Assessment of cytogenotoxic effects of diprofos drug on *Vicia faba* Plant

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**ABSTRACT**

Cytogenotoxic effects of diprofos drug with different concentrations (0.5, 1, 2 and 4 ml/100 ml water) were examined in *Vicia faba* plants. *Vicia faba* plants were sprayed with the four diprofos concentrations at the flowering stage, then after 15 days meiotic division behavior was examined and leaves protein was estimated using SDS protein electrophoresis. According to electrophoretic results, RAPD-PCR reaction was conducted on the M$_2$ *Vicia faba* plants which were treated with 2 ml/100 ml diprofos beside the control by using ten primers. Diprofos treatments induced highly significant total meiotic abnormalities % which were increased as the diprofos concentration increased. Abnormalities % in the second division were lower than those recorded in the first division in all treatments as a result of recovery in this cell age. Most abnormalities were observed in metaphase and anaphase stages in both the two meiotic divisions. Stickiness and disturbed chromosomes were the most dominant abnormalities in the two divisions. In addition, laggard, bridges, breaks and micronuclei occurred but with very low frequencies in some treatments. Diprofos treatment with 2ml/100ml caused disappearance of five protein bands (40, 50, 60, 80 and 100) KDa compared with the control via SDS-PAGE analysis. Also, it is obvious that diprofos treatment of M$_2$ *Vicia faba* plants showed a polymorphic genetic band, by using RAPD-PCR product, comparing with the control. Results strongly suggest that diprofos drug posses a cytogenotoxic effects and should be avoided if possible.

*Keywords,* cytogenotoxic effects; SDS-PAGE analysis; RAPD-PCR reaction and polymorphic genetic bands.

**INTRODUCTION**

Corticosteroids are contraindicated in patients with peptic ulcer, osteoporosis, psychoses or sever psyshonenroses. Because of the interference
with inflammatory and immunological response, corticosteroids should not be usually given in the presence of acute infections. Live vaccine should be avoided in patients receiving corticosteroids. Corticosteroid should not be given during pregnancy or lactation. Prolonged use of betamethasone in high doses may cause pituitary suppression, acute adrenal insufficiency, fluid and electrolyte disturbance, hyperglycemia and glucosuria, increased susceptibility to infections, peptic ulcers, osteoporosis, arrest of growth, cushin's habitus (moon-face, buffalo hump), behavioral disturbance and cataract \(^{(1)}\). Chromosomal aberrations induction and alteration of genetic material are the sensitive and important tests for evaluating genetic hazards of environmental mutagens, and/or carcinogens, because there is a clear association between chromosomal aberrations and certain types of cancer. Many investigators have suggested that the study of chromosomal aberrations in both mitotic and meiotic divisions, total DNA or RNA contents, changes in storage protein banding patterns and RAPD-PCR profile changes are suitable systems to detect the potential cytological and molecular effects caused by many chemicals. Chromosomal aberrations, alterations in protein banding pattern and DNA alteration by using RAPD-PCR analysis were observed by many cytogeneticists\(^{(2-18)}\) after many medical drugs and medicinal plants extracts treatments (drugs such as: piromicam, fenaton, diazepam, tetracyclines, macrolides, megazol, nitroimidazole, acetyl salicylic acid, cyclophosphoamide, clomide, hexamethyl phosoramidse, ibuprofen, cisplatin and 5-fluorouracil; medicinal plants extracts such as: Cryptolepis sanguinolenta, Rhaza stricta, Kockia indica and Cymbopogon citrates) on different biological systems (animals such as: mice, Drosophila melanogaster and human peripheral blood lymphocyte; plants such as: Pisum sativum, Vicia faba and Allium cepa ).

The purpose of the present study was to investigate the cytogenotoxic effects of diprofos drug on Vicia faba plant by estimating meiotic aberrations, changes in leaves protein banding patterns and screened genome DNA alteration by using the random amplified polymorphic DNA (RAPD-PCR) analysis.

**MATERIAL AND METHODS**

**1-Cytological analysis**

*Vicia faba* plants (Var. Giza 40) at the flowering stage were sprayed with four medical preparations of diprofos drug which produced by Schering Plough Company, USA. This drug is present in ampoule, form each one ml ampoule containing 7 mg betamethasone. The recommendation dose for this
drug depends on the disease type. Generally, Dosage for adults: short term (1-2) ml daily for the first few days, subsequently reducing the daily dosage by (0.50-1) ml every (2-5) days. Four diprofos concentrations: 0.50, 1, 2, 4 ml/100 ml water were used. Control plants were sprayed with distilled water. Ten flower buds from ten different plants were gathered after 15 days from spraying. For meiotic studies the appropriate flower buds were collected and fixed in carnoy's solution (ethyl alcohol absolute and glacial acetic acid with ratio 3:1) for 24h., and then transferred to 70% ethyl alcohol and kept in refrigerator. The cytological analysis were carried out by using 2% aceto carmine stain as described by Darlington and La Cour (1979)(19). The data recorded for different treatments were statistically analyzed using t-test for determining significantly of differences between these treatments.

2-SDS-PAGE protein electrophoresis

SDS- protein were performed on vertical slab (20 cm x 20 cm x 0.2 cm) using the gel electrophoresis apparatus (Manufactured by LABCONCO) according to Laemmli (1970)(20). The fresh leaves were taken from Vicia faba plants after 15 days from spraying with the four diprofos concentrations and the distilled water (control), these leaves were decoated and milled to fine powder. SDS-proteins were extracted over night using OX Tris-Hcl buffer of pH 6.8. Centrifugation was performed at 10000 rpm for 10 min. Then 40µl supernatant of SDS proteins were loaded in SDS-slab gel of 15% acrylamide containing 10% SDS. Gel was run at a current of 15 m. A. for 1 h followed by 25 m. A. for 4-5 h. Molecular weights of different bands were calibrated using the wide range protein marker ranged from 25-230 KDa according to Matta et al. (1981)(21). According to the electrophortic results, where only diprofos treatment with 2ml/100 ml caused disappearance of five protein bands: 40, 50, 60, 80 and 100 KDa compared with the control, thus remained to grown for seeding and then planted beside the control to obtain the M	extsubscript{2} generation to conduct the (RAPD-PCR) analysis.

3-RAPD-PCR analysis

*DNA extraction:

Isolation of DNA from leaves in M	extsubscript{2} Vicia faba plants which were sprayed with the third diprofos concentration 2ml/100ml beside the control was carried out. Protocol for the DNA isolation from plant leaves was applied according to Doyle and Doyle (1990)(22).
**Polymerase Chain Reaction (PCR):**

PCR reaction was conducted using Perkin Elmer (Germany) thermocycler and ten random 10-mer primers (Operon Tech. Inc., USA) were used with the following sequences (5’ → 3’) for RAPD analysis:

\{OP-A03(AGTCAGCCAC); OP-A18(AGGTGACCGT);
OP-A20(GTTGCGATCC); OP-B12(CCTTGACGCA);
OP-B16(TTTGCCCGGA); OP-C11(AAAGCTGCAG);
OP-C16(CACACTCCAG); OP-E18(GGACTGCAGA);
OP-G17(ACGACCGACA); OP-G18(GGCTCATGTG)\}.

The reaction conditions were optimized and mixtures consisted of the following: dNTPs (2.5 mM) 2.0 μl; Mg Cl₂ (25 mM) 1.5 μl; 10 x buffer 2.5 μl; primer (2.5 μM) 2.0 μl; template DNA (50 ng/ μl)20 μl; Taq (5 U/ μl)0.3 μl and ddH₂O 14.7 μl. The reaction mixtures were overlaid with a drop of light mineral oil per sample. Amplification was carried out in the thermocycler programmed for 40 cycles as follows: 94°C/ 4 min (1 cycle); 94°C/ 1 min, 37°C/ 1 min, 75°C/2 min(38 cycles); 72°C/12 min(1 cycle), 4°C (infinite)²³.

**Agarose gel electrophoresis:**

Agarose (1.2%) was used for resolving the PCR products. λ Phage DNA digested with Bst EII was used as a standard DNA (15 fragments). Molecular sizes in K bp of the resulted fragments of the standard DNA ranged from (2.64 to 0.16). The run was performed for one hour at 100 V in Pharmacia submarine apparatus (20cm X 20cm). Bands were detected on UV-transilluminator and photographed by a Polaroid camera. Results were documented with Gel Doc 2000 (Bio RAD).

**RESULTS AND DISCUSSION**

1- **Cytological studies**

A wide spectrum of meiotic abnormalities were recorded in ten flower buds from different plants after diprofos treatments. Data in Table (1) shows that all treatments induced highly significant total meiotic abnormalities% which increased as the diprofos concentration increased. Also, these abnormalities increased in most both the two meiotic divisions as the diprofos concentration increased.

On the other hand, total abnormalities% in the second division were
lower than those recorded in the first division in all treatments as a result of recovery in this cell age\(^{(24)}\).

Table (1): Numbers and percentages of abnormal PMCs in the 1\(^{st}\) & 2\(^{nd}\) meiotic divisions and total mean of meiotic abnormalities after 15 days from spraying *Vicia faba* plants with four concentrations of diprofos drug.

<table>
<thead>
<tr>
<th>Treatment (ml/100ml)</th>
<th>Control</th>
<th>0.50</th>
<th>1</th>
<th>2</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>%abnormal PMC, in 1(^{st}) division</td>
<td>dividing cells No.</td>
<td>204</td>
<td>172</td>
<td>226</td>
<td>259</td>
</tr>
<tr>
<td></td>
<td>abnormal cells No.</td>
<td>16</td>
<td>52</td>
<td>90</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>7.84</td>
<td>30.23</td>
<td>39.82</td>
<td>60.23</td>
</tr>
<tr>
<td>%abnormal PMC, in 2(^{nd}) division</td>
<td>dividing cells No.</td>
<td>216</td>
<td>196</td>
<td>196</td>
<td>269</td>
</tr>
<tr>
<td></td>
<td>abnormal cells No.</td>
<td>6</td>
<td>44</td>
<td>36</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>2.78</td>
<td>22.45</td>
<td>18.37</td>
<td>45.35</td>
</tr>
<tr>
<td>%abnormal PMC, in meiotic division</td>
<td>Total dividing cells No.</td>
<td>420</td>
<td>368</td>
<td>422</td>
<td>528</td>
</tr>
<tr>
<td></td>
<td>total abnormal cells No.</td>
<td>42</td>
<td>96</td>
<td>126</td>
<td>278</td>
</tr>
<tr>
<td></td>
<td>Mean of abnormal PMCs % ± SE.</td>
<td>5.23 ± 0.17</td>
<td>25.49 ± 4.24</td>
<td>29.59 ± 2.44</td>
<td>52.76 ± 0.52</td>
</tr>
</tbody>
</table>

PMCs: Pollen mother Cells. **highly significant (P < 0.01)**

Table (2) reveals that the highest percentages of abnormalities were recorded in both metaphase and anaphase stages in the two meiotic divisions\(^{(24)}\).

The induction of chromosomal abnormalities appeared to be common effects of many chemicals in many different biological systems\(^{(12-18,24-29)}\).

Most dominant of total abnormalities% were: stickiness and disturbed chromosomes in all meiotic divisions. Whereas, total abnormalities% after all treatments in the first and the second division ranged between 30.23% - 61.95% and 18.37% - 54.95% respectively, Table 1. However, stickiness and disturbed chromosomes percentages ranged between 13.96%-40.93%; 13.96%-22.57%; 11.23%-33.33%; and 4.08%-21.56% respectively, Table 3. In addition, laggards, bridges, breaks and micronuclei occurred but with very low frequencies in some treatments.

Table (2): Percentages of different abnormal meiotic phases after 15 days from spraying *Vicia faba* plants with four concentrations of diprofos drug.

<table>
<thead>
<tr>
<th>Treatments (ml/100ml)</th>
<th>Control</th>
<th>0.50</th>
<th>1</th>
<th>2</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frist meiotic division</td>
<td>Abnormal metaphase%</td>
<td>0.98</td>
<td>19.76</td>
<td>15.93</td>
<td>33.98</td>
</tr>
<tr>
<td></td>
<td>Abnormal anaphase%</td>
<td>6.86</td>
<td>9.30</td>
<td>17.71</td>
<td>19.31</td>
</tr>
<tr>
<td></td>
<td>Abnormal telophase%</td>
<td>-</td>
<td>1.16</td>
<td>6.19</td>
<td>6.95</td>
</tr>
<tr>
<td>Second meiotic division</td>
<td>Abnormal promphase%</td>
<td>-</td>
<td>2.04</td>
<td>1.02</td>
<td>5.20</td>
</tr>
<tr>
<td></td>
<td>Abnormal metaphase%</td>
<td>-</td>
<td>9.18</td>
<td>6.12</td>
<td>22.30</td>
</tr>
<tr>
<td></td>
<td>Abnormal anaphase%</td>
<td>2.78</td>
<td>9.18</td>
<td>10.20</td>
<td>14.13</td>
</tr>
<tr>
<td></td>
<td>Abnormal telophase%</td>
<td>-</td>
<td>2.04</td>
<td>1.02</td>
<td>3.72</td>
</tr>
</tbody>
</table>
Table (3): Types and percentages of meiotic abnormalities in the 1st and the 2nd meiotic divisions after 15 days from spraying *Vicia faba* plants with four concentrations of diprofos drug.

<table>
<thead>
<tr>
<th>Treatments (ml/100ml)</th>
<th>Control</th>
<th>0.50</th>
<th>1</th>
<th>2</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1st meiotic division</strong></td>
<td>Stickiness%</td>
<td>-</td>
<td>13.96</td>
<td>15.49</td>
<td>40.93</td>
</tr>
<tr>
<td>Disturbed%</td>
<td>7.84</td>
<td>13.96</td>
<td>22.57</td>
<td>16.22</td>
<td>17.51</td>
</tr>
<tr>
<td>Laggards%</td>
<td>-</td>
<td>2.33</td>
<td>0.88</td>
<td>0.77</td>
<td>2.02</td>
</tr>
<tr>
<td>Bridges%</td>
<td>-</td>
<td>-</td>
<td>0.88</td>
<td>2.32</td>
<td>2.02</td>
</tr>
<tr>
<td>Breaks%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.69</td>
<td></td>
</tr>
<tr>
<td><strong>2nd meiotic division</strong></td>
<td>Stickiness%</td>
<td>-</td>
<td>11.23</td>
<td>13.27</td>
<td>20.82</td>
</tr>
<tr>
<td>Disturbed%</td>
<td>2.78</td>
<td>6.12</td>
<td>4.08</td>
<td>21.56</td>
<td>14.42</td>
</tr>
<tr>
<td>Laggards%</td>
<td>-</td>
<td>1.02</td>
<td>-</td>
<td>-</td>
<td>1.80</td>
</tr>
<tr>
<td>Bridges%</td>
<td>-</td>
<td>2.04</td>
<td>-</td>
<td>0.74</td>
<td>-</td>
</tr>
<tr>
<td>Micronuclei%</td>
<td>-</td>
<td>2.04</td>
<td>1.02</td>
<td>2.23</td>
<td>5.40</td>
</tr>
</tbody>
</table>

Stickiness chromosomes observed in the most meiotic stages and the number of sticky cells increased in all stages of meiotic division as the diprofos concentration increased in most treatments, while the percentage of this abnormality decreased in the second division than those recorded in the first division as a result of recovery in this cell age (Table, 3; Fig. 1: a, b, c, d, e, f, g, h, l & r).

These results are in agreement with the results of many investigators\(^{26-28}\), who suggested that the chromosome stickiness may result from breakage and exchange between chromatin fibers on the surface of adjoining chromosomes.

Disturbed chromosomes observed in metaphase and anaphase in the two division after all treatments and the percentage of this abnormality generally increased by the increasing diprofos concentration, but it generally decreased in the second division than those recorded in first division as a result of recovery in this cell age (Table 3; Fig. 1: e, g, h, l, j & k).

This abnormality was observed by many researchers\(^{5-18; 24-29}\) after many chemicals treatments, they suggested that the disturbed chromosomes may result from the effect of the chemical treatments on proteins constituting the spindle apparatus.

Laggard chromosomes were observed in some meiotic stages after diprofos treatments with low percentages (Table 3; Fig. 1: d, f, l, m & n). Laggard chromosomes at metaphase could be attributed to the failure of the spindle apparatus to organize and function in a normal way\(^{29}\).

These laggards may be distributed randomly to either poles at both
anaphase and telophase I and II which result ultimately in aneuploidy\(^{26}\), or may give micronuclei at telophase\(^{17}\). The induction of laggard chromosomes could be attributed to irregular orientation of chromosomes\(^{24}\).

On the other hand, breaks occurred only in diprofos treatment 4ml/100 ml in the first division (Table 3; Fig. 1: r & s), while bridges were observed in most of meiotic divisions but with low percentages (Table 3; Fig. 1: e, j, o, p, q & s).

Bridges could be due to the breakage and reunion\(^{27}\) or due to the general stickiness of chromosomes\(^{28}\). While, micronuclei were also recorded in all treatments in prophase and telophase stages in the second meiotic division with low frequency (Table 3; Fig.1: c & t). These results are in agreement with the results of Fisun and Rasgele 2009\(^{29}\).

Finally, the induction of these chromosomal abnormalities pointed to the cytotoxicity potential of diprofos drug.
Figure (1): Different meiotic abnormalities produced after 15 days from spraying *Vicia faba* plants with four concentrations of diprofos drug. $M_1$, $A_1$, $T_1$: first (meta, ana, telo)phase; $P_2$, $M_2$, $A_2$, $T_2$: second (pro, meta, ana, telo)phase.
2-Biochemical and molecular studies

*SDS-protein electrophoresis:

SDS-PAGE banding patterns of proteins in leaves of *Vicia faba* plants after 15 days from spraying with four diprofos concentrations are shown in Figure 2. Diprofos treatment with 2ml/100ml caused disappearance of five bands with molecular weights of: 40, 50, 60, 80 and 100 kDa comparing with the control. The disappearance of some bands in soluble proteins of *Vicia faba* plant as a result of the effects of diprofos (2ml/100ml), could be explained on the basis of mutational event at the regulatory genes that prevent or attenuate transcription \(^{(30)}\). Induction of laggards, bridges and micronuclei by diprofos drug may lead to the loss of genetic materials. Therefore, disappearance of some electrophoretic bands were might due to the loss of their corresponding gene \(^{(31)}\).

![Figure(2): SDS-PAGE banding patterns of water soluble proteins in leaves of *Vicia faba* plants after 15 days from spraying with four concentrations of diprofos drug. {M: marker; C:control; 1-4: diprofos concentrations (0.50, 1, 2, 4ml/100ml water)}]
**Polymerase Chain Reaction (RAPD-PCR):**

RAPD profiles of genomic DNA from M₂ *Vicia faba* plants treated with 2ml/100 ml diprofos drug using 10 primers (A03, A18, A20, B12, B16, C11, C16, E18, G17 and G18) are shown in Figure, 3. RAPD-PCR reaction by the four primers (A03, A18, B16 and G17) didn't revealed any variation in DNA bands, but the other primers (A20, B12, C11, C16, G18 and E18) exhibited bands variation, whereas diprosos drug altered 23 DNA bands compared with the control (7 new bands appeared and 16 bands disappeared). Polymorphic bands of the six primers were scored as present (1) and absent (0) as indicated in Table (4).

**Table (4): RAPD profile alterations in DNA bands as detected with the six primers which gave polymorphic in M₂ treated Vicia faba plants with 2ml/100 ml diprofos drug comparing with the respective control.**

<table>
<thead>
<tr>
<th>Primer Code</th>
<th>Sequences(5’→3’)</th>
<th>Size of Polym. Bands(b.p.)</th>
<th>Control</th>
<th>Diprosos Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>OP-A20</td>
<td>GTTGCGATCC</td>
<td>2644 1608 1313 712</td>
<td>0 0 0 0</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td>OP-B12</td>
<td>CCTTGACGCA</td>
<td>1310 1295 1020 915 378</td>
<td>1 1 1 0</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>OP-C11</td>
<td>AAAGCTGCGG</td>
<td>1950 1830 1712 913 813</td>
<td>1 1 1 1</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>OP-C16</td>
<td>CACACTCCAG</td>
<td>1562 1355 688 214</td>
<td>1 1 0 0</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>OP-G18</td>
<td>GGCTCATGTG</td>
<td>592</td>
<td>1 0</td>
<td></td>
</tr>
<tr>
<td>OP-E18</td>
<td>GGACTGCAGA</td>
<td>1499 1328 1026 912</td>
<td>0 1 1 0</td>
<td></td>
</tr>
</tbody>
</table>

1: appearance of new bands, 0: disappearance of normal bands.
Diprofos treatment (2ml/100ml) induced the of 7 new polymorphic bands with molecular size of 2644, 1608, 1313, 712 bp by primer OP-A20; 915 bp by primer OP-B12; 214 bp by primer OP-C16 and 1499 bp by primer OP-E18 as compared with the control which lacked such bands (Table 4, Fig. 3).

On the other hand, this treatment caused disappearance of 16 polymorphic bands with molecular sizes of 1310, 1295, 1020, 378 bp by primer OP-B12; 1950, 1830, 1712, 913, 813 bp by primer OP-C11; 1562, 1355, 688 bp by primer OP-C16; 592 bp by primer OP-G18 and 1328, 1026, 912 bp by primer OP-E18 compared with the control, which these bands were present (Table 4; Fig. 3).

This results give good evidence to the ability of diprofos to induce variation may be mutation as a result of deletions compromising at least one nucleotide as revealed by the disappearance or appearance of many genetic bands as compared with control. Moreover, these results suggest that the RAPD technique is a useful biomarker assay to evaluate the genotoxic effects of diprofos drug on plant. These findings are in agreement with many researches\(^{(23;32-36)}\) who reported that the DNA polymorphism detected by RAPD analysis offered a useful biomarker assay to detect toxic chemicals genotoxicity in plant model systems.

In conclusion, RAPD-PCR was found to be more sensitive technique for detecting genetic alterations than SDS-PAGE protein profiles and can be used to detect genotoxic effects of pollutants. From this study, we concluded that diprofos drug have a cytogenotoxic effects and should be avoided if possible.
Figure (3): RAPD profiles of genomic DNA from M₂ *Vicia faba* plants treated with the 2ml/100m diprofos drug by using ten primers. (M: marker, C: control, D: diprofos drug); (Pr₁ → Pr₁₀ : primers).
REFERENCES


قياس التأثيرات السمية الخلوية الوراثية لعقار الديبروفوس على نبات الفول

أسماء أحمد محمود علي

المركز القومي لبحوث وتكنولوجيا الإشعاع – هيئة الطاقة الذرية – القاهرة - مصر

تم اختيار التأثيرات السمية الخلوية الوراثية لاربع تركيزات لعقار الديبروفوس (0.5, 1, 2, 4 مل/100 مل) مع ماء) على نبات الفول حيث تم تزويدها بالتركيزات السابقة بالإضافة إلى الرش بالماء (للمعينة الضابطة) لتوفير سلوك الإنقسام الميوزي بعد 15 يوم من الرش. كما تم تقدير بروتينات أوراق نبات الفول بعد 15 يوم من الرش بالتغليف الكهربائي للبروتينات SDS. بينما على نتائج التغليف الكهربائي للبروتين تم اختيار معالجة الديبروفوس (2/100 مل) لتقييم التغيير في الDNA لأوراق نبات الفول الهجين الثاني ؛ وذلك المعاملة وذلك بالإجراء تفاعل البليمرة المتسلسل (RAPD-PCR reaction) وكانت النتائج كالتالي:

- أظهرت المعاملات المختلفة زيادة عالية المعنوية بالنسبة الكلية للشذوذات الميوزية والتي تزيد بزيادة تركيز العقار.
- إنخفضت نسبة الشذوذات في الميوزي الثاني من مثلي تانى في المعاملة الأولى مما يدل على حدوث استشفاء بتقدم عمر الخلي.
- تركزت نسبة الشذوذات المختلفة في الطورين الإستوائي والإنسالي في كل الإنقسام الأول والثاني. وكذلك تركزت نسبة الشذوذات في اللوزة والتنية. كما ظهرت نتائج أخرى في بعض المعاملات بنسب قليلة كالكروكو. القنطرة والأدوية الصغرى التي ظهرت في بعض المعاملات بنسب قليلة.

- أظهرت نتائج التغليف الكهربائي للبروتينات لوراق نبات الفول أن معالمة الديبروفوس (4 مل/100 مل) أعطى خمس حزم بروتينية ذات وزن جزيئي (100, 80, 60, 50, 40) كيلو دالون مقارنة بالعينة الضابطة.
- إنخفضت نسبة الشذوذات في الفول والمعالمة بالتركيز (2/100 مل) لـ 23 حزمة DNA باستخدام RAPD-PCR (زيادة الشذوذات في نبات الفول). حيث أظهرت 16 حزمة DNA بينما ظهرت 7 حزمة DNA.
- ومن النتائج السابقة تؤكد السمية الخلوية الوراثية لعقار الديبروفوس ويجبر الحذر من تناول هذا المنتج إذا أمكن ذلك.