Persistent Cytomixis in *Ocimum basilicum* L. (Lamiaceae) and *Withania somnifera* (L.) Dun. (Solanaceae)

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Summary Persistent occurrence of cytomixis was recorded during microsporogenesis in control and mutagen treated M_1 plants/progenies of *Ocimum basilicum* L. ($2n=72$; family: Lamiaceae) and *Withania somnifera* (L.) Dun. ($2n=48$; family: Solanaceae). The phenomenon was observed in prophase I and metaphase I cells. Nature of cytomixis has been observed to be similar in both plant species and in all plant types of the species. Cytomixis was exhibited only in few groups of cells in a microsporophyll (4 to 8 clusters) and those cells in clusters (number of cells in clusters: 2 to 8 in *O. basilicum* and 2 to 4 in *W. somnifera*) were either anucleated or with deficient and with extra chromosomes than normal; while, most of the cells in microsporangium were not involved in cytomixis. Results obtained have been discussed.

Key words *Ocimum basilicum*, *Withania somnifera*, Persistent cytomixis, Secondary polyploids.

Cytomixis, a phenomenon relating to cell to cell migration of nuclear materials through cytoplasmic connections (Gates 1911) was first noted by Kornicke (1901) in PMCs of *Crocus sativus* and since then its occurrence has been reported more commonly during microsporogenesis in countless flowering plants. The phenomenon has also been recorded in root meristems (Bobak and Herich 1978, Brown 1947, Jacob 1941, Sarvella 1958, Tarkowska 1960), leaves and epidermal scales (Tarkowska 1960) and tapetal cells (Cooper 1952, Sapre and Deshpande 1987). In spite of its wide occurrence and reports, the validity and significance of the phenomenon is still ambiguous. Present investigation describes persistent occurrence of cytomixis in normal and mutagenized plant population of *Ocimum basilicum* L. (Sweet basil; family: Lamiaceae) and *Withania somnifera* (L.) Dun. (Ashwagandha; family: Solanaceae), important medicinal plants with antioxidant (*O. basilicum*: Juliani and Simon 2002; *W. somnifera*: Bhattacharya *et al.* 2001) and anticancerous (*O. basilicum*: Aruna and Sivaramakrishnan 1996; *W. somnifera*: Leyon and Kuttan 2004) properties, as a part of the research initiated on induced mutagenesis for genetic improvement of the species. In either plant species, normal and mutagen treated plants provided scope of collecting evidences from large and genetically diverse plant population so that a better precision could be reached in reporting the phenomenon of cytomixis. Further alteration if any, in cytotoxic behaviour of chromosomes under the mutational background can also be ascertained.

Materials and methods

Control (*O. basilicum*: Seeds obtained from Medicinal Plant Garden, Narendrapur Ramkrishna Mission, West Bengal, India; *W. somnifera*: seeds collected from Agri Horticultural Society of India) and mutagen treated (different doses of X-rays, gamma-rays, EMS, dES and hydroxylamine were employed) seeds were sown (month of May–June) in the Experimental plots of Kalyani Uni-

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versity to raise M_1 plant population. Cytomixis was detected during routine male meiotic studies performed in control and treated plants of either species (fresh and fixed flower buds) and consequently emphasis was given to analyze the phenomenon in a broader spectrum. For the purpose, inflorescences/flower buds of suitable sizes from 3 to 5 randomly selected plants from each dose of either species were fixed (August–October) in Carnoy's fluid (*O. basilicum*: 4 to 5 PM; *W. somnifera*: 6 to 7 AM) and preserved in 70% alcohol. PMCs (single microsporophyll squash preparation was made and 2 to 3 anthers were examined in each plant) and pollen grains were stained in 2% acetocarmine solution. Unstained and partially stained pollen grains were considered sterile. M_1 plant progenies (on an average 2 to 3 plants from each doses of treatments including controls) were also meiotically examined from inflorescences/flower buds fixed in the month of March–April. Photomicrographs were taken from temporary preparations.

Results

Cytomixis was evidenced from all microsporophyll squash preparations made in *Ocimum basilicum* ($2n=72$, Fig. 1) and *Withania somnifera* ($2n=48$, Fig. 6) and was found to occur only in prophase I (Figs. 2 and 4–5) and metaphase I (Figs. 3 and 7–9) cells. Nature of cytomixis was similar in both species and in all plant types examined in the species. Each squash preparation revealed that cytotoxic behaviour of chromosomes was only restricted in few groups of cells (4 to 8/anther) and those cells in clusters (number of cells in cluster: 2 to 8 in *O. basilicum* and 2 to 4 in *W. somnifera*) were either anucleated or with deficient and extra chromosomes than normal (Figs. 3–5) thereby demonstrating the degree and direction of chromosome transfer; while, scattered meiocytes always had normal chromosome numbers. Cell clusters involved in cytomixis tended to lie in close proximity to one another (Fig. 4). A total of 655 cytotoxic groups have been analyzed from 131 slides prepared in *O. basilicum* and those groups were of 2 (7.94%), 3 (9.31%), 4 (32.06%), 6 (11.15%), 7 (9.92%) and 8 (29.62%) PMCs; while in *W. somnifera* cell clusters were of 2 (46.34%), 3 (9.89%) and 4 (43.76%) meiocytes (889 groups observed from 127 slides). Cytotoxic connections between microsporocytes have been observed both as chromatin bridges (Figs. 2–5 and 7–9) and as direct cytoplasmic contact between adjacent cells with their common wall dissolved (Fig. 8). The chromosomes were found to be sticky and agglutinated during the process of cytomixis (Figs. 5 and 7–9).

Average pollen fertility in control (*O. basilicum*: 46.85%, range 30.14% to 59.66%; *W. somnifera*: 70.25%, range 67.90% to 72.63%) and treated samples (*O. basilicum*: 28.37% to 77.03%; *W. somnifera*: 24.93% to 81.82%) of both species was low to moderate.

Discussions

Results indicated that cytomixis was persistently present in *Ocimum basilicum* and *Withania somnifera* and the phenomenon was unaltered under the mutational background. Although cytomixis was persistent in the species and may be considered genotype specific, it was exhibited only by few groups of cells thereby suggesting that most of the meiocytes present in the microsporangium either vary in their cytotoxic potential or fail to get exposed to the specific condition required to trigger the process. Thus, cell clusters formed during early microsporogenesis have persisted only in few cases during the process of meiosis possibly signaling the phenomenon of cytomixis. The individual meiocytes within the compact group being closely addressed to facilitate chromatin/chromosome migration through cytoplasmic connections. The existence of cytoplasmic bridges between adjoining PMCs permitting the transfer of chromatin materials has been confirmed by several workers (Whelan 1974, Zheng *et al.* 1987, Yen *et al.* 1993, Falistocco *et al.* 1995, McLean *et al.* 1997, Boldrini *et al.* 2003, Tyagi 2003). Cytoplasmic bridges might have been formed before the onset of

Figs. 1–9. Meiosis in *O. basilicum* (1, normal PMC; 2–5, cytotoxicity) and *W. somnifera* (6, normal cell; 7–9, cytotoxicity). 1. diplotene showing 34II+4I ($2n=72$). 2. early prophase I showing cytotoxicity. 3. group of meiocytes showing cytotoxicity and chromosomal bridge formations at MI and cells in the group with deficient ($\rightarrow$) and enhanced ($\rightarrow$) chromosome numbers. 4. cytotoxic groups in close proximity to one another. 5. anucleated meiocyte ($\rightarrow$) in a group showing cytotoxicity. 6. 23II+2I ($\rightarrow$) at MI ($2n=48$). 7. chromatin bridges between meiocytes at MI and the chromosomes were sticky and agglutinated. 8. cytoplasmic connection ($\rightarrow$) between adjoining meiocytes. 9. metaphase cells showing cytotoxicity.

meiosis by non-synchronous premeiotic mitotic division where cytokinesis and karyokinesis were out of phase resulting in incomplete cell wall formation (de Nettancourt and Grant 1964).

Cytologically unbalanced plants like haploids, triploids, aneuploids and apomicts show cytotoxicity more often than normal diploids (Nirmala and Rao 1996). In the context it would be relevant to mention that *O. basilicum* and *W. somnifera* are not true diploids rather they are secondary polyploids as was evidenced from the analysis of secondary chromosome associations prevalent in their meiocytes. The species might have undergone cytological diploidization in course of evolution showing diploid like meiotic behaviour (*O. basilicum*, $n=36$, $x=12$: Mukherjee and Datta 2005, *W. somnifera*, $n=24$, $x=12$: Iqbal and Datta 2005). Further, euploid variations in chromosome number have also been observed from root tip cells of both species (*O. basilicum*, $2n=48$ and 72 : unpublished, *W. somnifera*, $2n=12, 18, 24, 36, 48$ and 72 , modal number $2n=48$: Iqbal and Datta 2005). Thus, due to disturbed genetical background of the species there might exist some sort of constraint in the genomes which may possibly be accounted for persistent cytotoxicity and with differential behaviour of the meiocytes in the microsporangium. Zheng *et al.* (1987) correlated cytotoxicity with different genotypic modifications. Soodan and Wafai (1987) presumed the involvement of specific genes for cytotoxicity which express only under particular environmental conditions.

Pollen fertility was variable in different plant types of *O. basilicum* and *W. somnifera* and cannot be accounted for more or less uniform cytotoxic behaviour of chromosomes prevailing in them. Aberrant meiocytes formed as the consequence of cytotoxicity may have reduced pollen fertility to some extent but low to medium pollen fertility recorded in the plant types of both species was more due to different mutagenic treatments and/or physiological factor(s). In progeny plants of both species no aneuploids could be recovered; however, the significance of cytotoxicity in the origin of aneuploids and polyploids has been emphasized (Sarvella 1958, Omara 1976, Bahl and Tyagi 1988, Falistocco *et al.* 1995). Datta and Biswas (1984) detected a trisomic from cytotoxic plant of *Nigella sativa*.

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