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Induced cytomictic variations through abiotic stresses in grasspea (*Lathyrus sativus* L.)

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Abstract

A cytogenetic study was undertaken to evaluate various types of cytomictic variations induced by different types of abiotic stresses viz. temperature, heavy metal (Pb) and water stresses on the meiotic cells of *Lathyrus sativus* L. Chromosome transfer and cytoplasmic channels between microsporocytes occurred from prophase-I to telophase-II stages of meiosis. The occurrence of cytomixis and other meiotic abnormalities were analyzed in comparison with pollen fertility. Due to cytomixis, various types of irregularities were noticed resulting into hypo- or hyperploids which may cause the origin of aneuploids or polyploids.

Key words: *Lathyrus sativus,* abiotic stresses, cytomixis, cytomictic variations

Introduction

In a sizeable proportion of cultivable area, the crops have to face various kinds of stresses which include heavy metals, adverse temperatures, deficit or excess of water, etc. Having achieved the remarkable success for relatively assured agriculture, the time has now come when greater emphasis should be given to improve agriculture in areas which suffer from the limitations of insecurity. Progress in agriculture, has exposed the human beings to heavy metal toxicity through different sources of soil, water and air. Though, these form an integral part of human life yet heavy metals are the foremost among the pollutants and suspected carcinogens [1, 2]. Polluted effluents discharged from the industries may accelerate the growth and development process in cultivated crops, or may show inhibitory effects [3] due to array of different organic and inorganic solutes and heavy metals. The cytological and genetical effects of some heavy metals like mercury, lead, cadmium and chromium in animals and plants have been studied by [4].

Besides heavy metals, increased variations in the temperatures of the earth surface are also another alarming situation. Sudden rise or fall in the temperature is considered as an environmental stress.

Drought is another most common water related stress experienced by crops. Agricultural drought may develop at any time of the cropping season. Its impact is usually most severe at the seedling and flowering stages [5, 6]. Besides drought stress, water logging is also one of the major abiotic stresses in rainfed ecosystems especially on soils with poor drainage. They can seriously reduce the yield [7].

Considering all the above mentioned abiotic stresses, the present study has been planned to evaluate the percentage of cytomixis induced by them in grasspea (*Lathyrus sativus* L.). The study reports an extensive occurrence of cytomictic variations induced by different types of stresses on grasspea. These variations have been discussed in the present text.

Material and methods

Healthy seeds of *Lathyrus sativus* of variety Pusa-24 were procured from National Bureau of Plant Genetic Resources, New Delhi. The experiment was planned into four different sets. In Set-1, the treatment of temperature stress was given for which the seeds were exposed to two different ranges of temperatures *viz.*, 55°C and 5°C for 24, 48 and 72 hours each. In set-2, different concentrations of $PbNO_3$ *viz.*, 25, 50, 100, 200 and 300 ppm were amended in the soil samples and seeds were sown alongwith the controls. In set-3, after 10 days of sowing, the plant was subjected to drought conditions for 15 days (no irrigation for 15 days). In set-4, water-logged conditions were imposed at the seedling stage (after 10 days of sowing) for 1-6 days, i.e. experimental pots were flooded with water for 6 days. Control set was separately maintained and treated as non-stressed set.

For cytological analyses, the floral buds at each set were fixed in Carnoy's fixative and later transferred into 70% alcohol. Slides were prepared using standard acetocarmine squash technique. Pollen fertility was assessed using 2% acetocarmine-glycerine staining technique.

Results and discussion

In nature plants are exposed to various kinds of stresses that affect their physiology, morphology and development. Every year environmental stresses cause considerable loss in the productivity of many crops. Among these stresses fluctuation in temperatures, water status of soil and intensity of light are the most crucial signals affecting the plant growth [8].

Grasspea is an annual pulse crop, belonging to family Fabaceae with chromosome number 2n = 14. It performs well under adverse agricultural conditions; hence was selected for the present study. During the present study, the effects of three different types of abiotic stresses *viz*., temperature (heat and chilling), heavy metal (Pb) and water stress (drought and waterlogging) were analysed on the meiotic cells of grasspea. A vast majority of chromosomal abnormalities were encountered, but the predominance of cytomictic variations was frequently noticed at each stress treated set.

The phenomenon of cytomixis was first described by Kornicke [9] in the PMCs of *Crocus vernus.* Gates [10] observed delicate threads of cytoplasm connecting adjacent PMCs in *Oenothera.* Gates [11] subsequently suggested that these connections must form an important avenue of exchange between the PMCs and described the transfer of nuclear material through them from one meiocyte to another, calling the process 'cytomixis'. This phenomenon has been widely described in angiosperms [12] including *Pisum sativum* [13]; *Cor chorus* [14]; *Pilocarpus pennatifolius* [15]; *Glycine max* [16].

Temperature stress has been studied under two categories: high (55°C) and low (5°C). Control plants showed almost regular meiosis, with no cytomixis (Fig. 1). However, cytomictic variations were observed extensively at all the four types of stresses studied. A duration-dependent cytomixis was exhibited at all the three durations (24, 48 and 72 h) of the temperature treatment. The cytomictic variations induced by 55°C ranged from 2.40% to 2.69% alongwith the increasing durations from 24 to 72 h; whereas, at 5°C the variations ranged from 2.20% to 2.54% (Table 1). Secondly, in metal treated set, the range was found from 2.88% to 6.22% (Table 2). The drought set showed the variations from 1.68% to 2.12% and in water logging set, the range was from 1.42% to 2.01 % (Table 3) and graph 1.

The phenomenon of cytomixis has been encountered at all the stages of meiosis and the chromosomal exchange predominantly occurred between the PMCs of same as well as different stages of divisions. Apart from cytomixis, another interesting feature to be noticed was the corroding of the cytoplasmic matter. The frequency of corroding showed a gradual increase alongwith the increasing duration 55°C treatment (Table 1) and at 300ppm of $PbNO_3$ (Table 2). Usually cytoplasmic connections (Fig. 2) and chromosomal migrations were observed at metaphase-I at each set of treatment but during the present investigation, Pb treated set showed a wide range of chromatin migration including prophase-I and metaphase-I (Fig. 3); anaphase-I and telophase-II (Fig. 4).

Table 1. Temperature stress induced cytomictic variations in grasspea

Dose of treatment (°C)	Duration (h)	No.of PMCs scored	Karyological abn. (%)	Cytoplasmic abn. (%)			Total Cytoplasmic abn.(%)+S.E.	Pollen fertility (%)
				cy	icm	co		
55	24	210	14.26	1.16	1.12	0.12	2.40±0.64	84.32
	48	232	18.12	1.21	1.18	0.18	2.57±0.72	80.11
	72	221	21.24	1.28	1.20	0.21	2.69±0.86	76.21
20 (control)	-	230	1.10	0.00	0.00	0.00	0.00±0.00	96.60
5	24	240	10.23	1.10	1.00	0.10	2.20±0.46	80.12
	48	238	12.12	1.12	1.11	0.16	2.39±0.58	74.46
	72	235	13.28	1.20	1.14	0.20	2.54±0.62	72.41

Abbreviations: cy-cytomixis, icm-inter cellular migration, co-corroding, abn.-abnormality.

Table 2. Heavy metal stress (Pb) induced cytomictic variations in grasspea

Dose (ppm)	No. of PMCs scored	Karyological abn.(%)	Cytoplasmic abn.(%)			Total Cytoplasmic abn.(%)±S.E.	Pollen fertility (%)
			cy	icm	co		
Control	450	1.00	0.00	0.00	0.00	0.00±0.00	96.88
25	455	9.26	1.26	1.10	0.52	2.88±0.81	88.12
50	451	13.12	1.48	1.01	1.36	3.85±0.93	80.43
100	460	22.43	2.23	0.47	1.51	5.21±0.62	74.28
200	463	26.25	2.74	1.42	1.34	5.50±0.76	70.12
300	468	32.16	3.02	1.16	2.04	6.22±0.98	67.25

Abbreviations: cy-cytomixis, icm-inter cellular migration, co-corroding, abn.- abnormality.

Another interesting feature to be noticed was the variation in the phenomenon of cytomixis itself. Three different types of pathways for chromatin migration were observed. The most frequent type was by the dissolution of the cell walls of the neighbouring PMCs (Fig. 2). A higher frequency fusion of PMCs was encountered at 72 h treatment of both 55°C and 5°C and at 200 and 300 ppm dose of $PbNO_3$.

Second method of migration was by cytoplasmic channels between distantly placed PMCs (Fig. 3). It was prevalent at lower doses (25, 50 and 100 ppm) of $PbNO_3$ and at drought conditions at seedling stage. The third type of pathway of chromatin migration was by beak formation between dissimilar stages of divisions (Fig.4). This type of migration was commonly found at water-logged conditions from 1-6 days.

Partial or total migration of chromatin material in one or several directions to the adjacent PMCs was noticed, resulting into hypo or hyperdiploid cells (Fig. 6). The content of one cell could be often seen into two or three neighbouring cells. The migration was not direction-oriented. In some PMCs, the location of some chromosomes was clearly an indication of imminent migration from a donor to its attached recipient cell.

The maximum frequency of cytomixis was found at 300 ppm of $PbNO_3$ (3.02%), followed by heat treatment of 55°C at 72 h (1.28%). Drought and water logging stresses were found to be moderate in inducing cytomictic variations (Table 3). Though chromatin migration was a rare phenomenon to be observed but cytoplasmic channels were frequently noticed at all the stages of divisions.

Besides cytomictic variations, other chromosomal abnormalities such as irregular seperation, tripolarity (Fig. 7) were also observed. The percentages of karyological abnormalities recorded at each stress conditions have been presented in their respective tables. Pollen fertility percentage was highest in control plants (96.6%) with darkly stained and large pollen grains (Fig. 8), whereas the unstained and slightly smaller sized pollen grains were considered as sterile (Fig. 9). Pollen fertility percentage recorded at temperature treated set, 55°C showed the range from 76.21% to 84.32%; whereas 5°C exhibited 72.41% to 80.12% (Table 1). In Pb treated set, pollen fertility ranged from 67.25% to 88.12% (Table 2). Drought stressed set showed the range from 77.24% to 84.86% and in water logged set pollen fertility ranged from 71.26% to 88.26% (Table 3).

Mostly all the higher doses and durations of each stress have shown a significant decline in the pollen fertility. According to Nirmala and Rao [24], a syncyte formation may be induced by genetic and environmental factors and generally lead to the formation of abnormal pollen grains. Cytomixis may have serious genetic consequences by causing deviations in chromosome number and may represent an additional mechanism for the origin of aneuploidy and polyploidy [25].

During the present study, metal (Pb) stress was found to be the most potent abiotic stress; inducing a great range of cytomictic as well as karyological abnormalities. Reports on carcinogenic activity of lead have been given by Maki-Paakkanen [17]. Following metal stress, heat stress has also exhibited extensive cytomixis. Although cytomixis has been reported in several plant species, its underlying cause has been assessed variously by different workers like abnormal pathological conditions [18], abnormal cell wall formation during pre-meiotic divisions, chemicals, radiations, temperature and of course genetic control [19]. In

Table 3. Water stress induced cytomictic variations in grasspea

Treatment	Duration (days)	No.of PMCs scored	Karyological abn. (%)	Cytoplasmic abn. (%)			Total cytoplasmic abn.(%)+S.E.	Pollen fertility (%)
				cy	icm	co		
Control	-	452	1.12	0.00	0.00	0.00	0.00±0.00	96.72
Drought (10 day plant)	15	454	3.32	1.02	0.98	0.56	2.46±0.41	77.24
Water logging (10 day plant)	1	458	3.12	1.10	1.01	0.21	2.32±0.53	88.26
	2	462	3.01	1.01	1.00	0.20	2.21+0.61	84.18
	3	463	2.86	1.00	0.96	0.19	2.15±0.57	80.21
	4	461	2.76	0.98	0.92	0.17	2.07±0.46	77.16
	5	458	2.72	0.98	0.84	0.16	1.98±0.41	74.28
	6	455	2.68	0.92	0.83	0.12	1.97±0.49	71.26

Abbreviations: cy-cytomixis, icm-inter cellular migration, co-corroding, abn-abnormality.

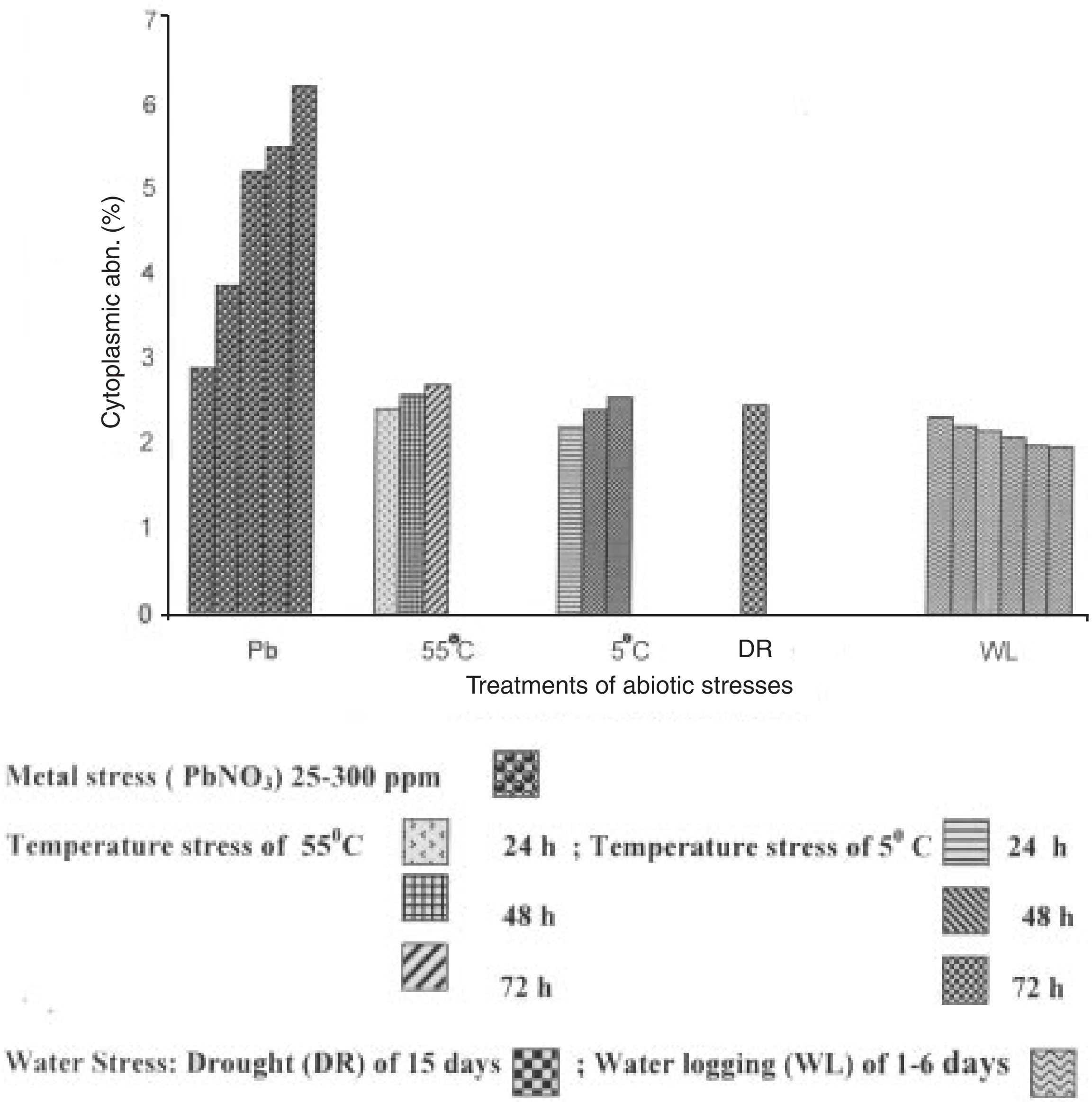

Fig. 1. A comparison of cytoplasmic abnormality (%) induced by different stresses

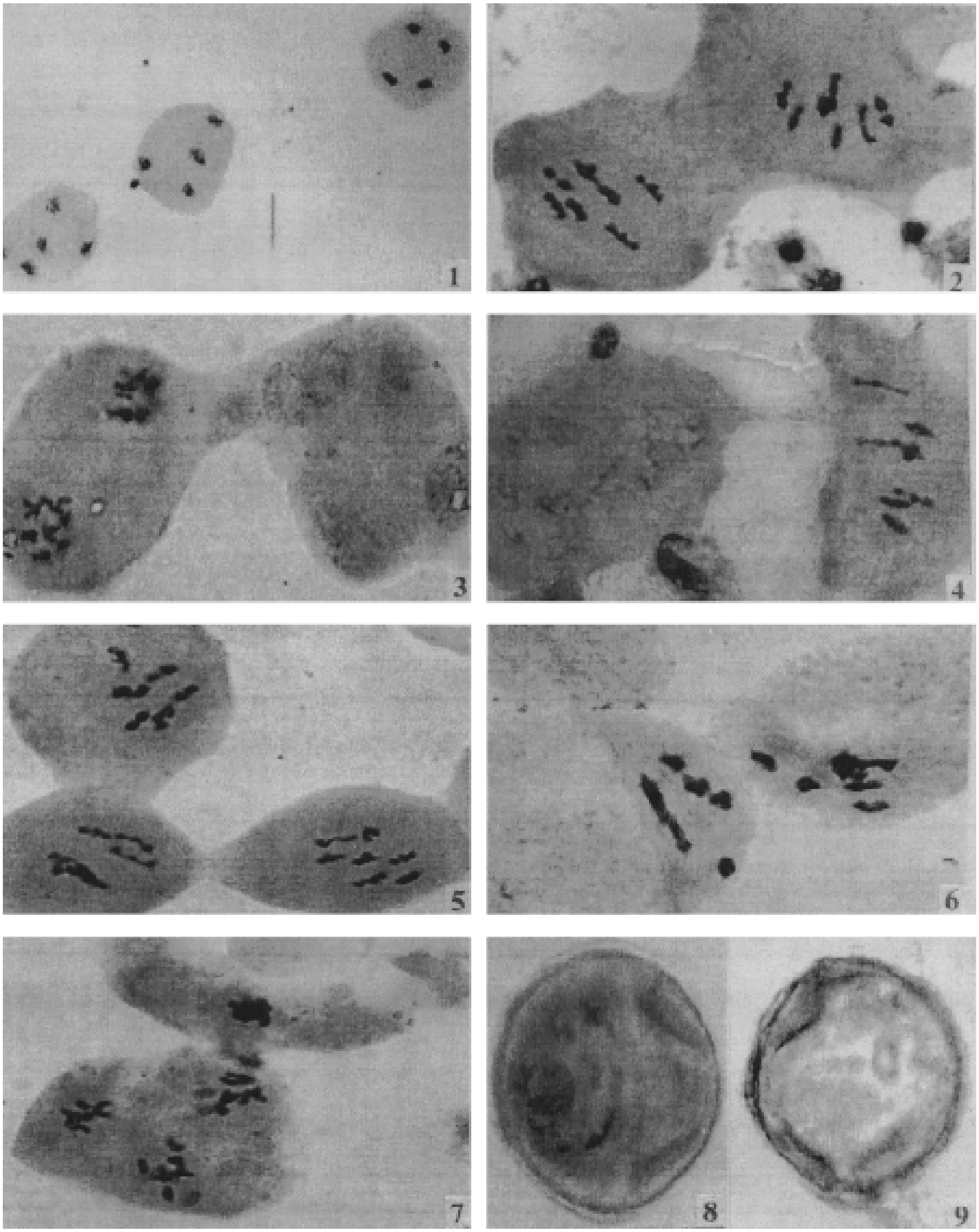

Fig. 2. **(1) Normal PMCs without cytomixis, (2) Cytomixis through dissolution of cell wall between similar stages of division (metaphase-I), (3) Cytomixis through the formation of cytoplasmic channels between dissimilar stages (anaphase-I and telophase-II), (4) Cytomixis through beak formation between dissimilar stages (prophase-I and metaphase-I) (5) Cytomixis between more than two PMCs of similar stages (metaphase-I), (6) Hypo and hyper ploid PMCs after cytomixis, (7) Tripolarity after cytomixis and corroding of cytoplasm, (8) Fertile pollen grains (Darkly stained and smooth), (9) Sterile pollen grains (lightly stained and shriveled)**

addition to genetic control of the phenomenon, stress factors such as high temperature and drought in certain periods of growing seasons have contributed to the incidence of cytomixis in *Diplotaxis harra* by Ghanima and Talaat [20].

The present study revealed that all the stages of meiosis were equally susceptible to cytomixis, contrary to general belief that early stages are more favourable [21]. Cell fusion leading to syncyte formation occurred between different stages of meiosis. Similar results have also been reported by different workers for example in prophase-I and metaphase-I [22]; metaphase-I and anaphase-I [23].

During the present investigations, comparative cytomictic analyses of stressed sets clearly indicate that $PbNO_3$ has imposed the highest percentage of cytomictic variations followed by heat stress treatment. In temperature stress, treatment of 55°C has caused more cytomictic variations than 5°C treatment, which indicates that higher temperature is a significant environmental mutagen. Besides lead and heat, drought stress has shown a lower frequency of cytomixis followed by water logging. Low temperature is one of the most important factors limiting the growth and distribution of plants worldwide [26]. Corroding of cytoplasmic matter may be the consequence of rupturing and damaging of the cell walls of neighbouring PMCs due to temperature elevation. Heat might have given rise to agglutination of the cytoplasm due to increased viscosity, leaving behind empty patches of cytoplasm.

According to Milajajev [27], the nuclear transfer may be attributed to nutritional deficiency of PMCs. However, whatever the probable cause behind this, it is gaining importance because of the formation of hypo or hyperdiploids giving rise to aneuploids or polyploids which can be exploited in plant breeding programmes.

Although the role of cytomixis in plant evolution is considered an additional mechanism for the origin of aneuploidy and polyploidy, the deviation in chromosome number affects pollen fertility. Induced cytomixis ultimately leads to high pollen sterility in comparison to that of controls due to genetically imbalanced pollen grains. The comparative analyses of the present study, clarifies that $PbNO_3$ induced cytomictic abnormalities may provide enough scope for further improvement in grasspea giving rise to aneuploids and polyploids under stressful conditions also.

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