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

Selma Tabur and Signem Onay*

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 [(NH₄)₂SO₄] and potassium sulphate (K₂SO₄) 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 (NH₄)₂SO₄. In addition, mitotic index of seeds germinated in media with K₂SO₄ 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 (NH₄)₂SO₄ and K₂SO₄ 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 (NH₄)₂SO₄ and K₂SO₄ were used 1, 10, 50, 100, 250, 500 and 1000 µM.

Seeds used in all applications were regularly put inside Petri dishes including (NH₄)₂SO₄ and K₂SO₄ 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 (NH₄)₂SO₄ and K₂SO₄ on mitotic index**

Mitotic index value in root meristems of seeds germinated in media with (NH₄)₂SO₄ and K₂SO₄ were compared with each other (Table 1). In parallel to
concentration rise, mitotic index of seeds in media with K$_2$SO$_4$ 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$_4$)$_2$SO$_4$ 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$_4$)$_2$SO$_4$ increased approximately to double as compared to K$_2$SO$_4$.

Table 1. Mitotic index in *V. hybrida* L. seed germinated in media containing (NH$_4$)$_2$ SO$_4$ and K$_2$SO$_4$.

<table>
<thead>
<tr>
<th>Treatment (µM)</th>
<th>(NH$_4$)$_2$SO$_4$</th>
<th>K$_2$SO$_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>0.16 ± 0.03$^{ab}$</td>
<td>0.16 ± 0.03$^{ab}$</td>
</tr>
<tr>
<td>1</td>
<td>0.15 ± 0.02$^{ab}$</td>
<td>0.17 ± 0.05$^{ab}$</td>
</tr>
<tr>
<td>10</td>
<td>0.20 ± 0.02$^{abc}$</td>
<td>0.18 ± 0.08$^{abc}$</td>
</tr>
<tr>
<td>50</td>
<td>0.25 ± 0.05$^{bc}$</td>
<td>0.23 ± 0.02$^{abc}$</td>
</tr>
<tr>
<td>100</td>
<td>0.21 ± 0.02$^{abc}$</td>
<td>0.11 ± 0.01$^{a}$</td>
</tr>
<tr>
<td>250</td>
<td>0.19 ± 0.02$^{abc}$</td>
<td>0.18 ± 0.02$^{abc}$</td>
</tr>
<tr>
<td>500</td>
<td>0.18 ± 0.02$^{abc}$</td>
<td>0.15 ± 0.03$^{ab}$</td>
</tr>
<tr>
<td>1000</td>
<td>0.11 ± 0.00$^{a}$</td>
<td>0.14 ± 0.04$^{ab}$</td>
</tr>
</tbody>
</table>

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$_4$)$_2$SO$_4$ and K$_2$SO$_4$ was noted in 50 µM, considering all concentrations. Minimum value was noted in (NH$_4$)$_2$SO$_4$ in 100 µM (0.11) and K$_2$SO$_4$ in 100µM (0.11) (Table 1). On the other hand, (NH$_4$)$_2$SO$_4$ became more effective than K$_2$SO$_4$ in high concentrations, considering effect of both applications on mitotic index (Table 1).

**Effects of (NH$_4$)$_2$SO$_4$ and K$_2$SO$_4$ on chromosome behaviour**

Seed germinated in distilled water at 20°C and subjected to (NH$_4$)$_2$SO$_4$ and K$_2$SO$_4$ 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 germinated in

*J. Agric. Res., 2009, 47(1)*
media containing \((\text{NH}_4)_2\text{SO}_4\).

<table>
<thead>
<tr>
<th>Treatment (µM) ((\text{NH}_4)_2\text{SO}_4)</th>
<th>Chromosome abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prophase</td>
</tr>
<tr>
<td>Control (C)</td>
<td>0.00</td>
</tr>
<tr>
<td>50</td>
<td>0.208</td>
</tr>
<tr>
<td>1000</td>
<td>0.350</td>
</tr>
</tbody>
</table>

Table 3. Ratios of chromosome abnormality in *V. hybrida* L. seeds germinated in media containing \(\text{K}_2\text{SO}_4\).

<table>
<thead>
<tr>
<th>Treatment (\text{K}_2\text{SO}_4) (µM)</th>
<th>Chromosome abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prophase</td>
</tr>
<tr>
<td>Control (C)</td>
<td>0.00</td>
</tr>
<tr>
<td>50</td>
<td>0.060</td>
</tr>
<tr>
<td>1000</td>
<td>0.339</td>
</tr>
</tbody>
</table>

![Fig. 1. Normal mitosis phases in *V. hybrida* L. seeds germinated in distilled water.](image)

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

*J. Agric. Res., 2009, 47(1)*
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

**Fig. 2.** Chromosome abnormalities in prophase of *V. hybrida* L. seeds germinated in media containing (NH₄)₂SO₄ and K₂SO₄ in various concentrations

**Fig. 3.** Chromosome abnormalities in metaphase of *V. hybrida* L. seeds germinated in media containing (NH₄)₂SO₄ and K₂SO₄ in various concentrations.
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 (NH₄)₂SO₄, not only in cells of seeds treated with (NH₄)₂SO₄ (Table 2) but also in all treated with K₂SO₄ (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 K₂SO₄ (0.547) than (NH₄)₂SO₄ (0.449).

Fig. 4. Chromosome abnormalities in anaphase of V. hybrida L. seeds germinated in media containing (NH₄)₂SO₄ and K₂SO₄ in various concentrations.

Fig. 5. Chromosome abnormalities in telophase of V. hybrida L. seeds germinated in media containing (NH₄)₂SO₄ and K₂SO₄ in various concentrations.
Effects of (NH₄)₂SO₄ and K₂SO₄ 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 (NH₄)₂SO₄ and in 100- 500- 1000 µM concentrations of K₂SO₄ 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 (NH₄)₂SO₄ and K₂SO₄. 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 (NH₄)₂SO₄ and K₂SO₄ showed various chromosome abnormalities (Fig. 2-5). It was determined that chromosome abnormalities in 1000 µM K₂SO₄ 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 (NH₄)₂SO₄. Similarly, chromosome abnormalities were noted in 418 cells (20.9%) out of 2000 divided seed cells treated with K₂SO₄.

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 N₂O). So, nitrogen-based fertilizers contribute directly to global warming. Ammonia gas (NH₃) 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 concludes 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

Effect of artificial fertilizers on mitotic index in *Vicia hybrida*.


