Induced cytomictic variations in pollen mother cells of *Sesbania cannabina* Poir.

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Abstract

Cytomixis has been reported in many plant species, but there is no published report in *Sesbania cannabina* spp. The cytological stability of any plant is an important consideration in view of its extensive use in genetics and plant breeding programmes. Present study reveals the occurrence of inter PMC (pollen mother cell) transfer of chromatin material. During present investigation, it was found that out of different doses of gamma rays + ethylmethane sulfonate, the highest dose displayed the highest instances of cytomixis. In present investigation, the phenomenon of cytomixis can be observed between 2 to 10 PMCs. During male meiosis, it occurs through narrow and broad cytoplasmic channels or through direct contact between PMCs from early prophase to late telophase stage. However, the frequency of its occurrence during late meiotic stages is rather low. It elucidates that in *Sesbania cannabina*, induced cytomixis results into possible sources for production of aneuploids and polyploids. This may be further useful in plant breeding programmes to improve genotypic and phenotypic characters of *Sesbania cannabina*.

Key words: Meiosis, cytoplasmic channels, polyploids, aneuploids

Introduction

The phenomenon of cytomixis was firstly described by Koernicke (1901) in PMCs of *Crocus vernus* and by Mieche (1901) in the epidermal layers of various monocotyledonous plants. Cytomixis and spontaneous fusion of pollen mother cells are reported in number of angiosperm species such as *Sorghum bicolor* (Ghaffari, 1970), *Nicotiana tobacum* (Sidorchuk et al., 2007), *Hippophae rhamnoides* (Signal et al., 2008) *Zea mays* (Rai et al., 2010) but there is still lack of any published report of cytomixis in *Sesbania cannabina*.

Cytomixis has been considered as a mechanism of evolutionary importance for plants (Srivastava and Raina, 1980; Zheng et al., 1987). While according to some other authors, it represents an unfavorable phenomenon with deleterious effects on fertility (Marecha, 1963) and may result in production of polyploid microspores.

Sesbania cannabina (Dhaincha) is a leguminous crop widely available in many tropical countries of Asia and Africa. Because of its ability to grow in heavy soils, withstand

waterlogging, and tolerate soil salinity, these are preferred as green manure crop and fodder for livestock. Due to vigorous growth and high potential to increase their biomass during rainy season, it is used as green manure for many important food crops such as rice, wheat and maize (Zheng et al., 1987). *Sesbania* seed contains 30-36% crude protein and is at present not being used for other agricultural or industrial purposes (Hossain et al., 2001). A very little attention has been paid for genetic improvement of this multipurpose crop which may lead into its widespread use among fodders and food resources. It may be a potential source of nitrogen for other plants and proteins for cattles as being a leguminous plant.

Meiosis is highly coherent and genetically programmed process and comprises pairing of homologous chromosomes, crossing over, and the reduction in chromosome number, the requirement of two cell divisions and the lack of s period between the two divisions (Latoo et al., 2006). The steps involved in meiosis, controlled by a large array of genes may lead into cytological aberrations which result in a loss of fertility and overall reproductive efficiency of plants (Rai et al., 2010).

The phenomenon of cytomixis consists of the migration of chromosomes between meiocytes through cytoplasmic connection. A cytomixis has been considered to be the characterstic of genetically unbalanced plants such as hybrids, mutants and aneuploids (Peng et al., 2003; Rai et al., 2010; Zhou, 2003). Though it is an infrequent cytological phenomenon, but it has been reported in a large number of plants (Cheng et al., 1975; Gattschalk, 1970; Omara, 1976).

Spontaneously or through induction, cytomixis may have serious genetic consequences, such as formation of PMCs with anomalous chromosome number or binucleate PMCs and of aberrant microspores (triads, pentads, and hexads), pollen sterility (Soodan and Waffai, 1987), Chromosome stickiness and syncythia (Patra et al., 1986).

Some of the factors for induced cytomixis may be the action of colchicine (Ghaffari, 2006), action of herbicides (Boback and Herich, 1978), the partial or total inhibition of cytokinesis during microsporogenesis (Risueno et al., 1969), changes in biochemical processes that involve microsporogenesis modifying the microenvironment of affected anthers (Koul, 1990) and action of gamma rays (Rai et al., 2010), influence of gene (Bedi, 1990).

In present investigation sequential treatment (gamma ray + EMS) results in phenomenon of cytomixis. The present investigation deals with the consequences of cytomixis in relation to meiotic behaviour and reproductive success of the species. The goal of this work is to conduct a comparative analysis of cytomictic frequency in PMCs of *Sesbania cannabina* and to elucidate, whether the transfer of nuclear material between PMCs constitutes an effective mechanism leading to the formation of 2n pollen or if it is only an abnormal phenomenon, which results in altered chromosome number in PMCs and does not generate significant modifications of the chromosome number.

Materials and method

Procurement of seeds: Seeds of *Sesbania cannabina* variety ND-1 were obtained from Sunn Hemp Research Station, Pratapgarh, U.P., India.

Gamma radiation and ethylmethane sulfonate treatment: Dry and healthy seeds of *Sesbania cannabina* variety ND-1 were irradiated at 4 doses of gamma rays viz. 10, 20, 30, 40, KR from ^{60}Co source at NBRI, Lucknow. The dose rate was 18 Krad /sec with gamma chamber-900 model.

After completion of gamma radiation treatment, same seeds were treated with 0.5% solution of ethylmethane sulfonate for 3 hr and sown in their respective pots in triplicate for germination. Alongwith the treated seeds, untreated seeds were sown for control set.

Cytogenetical analysis: On the onset of budding, the buds were fixed in Carnoy's fixative (1:3, glacial acetic acid: abs. Alcohol) in their respective bottles for 24 hrs and then stored in 70% ethyl alcohol in refrigerator and were used for cytogenetical analysis. Cytogenetical studies were carried out using 2% acetocarmine. A detailed study of pollen grains and pollen mother cells was done for each dose of treatment and control.

Results

Results of experiment have been presented in Table1. The study of pollen mother cells of control showed 12 perfect bivalents at metaphase I (n =12) and normal segregation (12:12) at anaphase I. At each dose, meiotic aberrations include cytoplasmic connections and chromatin or nuclei migration (Figure 1 and 2), while cytomixis was altogether absent in control plants. Meiotic phases which are most affected by the event of cytomixis were metaphase I and anaphase I which is shown in Table1 and the number of cells involved in one cytomictic event vary from 2 to 10 PMCs. Two types of connections were observed in between PMCs i.e. cytoplasmic channels (Figure 3) and direct fusion (Figure 4). Cytomixis through cytoplasmic channels was more frequent among PMCs (Figure 3 and 5). Partial or complete migration of chromosome or chromatin material in one or several directions to the neighboring cells was also noticed resulting into euploidy and aneuploidy in PMCs. In most of the cases, migration of chromatin material occurs between same stages of PMC (Figure 5 and 6) while cytomixis between PMCs with different meiotic stages (Figure 7) was also observed but in lesser frequency. Figure 8 shows migration of whole nuclei by direct fusion while Figure 9 shows cytomixis between cells in different phases. Cytomixis was more frequent at meiosis I than meiosis II. The ranges of frequency of cytomixis vary from 23.52 to 75.24 % at 10 Kr + 0.5% EMS and 40 Kr + 0.5% EMS doses, respectively. Highest frequency of cytomixis was observed during metaphase I which was found to be 15.29% followed by 14.32%, 12.13%, 11.40%, 8.98%, 6.55% and 6.55% at anaphase I, telophase I, prophase I,metaphase II, diakinesis and anaphase II, respectively.

As a general rule among the two participants in cytomixis one acted as a nuclear material donor while other as nuclear material recipient while in our study there were frequent cases where one cell was recipient for two or more neighboring cells. The migration of chromatin is observed to be partial as well as complete which results into the formation of anucleated (Figure 10), hypo and hyperploid PMCs (Figure 11).

increase in cytomixis and chromosomal abnormalities, and reduction in pollen fertility in *Sesbania cannabina* Poir.

Doses of treatment (gamma rays+0.5% EMS)	No. of total PMCs observed	No. of cells in cytomixis	% Of PMCs showing cytomixis	Types of cytomixis		% of cells showing cytomixis at various stages of meiosis							Other abnormalities (%)	Pollen fertility %
				CC	DF	D	P-I	M-I	A-I	T-I	M -II	A-II		
Control	370	-	-	-	-	-	-	-	-	-	-	-	-	92.56
10 Kr+EMS	340	80	23.52	58	22	1.17	3.52	5.0	4.70	2.94	2.64	2.94	15.29	81.76
20 Kr+EMS	393	175	44.52	105	70	2.54	7.73	9.92	9.16	4.83	5.34	4.07	19.33	74.16
30 Kr+EMS	440	240	54.54	160	80	4.09	8.86	11.81	10.45	8.18	6.59	4.54	23.18	65.39
40 Kr+EMS	412	310	75.24	190	120	6.55	11.40	15.29	14.32	12.13	8.98	6.55	28.88	56.01

*Abbreviations- CC = Cytoplasmic Channel, DF = Direct Fusion, D = Diakinesis, P-I = Prophase-I, M-I = Metaphase-I, A-I = Anaphase-I, T-I = Telophase-I, M-II = Metaphase-II, A-II = Anaphase-II.

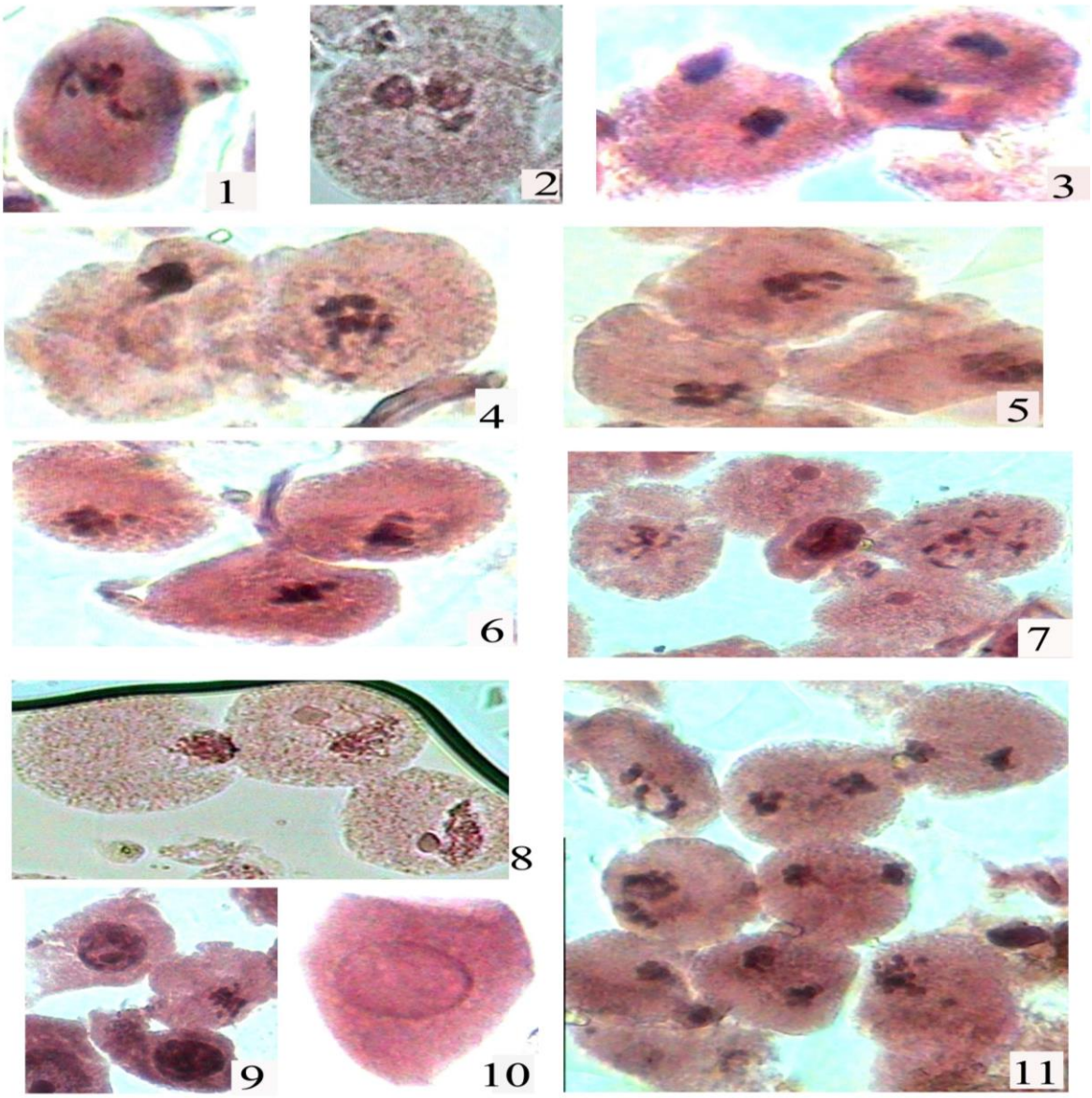

Figure 1. PMC showing formation of tube for cytomixis and chromatin migration, 2. PMC showing binucleate condition after nuclei migration, 3. Cytomixis between two PMCs through cytoplasmic channel, 4. Direct fusion between two PMC in different meiotic stages, 5. Cytomixis through direct fusion and tube formation between three PMCs, 6. Cytomixis between three PMCs at metaphase I, 7. Cytomixis between 5 PMCs in different meiotic stages, 8. Chromatin transfer between three PMCs, 9. Cytomixis between three PMCs in different meiotic stages, 10. Hollow PMC after nuclear migration, 11. Group of PMCs involved in cytomixis at anaphase I

As a result of cytomixis, rest meiotic courses and phenomenon of microsporogenesis is abnormal resulting into heterogeneous size of pollen grains or non fertile pollen grains. Pollen fertility for control plants was found to be 92.56% and it was reduced to 56.01% at highest dose of treatment i.e. 40 Kr + 0.5% EMS. The abnormal course is characterized by occurrence of various types of cytological aberrations such as stickiness, precocious, laggards, chromosomal bridges etc. The highest percentage of cytological aberrations were scored at highest dose (40Kr + 0.5% EMS) dose which was 28.88% and lowest at 10 Kr + 0.5% EMS i.e. 15.29%.

Discussion

Cytomixis in present investigation resulted in production of hypo and hyperploid and enucleated PMCs. Besides the phenomenon of cytomixis, PMCs showed various meiotic irregularities. Cytomixis may be the cause of these meiotic irregularities and formation of hypo, hyper and anucleated PMCs has also been reported in several plants (Signal et al., 2008)

Many authors reported cytomixis in different plant species irrespective of direction of movement of nuclei, their part or chromosomes and it is impossible to determine direction of their movement (Romanov and Orlova, 1971). But, present observation revealed that there is a certain tendency for the direction of intercellular migration. In present observation, the chains of cells are most often united by migrated chromosomes in sites of contacts. Similar observations were also revealed by Sidorchuk et al. (2007).

As a general rule the cytomixis may occur between the PMCs of similar or dissimlar meiotic stages which were reported by many authors. While in present investigation in most of the cases it has been observed that intercellular chromatin migration occurs between PMCs with similar stages of meiosis.

There are many factors which are proposed by different authors which are possible cause of cytomixis i.e. influence of gene (Kaul and Nirmal, 1991), abnormal formation of cell wall during premeiotic division (Kamra, 1960), action of chemical agents (Kumar and Sharma, 2002; Kumar and Srivastava, 2001; Ahmad et al., 2006), changes in biochemical processes that involve microsporogenesis modifying the microenvironment of affected anther(Koul, 1990), the presence of male stetrile genes and its frequency altered by environmental factors (Nirmala and Kaul, 1994) environmental stress and pollution (Bellucci et al., 2003), pathological conditions (Boback and Herich, 1978) etc.

Kamra (1960) reported that no amount of defective squashing or application of pressure could produce such small protrusion so close to another, or to form PMCs with extra fragments or increase the number of bivalents in them especially at metaphase. The comparative study of control and treated set clearly elucidates that the cause of cytomixis might be abnormal genetic behavior due to treatment of gamma rays and EMS.

Cytomixis is believed to play an important role in the evolution of plants. In case of cytomixis the number of chromosomes may be lower or higher than expected. As a consequence of cytomixis during the course of meiosis aneuploidy or polyploidy may

occur in the next generation. More or less viable gametes carry an unbalanced chromosome number. Such aneuploid and polyploid gametes can be used in further plant improvement programme of *Sesbania cannabina* to produce genetic variability through altered chromosome number.

Acknowledgements

The authors are grateful to the Sunn Hemp Research Station, Pratapgarh, U.P.,India for providing inbred seeds of *Sesbania cannabina* and University grant commission New Delhi for providing financial assistance Thanks are also due to the Head, Department of Botany, University of Allahabad, for providing the necessary facilities.

References

Ahmad, B. T., Parveen, S., Khan, A. H., (2006) MMS-induced cytomixis in pollen mother cells of Broad Bean (*Vicia faba* L.). Turk. J. Bot., 30, 273-279.

Bedi, Y. S., (1990) Cytology in woody species, Proc. Indian Acad. Sci. (Plant Sci.), 100, 233-238.

Bellucci, M., Roscini, C., Mariani, A., (2003) Cytomixis in pollen mother cells of *Medicago sativa* L. J. Hered., 94, 512-516.

Boback, M. and Herich, R., (1978) Cytomixis as a manifestation of pathological changes after the application of trifluralin. Nucleus, 20, 22-28.

Cheng, K. C., Nich, H. W., Yang, C. L., Wang, I.M., Chou, I.S., Chen, J. S., (1975) Light and electron microscopical observations on cytomixis and the study of its relation to evolution. Acta Botanica Sinica, 17, 60-69.

De, M. and Sharma, A. K., (1983) Cytomixis in pollen mother cells of an apomictic ornamental Ervantamia divericata (Linn) Alston. Cytologia, 48, 201-207.

Dwivedi, N. K., Sikdar, A. K., Jolly, M. S., Suseelamma, B.N., Suryanarayana, N., (1988) Induction of tetraploidy in colchicines-induced mutant of mulberry. I. Morphological and cytological studies in cultivar Kanva – 2. Indian Journal of Genetics, 48, 305-311.

Ghaffari, S. M., (2006) Occurrence of diploid and polyploid microspores in *Sorghum bicolor* (Poaceae) is the result of cytomixis. African Journal of Biotechnology, 5(16), 1450-1453.

Gttschalk, W., (1970) Chromosome and nucleus migration during microsporogenesis of *Pisum sativum*. Nucleus, 13, 1-9.

Hossain, M. A., Focken, U., Becker, K., (2001) Evaluation of an unconventional legume seed, *Sesbania aculeata*, as a dietary protein source for common carp, *Cyprinus carpio* L. Aquaculture, 198, 129-140.

Kamra, O. P., (1960) Chromatin extrusion and cytomixis in pollen mother cells of *Hordeum*. Hereditas (Lond.), 46, 592-600.

Kaul, M. L. H. and Nirmal, C., (1991) Male sterile gene action diversity in Barley and Pea. Nucleus, 34, 32-39.

Koernicke, M., (1901) Uber ortsveranderung von Zellkarnern SB Niederhein, Ges. Natur-U Heikunde Bonn A., 14-25.

Koul, K. K., (1990) Cytomixis in pollen mother cells of *Alopecurus arundinaecus* Poir. Cytologia, 55, 169-173.

Kumar, G. and Sharma, V., (2002) Induced cytomixis in Chickpea (*Cicer arietinum* L.). Nucleus, 45, 24-26.

Kumar, G. and Srivastava, U., (2001) Cytomixis variation in Isbgol (*Plantago ovata* forsk). Nucleus, 44, 180-182.

Latoo, S. K., Khan, S., Bamotra, S., Dhar, A. K., (2006) Cytomixis impairs meiosis and influences reproductive success in Chlorophytum comsum (Thumb) Jacq.-an additional strategy and possible implications, J. Biosci., 31, 629-637.

Marechal, R., (1963) Quelques observations sur le phenomene de cytomixie chez Gossypium. Bull. Inst. Agron. Gembloux, 31, 58-67.

Miehe, H., (1901) Uberdie Wanderung des Pflanzlichen Zellkarnes Flora, 88, 105-142.

Nirmala, C. and Kaul, M., (1994) Male sterilitry in Pea. Cytologia, 59, 195-201.

Omara, M. K., (1976) Cytomixis in *Lolium perenne* . Chromosoma, 55, 267-271.

Patra, N. K., Chauhan, S. P., Srivastava, H. K., (1986) Syncites with premeiotic mitotic and cytomictic comportment in opium poppy (Papaver somniferum L.). Indian Journal of Genetics, 47, 49-54.

Peng, Z. S., Yang, J., Zheng, G. C., (2003) Cytomixis in pollen mother cells of new synthetic hexaploid amphiploid (*Aegilops tauchii Triticum turgidum*). Cytologia, 68, 335-340.

Premchandran, M. N., Sachan, J. K. S., Sarkar, K. R., (1988) Cytomixis in Maize Trisomic. Current Sciences, 57, 681-682.

Rai, P. K., Kumar, G., Tripathi, A., (2010) Induced cytomictic diversity in Maize (*Zea mays* L.) inbred. Cytology and Genetics, 44(6), 334-338.

Risueno, M. C., Gimenez-Martin, G., Lopez-Saez, J. F., R-Garcia, M. I., (1969) Connexions between meiocytes in plants. Cytologia, 34, 262-272.

Romanov, I. D., Orlova, I. N., (1971) Cytomixis and its consequences in microsporocytes Triticale. Genetika, 7(12), 5-13.

Sidorchuk, Yu. V., Deineko, E. V., Shumny, V. K., (2007) Peculiarities of cytomixis in pollen mother cells of transgenic tobacco plants (*Nicotiana tobaccum* L.) with mutant phenotype. Cell and Tissue Biology, 6, 570-576.

Signal, V. K., Kaur, D., Kumar, P., (2008) Effect of cytomixis on pollen size in 'Seabuckthorn' (*Hippophae rhamnoides* L., Elaeagnaceae). Cytologia, 73(2), 167-172.

Soodan, A. S. and Waffai, (1987) Spontanoeus occurrence of cytomixis during microsporogenesis in almond (*Prunus amygidalus* Batsch) and peach (*P. persica* Batsch). Cytologia, 52, 361-364.

Srivastava, P. K. and Raina, S. N., (1980) Cytomixis in Clitoria ternantea L. var. pleniflora Fants. Current Sciences, 49, 824-835.

Ye, Z. H., Yang, Z.Y., Chan, G. Y. S. Wong, M. H., (2001)Growth response of *Sesbania rostrata* and *S. cannabina* to sludge-amended lead/zinc mine tailings: A greenhouse study. Environment International, 26, 449-455.

Zheng, G. C., Yang, Q., Zheng, Y., (1987) The relationship between cytomixis, chromosome mutation and karyotype evolution in Lily. Caryologia, 40, 243-259.

Zhou, S.Q., (2003) Viewing the difference between diploid and polyploidy in the light of the upland cotton aneuploidy. Hereditas, 138, 65-72.