cytoplasmic channels (arrows); i- simultaneous transfer of chromatin material from 1 PMC to 2 other PMCs; j- chromatin transfer through direct fusion of PMCs (arrow shows cytoplasmic tube carrying transmigrating bivalent). Scale bar: 4.2 µm.

± 1.15% at 25 kR. Based on comparison of frequencies of syncytes at various meiotic stages (Table 1), it has been established that the frequency of the syncyte manifestation in PMCs decreases in later stages of meiotic division.

Heterogeneously sized pollen grains were also observed in gamma-treated sets. The mean and SD of relative frequency of large pollen grains to that of smaller (normal) pollen grains, along with pollen fertility and abnormal tetrad percentage, are presented in Table 2. The mean diameter of normal (reduced) pollen grains was 38.20 µm, while the mean diameter of unreduced pollen grains was 58.63 µm, taken as an average of the 3 treated sets. The pollen fertility was found to be 93.17 ± 0.18% for the control, while it was 88.37 ± 1.35%, 80.91 ± 0.83%, and

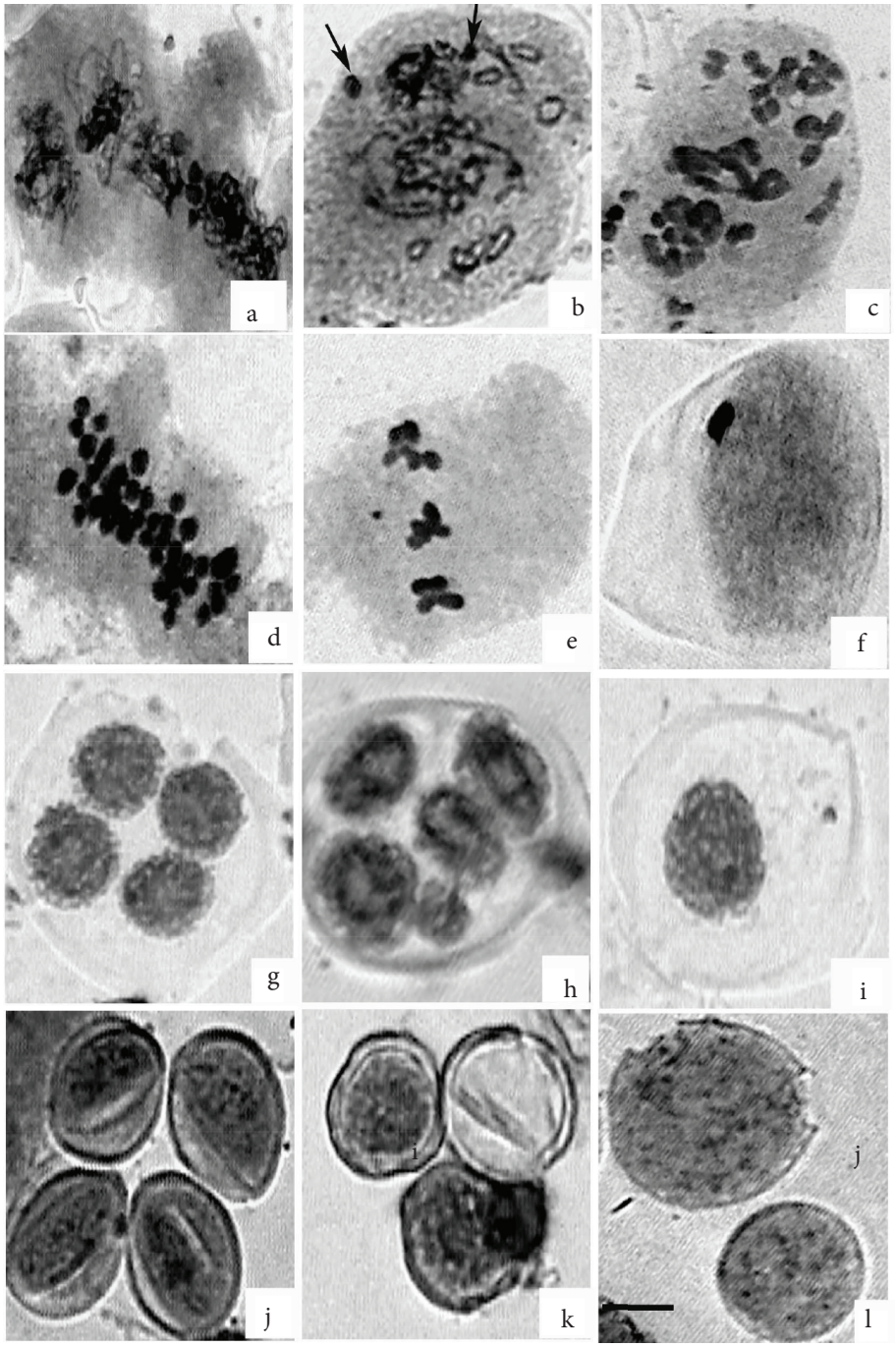

Figure 2. a- Syncyte with 2 synchronised nuclei at pachytene stage of prophase I; b- PMC with double chromosome complement (arrow showing 2 nucleoli); c- polyploid PMC; d- syncyte (tetraploid) with 44 bivalents at metaphase I plate; e- hypoploid PMC; f- enucleated PMC with a single bivalent; g- normal tetrad; h- polyad; i- monad; j- fertile pollen grains; k- sterile pollen grain (transparent); l- fertile heterosized pollen grains. Scale bar: 4.2 µm.

73.97 ± 1.79% for 5, 15, and 25 kR, respectively. For the other chromosomal abnormalities, the percentage mean rose essentially from 16.93 ± 0.35% to 31.76 ± 0.58% with the increase in gamma dose.

4. Discussion

Cytomixis, the phenomenon of inter-PMC transfer of chromatin material, has a profound effect on the meiotic course and postmeiotic products. According to the

Table 1. Effect of an increasing dose of gamma on the frequency of cytomixis and syncyte manifestation during meiosis of poppy.

Treatment doses	Total number of PMCs observed	Cytomixis			Syncytes								Other chromosomal abnormalities (%)
		Frequency of PMCs involved (%)	Type (%)		Frequency of syncytes (%)	Meiotic stage (number)							
						PI			MI	AI	TI	Meiosis II	
			CC	DF		Pt	Dp	Di					
Control	356	-	-	-	-	-	-	-	-	-	-	-	-
5 kR	372	12.09 ± 1.24	71.11	28.88	2.15 ± 0.82	3	-	4	1	-	-	-	16.93 ± 0.35
15 kR	336	32.44 ± 2.55	64.22	35.77	3.77 ± 1.20	3	2	4	3	-	-	-	28.27 ± 0.62
25 kR	318	36.79 ± 2.30	66.68	33.31	4.16 ± 1.15	4	2	5	2	1	-	-	31.76 ± 0.58

Abbreviations: PMCs = pollen mother cells, CC = cytomictic channel, DF = direct fusion, PI = prophase I, MI = metaphase I, AI = anaphase I, TI = telophase I, Pt = pachytene, Dp = diplotene, Di = diakinesis.

Table 2. Effect of an increasing dose of gamma on postmeiotic products, i.e. sporads and pollen grains.

Treatment doses	Pollen			Abnormal sporads (%)
	Fertility (%)	Diameter (μm)	Relative frequency of heterosized pollen	
Control	88.30–94.52	36.60–41.00	100	
	(93.17 ± 0.18)	(38.20 ± 0.34)		
5 kR	75.16–91.45	56.80–60.50	2.06–2.63	
	(88.37 ± 1.35)	(58.20 ± 0.80)	(2.41 ± 1.13)	
		36.40–42.00	95.20–98.85	12.40 ± 3.40
		(38.50 ± 0.04)	(97.56 ± 0.64)	
15 kR	66.89–82.60	56.20–60.60	2.51–3.21	
	(80.91 ± 0.83)	(58.60 ± 0.21)	(2.94 ± 0.75)	
		36.20–41.20	95.43–98.55	23.45 ± 2.12
		(38.40 ± 0.02)	(97.06 ± 0.43)	
25 kR	59.10–80.54	56.80–60.20	2.56–3.58	
	(73.97 ± 1.79)	(58.50 ± 0.63)	(3.04 ± 1.26)	
		36.00–41.40	95.32–98.07	28.10 ± 3.07
		(38.20 ± 0.29)	(96.96 ± 0.40)	

results revealed via histochemical methods, the one-way transport of nutritious substances and several organelles from the actively functioning PMCs to the weaker ones occurs through the cytomictic channels, originating in the preexisting system of plasmodesmata. Migration of chromatin material or chromosomes among the proximate PMCs occurs through cytoplasmic connections and cytomictic channels as well as through cell wall dissolution (Falistocco et al., 1995). Cytomixis through cytoplasmic channels was more frequently observed (Bahl & Tyagi 1988; Haroun et al., 2004; Gulfishan et al., 2010). The transfer of the nucleus and nuclear material occurred both through single channels as well as through multiple cytomictic channels simultaneously. While the maximal number of cytomictic channels was observed at the early prophase stages, they were closed by the callose by the end of the meiosis I. Similar observations have been reported by many researchers (Bahl & Tyagi, 1988; Seijo, 1996; Haroun et al., 2004).

Regarding the origin of cytomixis, the process remains enigmatic. From among the causes projected for cytomixis, gamma radiation is proposed to be effective, resulting in the production of an imbalanced and sterile genetic system (Amma et al., 1990). Different cytological mechanisms are responsible for the production of 2n gametes (Bretagnolle & Thompson, 1995), which includes premeiotic doubling of the chromosomes, omission of the first and second meiotic division, postmeiotic division, abnormal spindle geometry, abnormal cytokinesis, and desynapsis of the meiocytes during sporogenesis (Veilleux, 1985). Several researchers suggested that cytomixis introduced a distinct impact on microsporogenesis, since the transport of fragments or the whole nucleus between generative cells through the cytomictic channels could lead to the formation of aneuploid and polyploidy gametes. Unreduced gametes are known to produce individuals with higher ploidy level through a process known as sexual polyploidisation (Veilleux, 1985); this is considered to be the major course for the formation of polyploids (Kim et al., 2009).

Detailed cytological study of a gamma-induced poppy population showed that the occurrence of cytomixis might be considered to be one of the possible mechanisms of syncyte formation. Cytomictic transmigration occurring through cell wall dissolution among the neighbouring PMCs generally leads to the formation of syncytes (Falistocco et al., 1995). Syncyte manifestation has been reported from Fabaceae (Sarbhoy, 1980), Poaceae (Levan, 1941; Caetano-Pereira et al., 1999; Mendes-Bonato et al., 2003; Boldrini et al., 2006), Solanaceae (Padmaja, 1988), and Asteraceae (Kim et al., 2009) and thus may be a common phenomenon in angiosperms. Syncyte formation in diploid individuals has great significance in the initiation of low-level polyploidy and is very likely to play a major role in producing infraspecific polyploids (Kim et al., 2009). To our knowledge this is the first report on the occurrence of syncyte formation in the species *Papaver somniferum*.

As far as meiotic diploidisation leading to the formation of 2n or unreduced pollen is concerned, it is widely seen in various plant species (Veilleux, 1985); those produced by syncytes formed through cytomixis may be included (Mendes-Bonato et al., 2001; Kim et al., 2009). The fusion of 2 PMCs always occurs early in the meiotic division, to produce 2n pollen grains (Kim et al., 2009). The presence of giant pollen grains has been used as an indication of the production of 2n pollen (Bretagnolle & Thompson, 1995). Similar effects of cytomixis on pollen size have also been reported in *Vicia faba* L. (Haroun et al., 2004) and in *Caltha palustris* L. (Singhal & Kumar, 2008). The other consequences of cytomixis, along with the associated chromosomal abnormalities in gamma-irradiated poppy populations, were the formation of abnormal sporads and the occurrence of partial pollen sterility. During the process of cytomixis, the donor PMC chromatin material was reduced and drawn towards the point of the cytomictic contact, and then moved to the recipient cell through cytomictic channels. In keeping with the observations made, such chromatin materials were eliminated in the form of pyknotic chromosomes (Singhal & Kumar, 2008). Anomalies could be attributed to the formation of genetically imbalanced cells that lead to degeneration of cells (Vardar et al., 2012) and infertile pollen grains.

Decisively, the present research clearly elucidates that, in poppies, induced cytomixis through gamma ray treatments may be considered to be a possible source of production of polyploid gametes through syncyte manifestation. Such gametes can be used in breeding programs to create genetic variability through altered chromosome numbers. Thus, syncytes may have potential applications in improving poppy diversity and polyploidy formation.

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