EFFECT OF ARTIFICIAL FERTILIZERS ON MITOTIC INDEX AND CHROMOSOME BEHAVIOUR IN *VICIA HYBRIDA* L.

*Selma Tabur and Signem Oney**

ABSTRACT

This study was conducted in Suleyman Demirel University, Arts and Sciences Faculty, Department of Biology, Isparta, Turkey during 2008 to determine whether ammonium sulphate [$(NH_4)_2SO_4$] and potassium sulphate (K_2SO_4) are genetically harmful or not. Ammonium sulphate and potassium sulphate were used as artificial fertilizer in various concentrations (1, 10, 50, 100, 250, 500 and 1000 µM) to see their effect on mitotic index and chromosome behaviour in *Vicia hybrida* L. Levels of 1, 10 and 50 µM of both chemicals led to a significant increase in mitotic index. For example, mitotic index in root- tips of seeds germinated in distilled water was 0.16 while it was 0.15 at 1 µM, 0.20 at 10 µM and 0.25 at 50 µM in seeds germinated in media with $(NH_4)_2SO_4$. In addition, mitotic index of seeds germinated in media with K_2SO_4 was 0.16 at seeds control, 0.17 at 1 µM, 0.18 at 10 µM and 0.23 at 50 µM. High concentrations caused gradual decreases in mitotic index. Various chromosome abnormalities were also observed in high concentrations.

KEYWORDS: ***Vicia hybrida;*** **ammonium sulphate; potassium sulphate; mitosis; chromosomes; Turkey.**

INTRODUCTION

Food availability for mankind is rapidly declining because of overgrowth in world population and decrease in agricultural area. To deal with problem producers focus on genetic improvement and artificial fertilization for increasing per unit plant yield. However, excessive fertilizer applications can cause lot of problems (9). Though fertilizers have increased productivity, yet these can become lethal to some of beneficial insects and animals (11).

To increase crop yields and soil fertility producers use agricultural chemicals and artificial fertilizers. In this case plants become prone to fertilizer more and more and soil infertility increases. However, these fertilizers also have caused serious soil, water and air pollution. It was realized that agricultural chemicals caused health problems affecting immune system in living organisms (4, 5, 17). As a result of all these, gene transplantation generated

***Suleyman Demirel University, Arts and Sciences Faculty, Department of Biology, Isparta, Turkey.**

wild plants, harmful insects and disease resistant products (6, 10, 14, 15). Consequently, mankind was familiar with organisms whose genes were changed as well. In brief, artificial fertilizers created various problems although these were previously used to increase crop yields.

The present study was conducted to determine effects of $(NH_4)_2SO_4$ and K_2SO_4 on mitotic index and chromosome behaviour and also to know how far these are genetically harmful.

MATERIALS AND METHODS

This study was conducted in Department of Biology, Arts and Sciences Faculty, Suleyman Demirel University, Isparta, Turkey. *Vicia hybrida* seeds were surface sterilized with 1 percent sodium hypochloride according to the method of Braun and Khan (1). Concentrations of $(NH_4)_2SO_4$ and K_2SO_4 were used 1, 10, 50, 100, 250, 500 and 1000 µM.

Seeds used in all applications were regularly put inside Petri dishes including $(NH_4)_2SO_4$ and K_2SO_4 in different concentrations (20 ml). Germination tests were carried out in constant temperature (20°C), in incubator and continuous darkness.

Cytotaxonomical observations: When root tips reached 1-1.5 cm at end of germination these were cut off and pretreated with paradichlorobenzene for four hours and then fixed in an ethanol-acetic acid (1:3) solution for at least 24 hours at 4°C. Hydrolysis processes were made at 60°C and in 1 N HCl for 15-18 minutes (3). Feulgen method was used to stain (13). After root tips stained, squash preparates were arranged and permanented with alcohol vapour exchange method (2). These preparates were observed under microscope (100 X) and cells in mitosis were counted. Hereby, mitotic index was evaluated by analyzing at least 2000 cells per treatment. Chromosomal abnormalities were calculated for each concentration as percentage of 350-400 dividing cells counted. According to Duncan's multiple range test using SPSS 14.0 program, all parameters relating to statistical evaluation were carried out.

RESULTS AND DISCUSSION

Effects of $(NH_4)_2SO_4$ and K_2SO_4 on mitotic index

Mitotic index value in root meristems of seeds germinated in media with $(NH_4)_2SO_4$ and K_2SO_4 were compared with each other (Table 1). In parallel to concentration rise, mitotic index of seeds in media with K_2SO_4 increased until certain level (50 µM). For example, while mitotic index value was 0.16 in seeds of control groups, it was 0.17 at 1 µM, 0.18 at 10 µM and 0.23 at 50 µM. In parallel to concentration rise (except for 1 µM), $(NH_4)_2SO_4$ also increased mitotic index until 50 µM concentration. In addition, concentrations of 1 µM of both chemicals caused to unheeded decrease in mitotic index. Mitotic index value of seeds treated with 100 µM $(NH_4)_2SO_4$ increased approximately to double as compared to K_2SO_4 .

Table 1. Mitotic index in *V. hybrida* L. seed germinated in media containing $(NH_4)_2$ SO_4 and K_2SO_4.

Treatment (µM)	Mitotic index (MI)	
	$(NH_4)_2SO_4$	K_2SO_4
Control (C)	0.16 ± 0.03^{ab}	0.16 ± 0.03^{ab}
1	0.15 ± 0.02^{ab}	0.17 ± 0.05^{ab}
10	0.20 ± 0.02^{abc}	0.18 ± 0.08^{abc}
50	0.25 ± 0.05^{bc}	0.23 ± 0.02^{abc}
100	0.21 ± 0.02^{abc}	0.11 ± 0.01^{a}
250	0.19 ± 0.02^{abc}	0.18 ± 0.02^{abc}
500	0.18 ± 0.02^{abc}	0.15 ± 0.03^{ab}
1000	0.11 ± 0.00^{a}	0.14 ± 0.04^{ab}

Values in a column followed by same letters do not different significantly ($P<0.05$).
± Standard deviation.

The most positive effect on mitotic index in seeds treated with $(NH_4)_2SO_4$ and K_2SO_4 was noted in 50 µM, considering all concentrations. Minimum value was noted in $(NH_4)_2SO_4$ in 1000 µM (0.11) and K_2SO_4 in 100µM (0.11) (Table 1). On the other hand, $(NH_4)_2SO_4$ became more effective than K_2SO_4 in high concentrations, considering effect of both applications on mitotic index (Table 1).

Effects of $(NH_4)_2SO_4$ and K_2SO_4 on chromosome behaviour

Seed germinated in distilled water at 20°C and subjected to $(NH_4)_2SO_4$ and K_2SO_4 in different concentrations, showed various chromosome abnormalities (Fig. 1-5). Percentages of these chromosome abnormalities were counted dividing abnormal cell numbers by cell numbers in mitosis (Table 2-3).

Chromosome abnormality could not be determined in root tip cells of seeds germinated in distilled water during microscopic examination. It means that all phases of mitosis were observed as normal (Fig. 1).

Table 2. Ratios of chromosome abnormality in *V. hybrida* L. seeds germianted in

media containing $(NH_4)_2SO_4$.

Treatment (μM) $(NH_4)_2SO_4$	Chromosome abnormalities				
	Prophase	Metaphase	Anaphase	Telophase	Total
Control (C)	0.00	0.00	0.00	0.00	0.00
50	0.208	0.000	25.00	0.006	0.150
1000	0.350	0.533	87.00	0.046	0.299

Table 3. Ratios of chromosome abnormality in *V. hybrida* L. seeds germinated in media containing K_2SO_4.

Treatment K_2SO_4 (μM)	Chromosome abnormalities				
	Prophase	Metaphase	Anaphase	Telophase	Total
Control (C)	0.00	0.00	0.00	0.00	0.00
50	0.060	0.260	36.666	0.002	0.160
1000	0.339	1.142	25.00	0.005	0.387

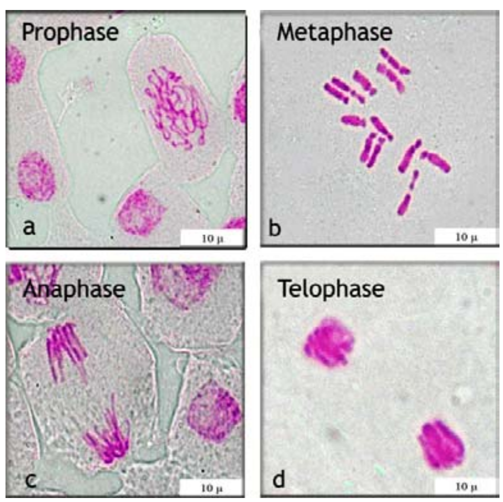

Fig. 1. Normal mitosis phases in *V. hybrida* L. seeds germinated in distilled water.

However, lot of chromosome abnormalities were identified such as disorderly prophase (Fig. 2 a-d), adherent chromosomes (Fig. 3 a-b), separation of metaphase plane to two (Fig. 3 c), fragment (Fig. 3 d), anaphase bridges (Fig. 4 a-c), lagging chromosomes in anaphase (Fig. 4 d-e) and telophase (Fig. 5 d), fault polarization in anaphase (Fig. 4 f-g), telophase (Fig. 5 a-b), disorderly anaphase (Fig. 4 h), and telophase bridges (Fig. 5 c) in cells of seeds treated with $(NH_4)_2SO_4$ and K_2SO_4. Ratios of chromosome abnormality were detected by counting preparates in concentrations of the highest (1000 μM) and the least (50 μM) of mitotic index value (Table 2-3).

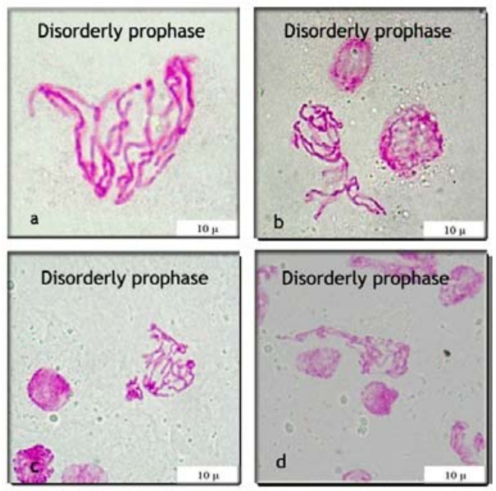

Fig. 2. Chromosome abnormalities in prophase of *V. hybrida* L. seeds germinated in media containing $(NH_4)_2SO_4$ and K_2SO_4 in various concentrations

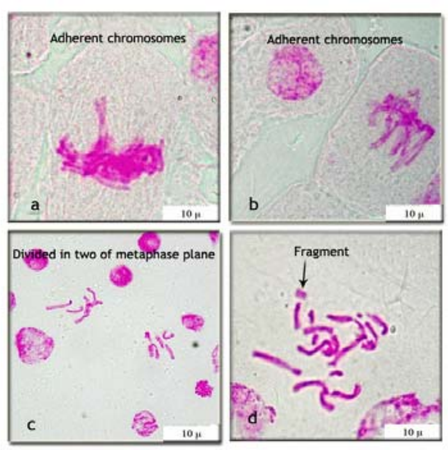

Fig. 3. Chromosome abnormalities in metaphase of *V. hybrida* L. seeds germinated in media containing $(NH_4)_2SO_4$ and K_2SO_4 in various concentrations.

Chromosome abnormalities were evaluated considering phases of mitosis in both chemicals. In addition, it was determined that chromosome abnormality was higher in anaphase and minimum in telophase in concentrations of both

50 µM and 1000 µM in seeds treated with above mentioned chemicals (Table 2-3).

Considering all these ratios, it was detected that anaphase had higher chromosome abnormality (87) in 1000 µM $(NH_4)_2SO_4$, not only in cells of seeds treated with $(NH_4)_2SO_4$ (Table 2) but also in all treated with K_2SO_4 (Table 3). Abnormalities in anaphase (Fig. 4) were defined as anaphase bridges (a - c), lagging chromosomes (d - e), fault polarizations (f - g) and disorderly anaphase (h). In general, it was observed that chromosome abnormalities were more in K_2SO_4 (0.547) than $(NH_4)_2SO_4$ (0.449).

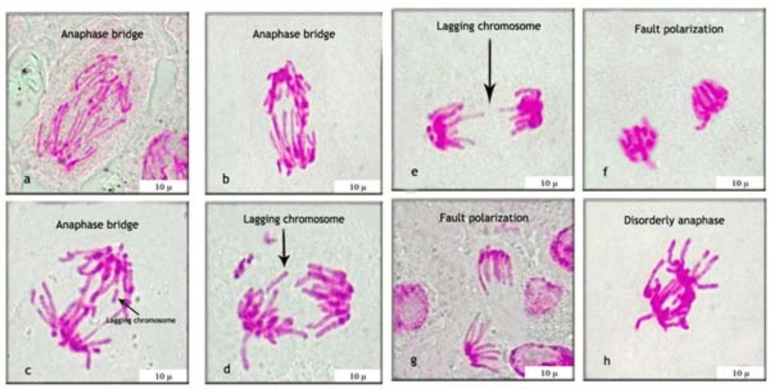

Fig. 4. Chromosome abnormalities in anaphase of *V. hybrida* L. seeds germinated In media containing $(NH_4)_2SO_4$ and K_2SO_4 in various concentrations.

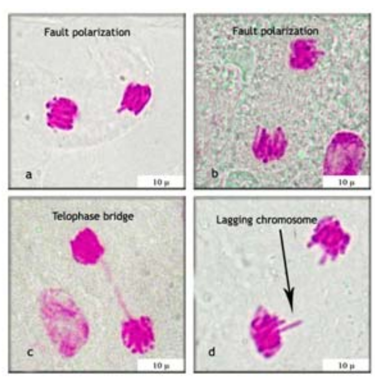

Fig. 5. Chromosome abnormalities in telophase of *V. hybrida* L. seeds germinated in media containing $(NH_4)_2SO_4$ and K_2SO_4 in various concentrations.

Effects of $(NH_4)_2SO_4$ and K_2SO_4 , on mitotic index and chromosome behaviour originating from artificial fertilizers and emerging in parallel to crop rise in 20th century, were also compared.

According to present findings, mitotic index value in 1 - 1000 µM concentrations of $(NH_4)_2SO_4$ and in 100- 500- 1000 µM concentrations of K_2SO_4 could not excel control group (Table 1). The study also revealed that mitotic index value rises in parallel to cell division. It can be said that cell division decreases depending on the increasing concentration in both $(NH_4)_2SO_4$ and K_2SO_4. This indicates that artificial fertilizers should be used by suitable methods and in recommended doses.

As a result of cytological studies, chromosome abnormalities were not observed in *Vicia hybrida* L. seeds germinated in distilled water (Fig. 1). However, seeds germinated in media containing $(NH_4)_2SO_4$ and K_2SO_4 showed various chromosome abnormalities (Fig. 2-5). It was determined that chromosome abnormalities in 1000 µM K_2SO_4 were more than all applications studied (Table 2-3). Chromosome abnormalities were observed in 363 cells (18.2%) out of 2000 divided cells in seeds treated with $(NH_4)_2SO_4$. Similarly, chromosome abnormalities were noted in 418 cells (20.9%) out of 2000 divided seed cells treated with K_2SO_4.

Artificial fertilizers become injurious by accumulating in plant structure. These pass from plant to animal and human directly or indirectly. Recently, artificial fertilizers unconsciously used, have threatened human health (16).

Excessive nitrogen fertilizer applications can also lead to pest problems by increasing birth rate, longevity and fitness of certain pests (7, 8, 11). Excessive fertilization is primarily associated with the use of artificial fertilizers because of massive quantities applied and destructive nature of chemical fertilizers on soil. High solubility of chemical fertilizers also exacerbates their tendency to degrade ecosystems.

Storage and application of some fertilizers in certain weather or soil conditions can cause emissions of greenhouse gas (nitrous oxide N_2O). So, nitrogen-based fertilizers contribute directly to global warming. Ammonia gas (NH_3) may be emitted following application of inorganic fertilizers, or manure or slurry. Besides nitrogen, ammonia can also increase soil acidity.

For these reasons, it is recommended that knowledge of nutrient content of soil and nutrient requirements of crop are carefully balanced with nutrients application in inorganic fertilizer especially. By careful monitoring of soil conditions, farmers can avoid wasting expensive fertilizers, and also avoid the potential costs of cleaning up any pollution created as a byproduct of their farming.

Consequently, organic farming is proposed to protect environment and natural sources, to repair harmful ecological balance, to protect flora and fauna and to maintain biological diversity. In order to develop organic farming, one of traditional ways of agriculture and to protect environment and human health all over the world, remarkable studies must be conducted at various levels in different countries.

The study condludes that artificial fertilizers proved harmful unless these are used by suitable methods, dose or amounts advised. As the effect of mentioned artificial fertilizers on mitotic activity and chromosome behaviour has not been studied sufficiently, we believe that present findings will provide base for subsequent studies in future.

REFERENCES

1. Braun, J. and A. Khan. 1976. Alleviaton of salinity and high temperature stress by plant growth regulators permeated into Lettuce seeds via acetone. J. Amer. Soc. Hortic. Sci. 101: 716- 721.
2. Elçi, Ş. 1982. Sitogenetikte gözlemler ve araştırma yöntemleri. Fırat Üniv. Fen Edb. Fak. Yay. Elazığ, Türkiye, 166 pp.
3. Fox, D.P., 1969. Some characteristics of the cold hydrolysis technique for staining plant tissues by the feulgen reaction. J. Histochem. Cytochem. 17: 266- 272.
4. Gill, D.S. 1995. Development of herbicide resistance in annual ryegrass populations in the cropping belt of western Australia. Aust. J. Exp. Agr. 3: 67- 72.
5. Goldburg R.J. 1992. Environmental concerns with the development of herbicide- tolerant plants. Weed Technol. 6: 647- 652.
6. Holt, J.S., S.B. Powles and J. A. M. Hotlum 1993. Mechanisms and agronomic aspects of herbicide resistance. Annu. Rev. Plant Physiol. Plant Mol. Biol. 44: 203- 229.
7. Jahn, G.C. 2004. Effect of soil nutrients on the growth, survival and fecundity of insect pests of rice: An overview and a theory of pest outbreaks with consideration of research approaches. Multitrophic interaction in soil and integrated control. IOBC/WPRS Bulletin. 27 (1): 115- 122.
8. Jahn, G.C., L.P. Almazan and J. Pacia. 2005. Effect of nitrogen fertilizer on the intrinsic rate of increase of the rusty plum aphid, *Hysteroneura*

setariae (Thomas) (Homoptera: Aphididae) on Rice (*Oryza sativa* L.). Environ. Entomol. 34 (4): 938- 943.
9. Mason, M.G. 1980. An investigation of reduction in wheat yields after use of a high level of ammonium sulphate for a number of years. Aust. J. Exp. Agric. Anim. Husb. 20: 210- 219.
10. Mikkelsen, T.R., B. Andersen and R.B. Jorgensen. 1996. The risk of transgene spread. Nature (London). 380, 31.
11. Preap, V., M.P. Zalucki, H.J. Nesbitt and G.C. Jahn. 2001. Effect of fertilizer, pesticide treatment, and plant variety on realized fecundity and survival rates of *Nilaparvata lugens* (Stål); generating outbreaks in Cambodia. J. Asia Pac. Entomol. 4 (1): 75- 84.
12. Preap, V., M.P. Zalucki and G. C. Jahn. 2002. Effect of nitrogen fertilizer and host plant variety on fecundity and early instar survival of *Nilaparvata lugens* (Stål): Immediate Response. Proc. 4th Int. Workshop on Inter-Country Forecasting System and Management for Planthopper in East Asia. November 13- 15. Guilin China. Pub. Rural Development Administration (RDA) and FAO. 163: 180, 226.
13. Sharma, P.C. and P.K. Gupta. 1982. Karyotypes in some pulse crops. The Nucleus. 25: 181- 185.
14. Steinbrecher, R.A. 1996. From green to gene revolution: "The experimental risk of genetically engineered crops". The Ecologist. 26 (6): 275.
15. Tabashnik, B.E. 1994. Evolution of resistance to *Bacillus thuringenesis*, Annu. Rev. Entomol. 39: 47- 79.
16. Verhoog, H. M. Matze, E. Van Bueren and T. Baars. 2003. The role of the concept of natural (naturalness) in organic farming. J. Agric. Environ. Ethics. 16:29-49.
17. Wolfe D.W. 2001. Tales from the Underground: A Natural History of Subterranean Life. Perseus Publishing. Cambridge. Pp. 221.