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Cytogenetic Effects of Magnesium Sulphate on The Root Tip Cells of Pea (*Pisum sativum* L.cv.araka)

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The effects of magnesium sulphate on mitosis were investigated in *Pisum sativum* L.cv.araka. developed *in vitro* conditions. The roots of pea (*Pisum sativum* L.cv.araka) were treated with $MgSO_4$ concentrations of 1 -0.1 -0.01 mg/L for 3-6 and 12 h treatment periods. It was found that $MgSO_4$ has a marked mitodepressive action on mitosis. Magnesium sulphate significantly decreased the mitotic index at all concentrations and treatment periods. Mitotic abnormalities were increased and the mitotic index was decreased depending on the concentration and duration time of magnesium sulphate applied.

Key Words: Cytogenetic effects, Magnesium sulphate, *Pisum sativum*.

INTRODUCTION

Plants require nutrients in order to grow, develop and complete their life cycle. The supply of nutrients to the plants should be balanced in order to maximise the efficiency of the individual nutrients so that these meet the needs of the particular crop and soil type. The application of fertilizers can considerably increase the harvested yield per hectare of land. This will probably be a necessity for the increasing global population. However, if too much fertilizer is used, there is a risk of over fertilizing the soil[1].

In developed agricultural systems, inorganic fertilizers are applied to the soil to supply the essential nutrients required for the growth of plants. Many cytological studies have been carried out to detect the harmful effects of fertilizers including heavy metals on different plants[2-8]. Njagi and Gopalan[2] reported that the food preservatives sodium benzoate and sodium sulfite inhibit DNA synthesis and cause the formation of anaphase bridges, premature chromosome condensation heading to pycnotic nuclei and chromatin erosion in interphase nuclei in *Vicia faba* root meristems. Magnesium is a macronutrient element for plants and $MgSO_4$ form have been used extensive for fertilizers. As heavy weight nutrients,sulphur and magnesium contained in magnesium sulphate important role in increasing output and improving quality due to favourable condition, time and inadequate fertilizers[1]. When the fertilizers ample in the soil the element dose increases such as calcium, magnesium and potassium. But this can be harmful which effect to change ion mechanism[9].

In recent years, different chemical substance have investigated on the plant chromosome structure[10]. Some studies have been shown that inorganic compounds such as ammonium sulphate, ammonium phosphate, potassium chloride, copper chloride, sodium metabisülfite, potassium dichromate caused various abnormality on cell divisions[11].

Furthermore for *in vitro* culture initiation was supplemented with 0.5 mg/L GA_3 as plant growth regulators (PGRs)[12]. Gibberellins provide internode elongation by means of increase cell division. Since 1950 it has known that during the germination gibberelin's activity takes action to nutrient substance[13]. All of known Gibberelin's equal activity that to increase and stimulating growth[14].

The purpose of the present study is to investigate the cytogenetic effects of $MgSO_4$ in pea (*Pisum sativum* L.cv.araka) root tip cells.

EXPERIMENTAL

In present study, the mature seeds of pea (*Pisum sativum* L.cv.araka) was used as source of plant material. The seed of pea (*Pisum sativum* L.cv.araka) used as material were first washed with tapwater for 15 min. Then, depending upon the development of the material, they were kept in 70 or 96 % ethanol for 15-20 s, in 5 or 10 % sodium hypochlorite (NaOCl) for 20 min. Later they were cleaned from NaOCl by rinsing them in sterilized water 5 times for 5 min. The seeds were transferred in Magenta GA-7 vessels containing 1/1 MS medium[12] supplemented with 0.5 mg/L GA_3 gibberellic acid). The basal MS medium contained 30 g/L sucrose (w/v) and 5.4 g/L agar (w/v). The pH of the medium was adjusted to 5.7 prior to autoclaving (120 ºC for 20 min). The seeds were left to grow in the culture room under 3000 lux 16/8 photoperiod at 25 ± 2 ºC.

The rooting response was scored after 15-20 days culture. The root tips of *in vitro* rooted shoots of pea (*Pisum sativum* L.cv.araka) were treated for 3, 6, 12 h at concentrations of 1, 0.1 and 0.01 mg/L of $MgSO_4$ diluted with distilled water. For the control group, the seeds of pea (*Pisum sativum* L.cv.araka) were rooted in distilled water at room temperature (20 ± 2 ºC). Then both the experimental and control root tips (1.5-2.0 cm) were excised from the shoots and treated for 4 h in *p*-dichlorobenzene, fixed in absolute ethanol:glacial acetic acid (3:1) for 24 h in room temperature and stored in 70 % ethanol at 4 ºC. The roots washed for 15 min, hydrolyzed for 5-12 min in 1 N HCl at 60 ºC and stained in acetocarmine for 2 h. Then each root tip squashed and prepared for count the mitotic cells. They were covered with Canada balsam for permanent slides and made for each concentration and treatment period.

On the slides selected by change 5 region for cytogenetic examine and the cells in this regions counted for estimating total mitotic cells. It was determined chromosomal abnormalities in counting cells which different phases in mitotic divisions. Comparision of control and abnormality cell in the mitotic division was estimated[15]. The significance between the mean results and control was determined by variance analyses ($p = 0.05$).

For each variable 4-5 root tip squashes were prepared and a minimum of 300-400 mitotic cells were counted. The number of cells in the mitosis divisions were scored and the mitotic index (MI) was determined. The cytological abnormalities were scored in the mitotic cells and the results are shown in the Table-1. The most common abnormalities were fixed by microphotography.

RESULTS AND DISCUSSION

The cytogenetic effects of different $MgSO_4$ treatments on mitotic divisions in the root tip cels of *Pisum sativum* L. cv.araka given Table-1. Magnesium sulphate effected the mitotic index in the treatment group at all concentrations and in all treatment periods. Mitotic index decreased with increasing concentrations of $MgSO_4$. It was observed that $MgSO_4$ inhibited mitosis and also blocks it at the metaphase and chromosomal aberrations induced by $MgSO_4$. Chromosomal aberrations were observed to have increased in frequency with increasing concentration (Fig. 1). The most common types of observed anomalies were condensed chromatine, sticky chromosomes, chromatine bridge and ring chromosome.

TABLE-1
EFFECTS OF MAGNESIUM SULPHATE ON MITOTIC CELL DIVISION AND SOMATIC CHROMOSOMAL ABNORMALITIES IN *Pisum sativum* L.cv.araka

Time (h)	Content (mg/L)	Total cells numbers	Division cell numbers	MI	Lagging chrom. In anaphase (%)	Bridge in anaphase (%)	Ring chrom. (%)	Sticky chrom. (%)	Disturbed chrom. (%)	Condensed chrom. (%)
3	0.1	893	81	9.07	0.27	0.13	0.36	0.16	1.02	0.19
	1.0	642	69	10.74	–	0.19	1.43	0.11	1.36	0.40
	10.0	766	63	8.22	2.79	1.07	2.09	1.21	1.15	1.13
6	0.1	933	86	9.21	0.17	0.25	1.18	0.33	1.23	0.32
	1.0	849	71	8.36	1.82	1.15	1.28	1.42	1.05	1.04
	10.0	627	50	7.92	3.31	2.00	2.75	0.27	2.18	1.56
9	0.1	726	62	8.53	2.51	1.84	1.25	1.89	2.33	1.68
	1.0	658	49	7.44	2.89	1.19	2.55	1.51	2.37	2.34
	10.0	617	45	7.29	3.18	2.27	3.01	2.22	2.52	3.07
Control	802	97	12.94	–	–	–	0.07	–	–	–

MI = mitotic index

There are a variety of studies have been reported relating to this sudy. For instance, Rencüzogullari *et al.*[7], investigated effects of sodium metabisulfite on mitosis in *Allium cepa* L. Inceer *et al.*[8], investigated effects of copper chloride on root tips cells of *Helianthus annuus* L. Arkhipchuk *et al.*[6], studied cytogenetic effects inorganic toxic substances on *Allium cepa*, *Lactuca sativa* and *Hydra attenuata* cells. All of them found that mitotic abnormalities were increased and the mitotic index was decreased depending on the concentration of type of cytotoxic substance.

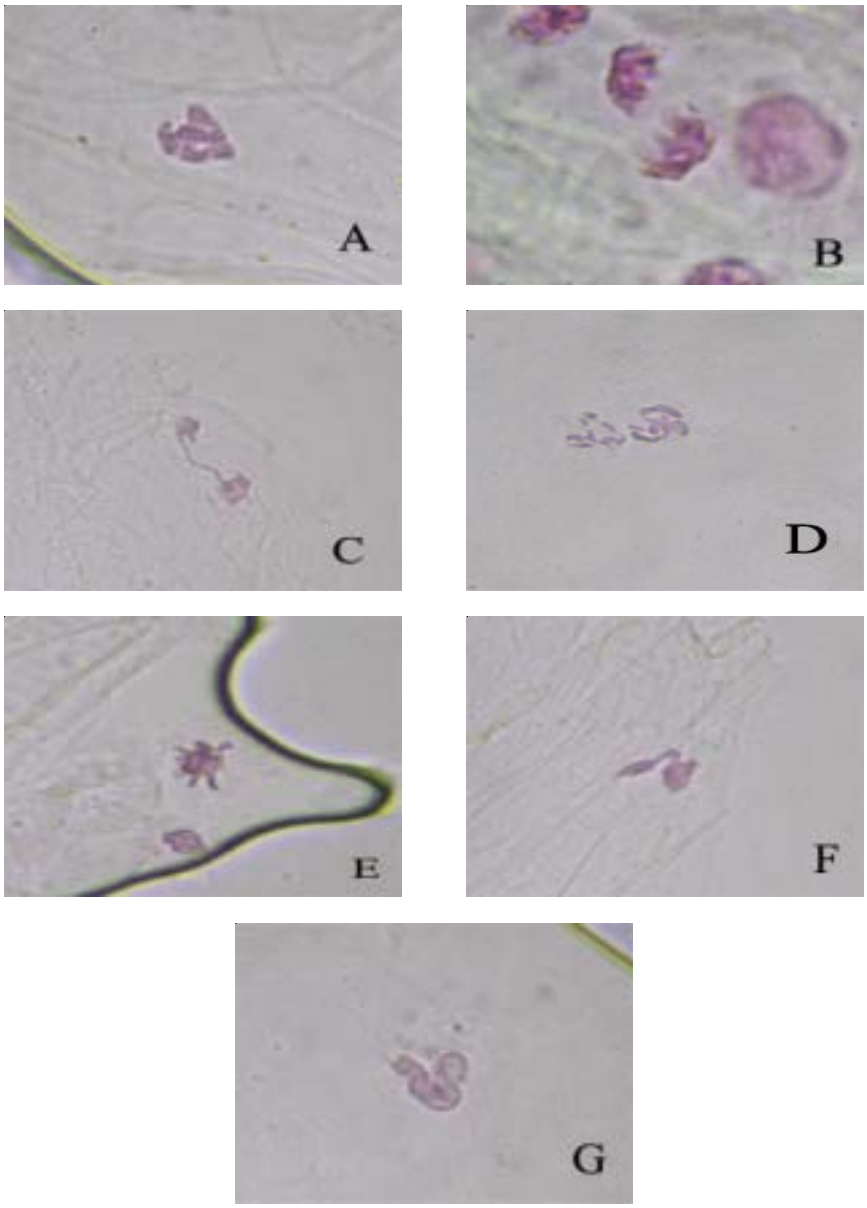

Fig. 1. (A) Sticky chromosomes (B) Lagging chromosome in anaphase (C) Bridge in anaphase (D) Disturbed chromosome (E-F) Condensed chromosome (G) Ring chromosome

In conclusion, as has been stated above, $MgSO_4$ has harmfull effects on the root tip cells of *Pisum sativum* L.cv.araka. In addition to these findings, the increase in soil and water pollution can lead to certain irreversible cytogenetic effects in plants and even in higher organisms.

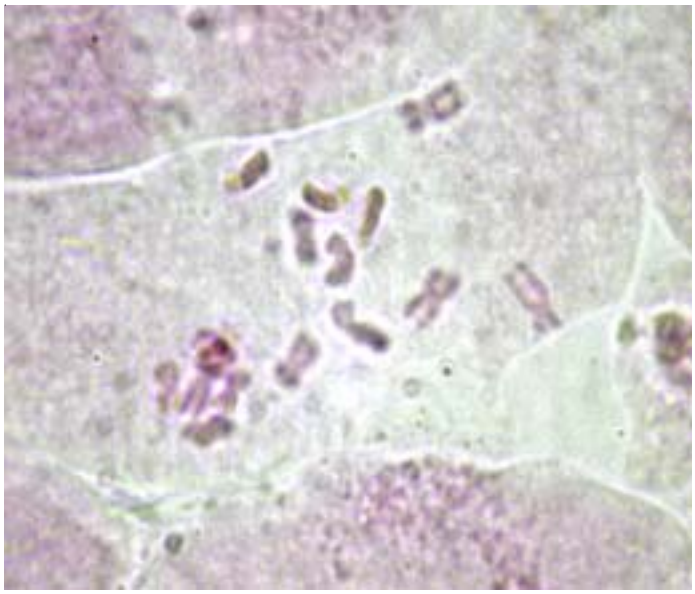

Fig. 2. C-mitosis

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