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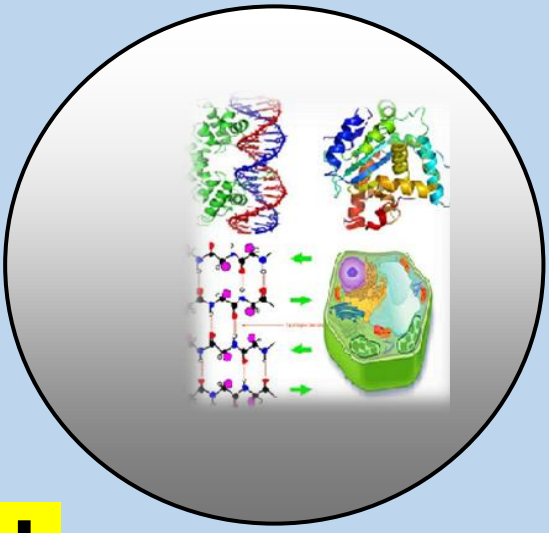

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A Case of Sodium Azide induced Cytomixis in PMCs of Pearl Millet

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ABSTRACT

Mutagenesis provides easy and effective means of bringing about genetic changes in the chromosomes of valuable crop plants for breeders to select from. These wide arrays of genetic changes are marked with chromosomal aberrations before the plants are able to adjust themselves to the changed genetic architecture. Some of these abnormalities are very interesting and give us an insight into the mechanism of working in the chromosomal divisions. Cytomixis is one such phenomenon which although is less common than other cytogenetic anomalies but is of great significance in bringing about massive transfer of genes from one cell to another. At times this may cause a genetic burden on the plants and may lead to reduction of pollen fertility but at the same time its apparent ability to induce mutations cannot be disregarded. The present investigation comes from an experimental setup for assessment of effects of Sodium Azide on the PMCs of Pennisetum glaucum (L) RBr where in some plants predominant cytomixis was recorded. The effects and probable causes of the same have been discussed in the paper.
Keywords: Pearl millet, Sodium Azide, Mutagenesis and Cytomixis.

INTRODUCTION

The phenomenon of cytomixis was firstly described by Koernicke (1901) in PMCs of *Crocus vernus* and later by Gates (1911) in *Oenothera gigas* and *O. biennis*. Cytomixis pre-exists in the meiocytes in for cytoplasmic connections called plasmodesmata in the syncytium but later on they get obliterated by the deposition of callose (Heslop-Harrison 1966). However under certain conditions (natural or induced) they may persist and enlarge to enable the transfer of not only cytoplasmic organelles but also the chromatin material (Resuino et al 1969). Cytomixis may also occur due to spontaneous fusion of pollen mother cells. It has

been reportedby a number of workers in different plants (Levan 1941; Sarvella 1958; Heslop-Harrison 1966; Gottschalk 1970; Saggoo and Bir 1983; Sen and Bhattacharya 1988; Wang 1988; Bedi 1990; Haroun 1995; Bione *et al.* 2000; Wu *et al.,*2003; Datta *et al.,* 2005; Ghaffari 2006; Singh *et al .,*2007;Singhal and Kumar 2008; Song and Li 2009; Kumar *et al*. 2010; Mursalimov and Deineko2011; Guan *et al.,* 2012; Kumar and Srivastava, 2013).

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 syncytia (Patra et al., 1986).

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 unfavourable phenomenon with deleterious effects on fertility (Marecha, 1963) and may result in production of polyploid microspores.

The present investigation is a part of a large experimental set up to assess the effects of various chemical mutagens on the meiosis of Pearl Millet (*Pennisetum glaucum* (L)RBr) during which, a number of cytological anomalies were encountered and cytomixis was one of them. Although less frequent, the effect was elicited by a Sodium Azide which is one of the most potent mutagen known for this plant.

MATERIAL AND METHODS

In present investigation dry and healthy seeds of *Pennisetum glaucum* (L) RBr were pre-soaked in distilled water for 12 h and they soaked in 4 different concentrations of Sodium Azide viz. 0.25%, 0.5%, 1.0% and 1.5% for 12 h. The doses were selected on the basis of the LD_{50} for the chemical on the plant.

Table 1. The parameters studied in the plants showing cytomixis (pooled and averaged data).

S. No	Character Studied	Values recorded (Mean ± SE)
1.	No. plants studied	14
2.	No. of PMCs studied	2500
3.	No. of PMCs with cytomixis	458
4.	% of PMCs with cytomixis	18.33±1.67
5.	% of PMCs with Cytoplasmic connections	72.4±1.07
6.	% of PMCs with direct Fusion	27.6±1.47
7.	% of PMCs in different stages of meiosis	
i.	Metaphase I	39.21±1.55
ii.	Anaphase I	25.45±1.12
iii.	Metaphase II	19.12±2.76
iv.	Anaphase II	12.50±0.63
v.	Others	3.72±1.08
8.	% of cases with cytomixis between 2 PMCs	76.4±1.45
9.	% of cases with cytomixis between more than 2 PMCs	23.6±0.61
10.	Pollen Fertility	63.41±2.54

The seeds were then washed thoroughly and sowed to rise M1 generation. At the time of flowering unopened buds from the cobs were fixed in Carnoy's fluid and stored at $4^{O}C$.
These were later examined cytologically using standard acetocarmine squash technique. Pollen Fertility was recorded using Acetocarmine-Glycerin Jelly Staining Techniques where unstained and undersized pollen grains were considered as Sterile and well-formed and stained pollen grains as fertile.

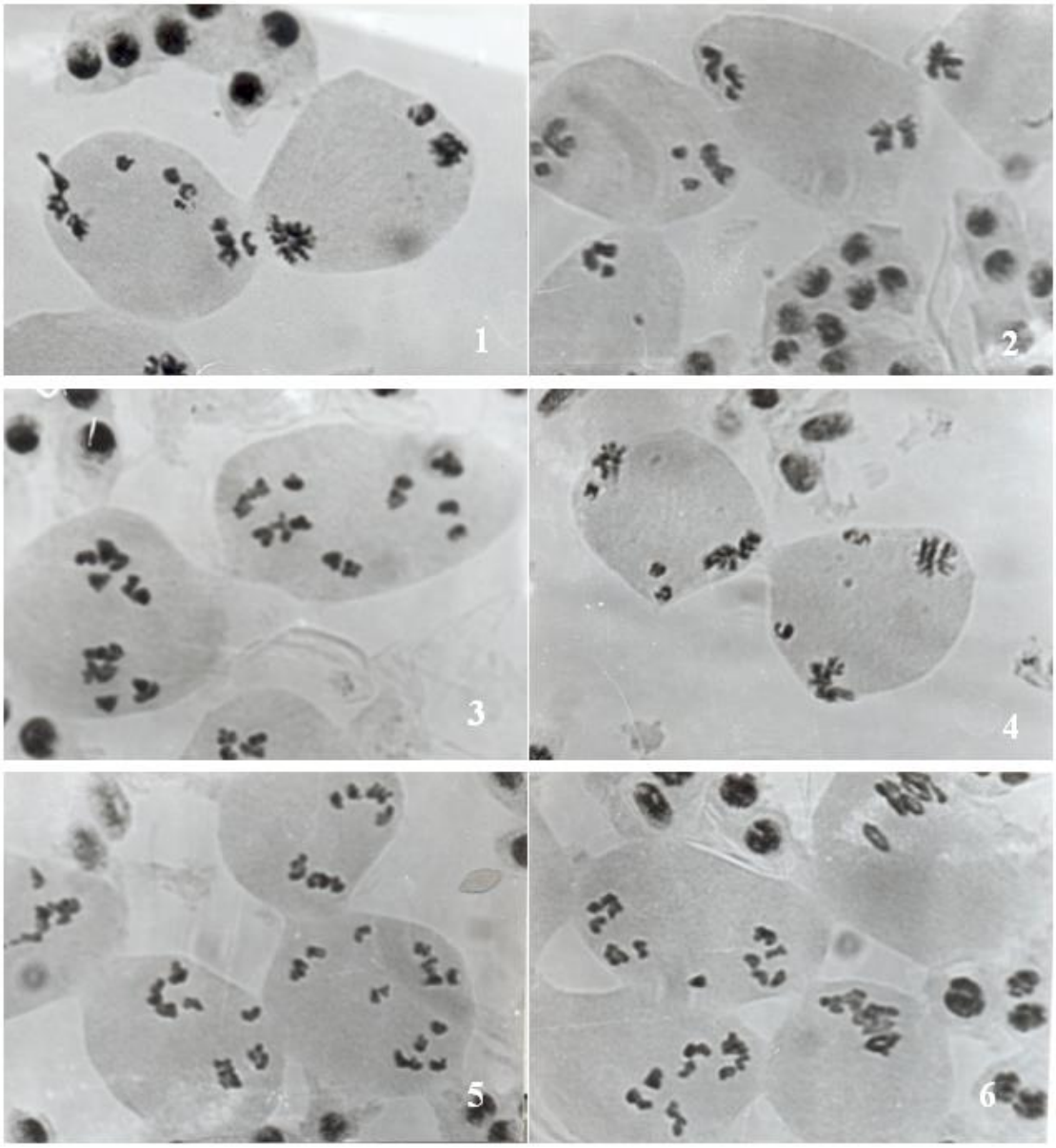

Plate 1: Fig 1-6: Various types of cytomictic connections found in the PMCs. 1. Cytoplasmic connection between 2 PMCs at Anaphase I. 2. Connections between 4 PMCs at Anaphase I. 3. Disturbed Metaphase I and formation of connections. 5. Connections between 4 PMCs with two at Metaphase and two at Anaphase. 6. Fusion of walls and transfer of chromatin.

RESULTS

Among various abnormalities recorded in the Pollen Mother Cells of the treated plants, one abnormality caught our attention due to its sheer dominance in a few plant PMCs of sets treated with 1.0% SA. Results of experiment have been presented in Table 1. The study of pollen mother cells of control showed 7 perfect bivalents at metaphase I (n =7) and normal segregation (7:7) at anaphase I.

At the given dose, cytoplasmic connections and chromatin or nuclei migration were frequent 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 Table 1 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 and direct fusion. Cytomixis through cytoplasmic channels was more frequent among PMCs. Partial or complete migration of chromosome or chromatin material in one or several directions to the neighbouring 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 while cytomixis between PMCs with different meiotic stages was also observed but in lesser frequency. Cytomixis was more frequent at meiosis I than meiosis II. 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 neighbouring cells. The migration of chromatin is observed to be partial as well as complete which results into the formation of anucleated, hypo and hyperploid PMCs.

As a result of cytomixis, abnormal genetic load of the cells leads to an abnormal phenomenon of micro sporogenesis 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 63.41% in the treated set.

DISCUSSION

Migration of chromatin material or chromosomes among the adjacent meiocytes occurs through cytoplasmic connections and cytomictic channels as well as through cell wall dissolution / fusion (Falistocco et al., 1995). Cytomixis in present investigation resulted in production of hypo and hyperploid and enucleated PMCs. Besides the phenomenon of cytomixis, PMCs showed various meiotic irregularities and predominant among them being stickiness. Cytomixis may be the cause of these meiotic irregularities and formation of hypo, hyper and anucleated PMCs has also been reported in several plants.

As a general rule the cytomixis may occur between the PMCs of similar or dissimilar 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; 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 behaviour due to treatment of gamma rays and EMS.

Results of the study determined that among various cytogenetic abnormalities observed in the given plant PMCs the greater role is played by cytomixis in induction of unreduced pollen grains followed by tripolar cell formation and chromosome stickiness. More over these results indicate that all meiotic abnormalities may cause pollen sterility of the plants showing them.

The presence of giant pollen grains has been used as an indication of the production of 2n pollen. Such gametes resultfrom abnormalities during either micro sporogenesis (2n spores) or megasporogenesis (2 n spores, Villeux, 1985).

Unreduced pollen grains are known to produce individuals with higher ploidy level through a process known as sexual polyploidization (Falistocco et al., 1995), which has been considered as the major route to the formation of naturally occurring polyploids.

Different cytological mechanisms are responsible for the production of 2n gametes, including premiotic doubling of the chromosomes, omission of the first and secondmeiotic division, post-meiotic division, abnormal spindle geometry, abnormal cytokinesisand desynapsis of the meiocytes during the sporogenesis (Villeux, 1985).

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.

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