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Cytogenetic and Molecular Evaluation of Genetic effect of the Clomid Drug on *Vicia faba* Plant

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ABSTRACT

The cytogenetic effects of four clomid drug concentrations, i.e: (25, 50, 100 and 150 mg/100 ml water) were studied on *Vicia faba* plants. However, faba bean plants were sprayed with clomid concentrations at the flowering stage and meiotic division behavior was studied after 24, 48 hours and 15 days from spraying. SDS-protein electrophoresis was used to estimate leaves protein in the treated *Vicia faba* plants after 15 days of spraying. RAPD-PCR reaction was conducted in M_2 faba bean plants which were treated with the highest clomid concentration 150mg/100ml. All clomid treatments caused highly significant total meiotic abnormalities, which were increased as clomid concentrations increased in all period durations except for 150mg/100ml at 48 h. However, these abnormalities increased as period duration increased from 24 h to 48 h, but it decreased at 15 days period compared with 48 h at the three clomid concentrations (25, 50, 100 mg/100ml) as a result of recovery in this period. Similarly, meiotic 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. The most abnormalities were seen in metaphase and anaphase stages in the both two meiotic divisions. On the other hand, stickiness and disturbed chromosomes were the most dominant of abnormalities. In addition laggards chromosomes, bridges, fragments, breaks, micronuclei occurred but with very low percentages in some treatments. All clomid treatments increased intensity of four bands of SDS-protein electrophoresis with molecular weights: 40, 60, 90 and 150 KDa. The highest clomid concentration (150 mg /100ml) showed a polymorphic genetic bands by using RAPD-PCR product compared with control. The results of this experiment show that clomid drug have toxic effects

in the cytological and genetic levels and must be take this drug under control.

Keywords: Cytogenotoxic effects; SDS-PAGE analysis; RAPD-PCR reaction and polymorphic genetic bands.

INTRODUCTION

Clomiphene belongs to the triphenylethlene class compounds derived from the same stilbene nucleus as diethylstilbestrol, compounds of this class display a variety of estrogenic and anti estrogenic activities. It has an ovulation inducer, competitive inhibiting action on estrogen retro control at the level of hypothalamus causing an increase in the follicular stimulating hormone (FSH), resulting in follicle maturation, in turn increasing in luteinizing hormone (LH) peak which stimulates the ovulation and the formation of corpus leteum. Side effects of clomiphene may occur like; CNS disturbance include vertigo, insomnia and depression. There is a risk of multiple birth, with clomiphene which also may affect patients with liver diseases, endometrial carcinoma and ovarian cysts [(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 researches demonstrated that various drugs caused a cyto- and geno-toxicity effects in different biological systems. Where, docetaxel, vencristine, vinoorelbine, vinlastine and cyclophosphamide (anti carcinogenic agents) induced aneuploidy mutations, sex-linked recessive lethal mutations and increased chromosomal aberrations in *Drosophila melanogaster* and rat peripheral lymphocytes. Also, chemotherapy treatments with epirubicin, cisplatin and 5-fluorouracil for gastric cancer patients increased pattern in the frequency of chromosomal aberrations[(2-6)]. On the other hand, antiepileptic drugs such as; fenaton induced structural chromosomal aberrations, but diazebam caused numerical chromosomal aberrations (aneuploidy) and altered protein bands in mice-bone marrow cells and human-cell culture. However, phenoparpital caused cancer in various types of human cell cultures: liver, lung, brain, bone marrow and nervous cells[(7-9)]. Meanwhile, megazole (anti-chages disease drug) induced DNA damage in mice lymphocytes[(10)]. On the other side, anti bacterial and parasites drugs; nitroimialazol and arnadazol reduced mitotic activity and increased sister chromatid exchanges in human blood lymphocytes culture [(11;12)]. Also, antibiotics drugs; fluoroquinolones, tetracyclines and macrolides induced sister chromatid exchanges in mice germ cells. While,

tetracycline, amoxicillin and streptomycin caused mitotic and meiotic aberrations in *Pisum sativum* [13;14]. However, anti-inflammatory drugs; nimesulide and ibuprofen caused mitotic depression, induced structural and numerical chromosomal aberrations in mice bone marrow cells[15;16]. Similarly, acetyl salicylic acid and metamizole sodium reduced mitotic activity and increased micronuclei rate in *Allium cepa* plant[17]. Meanwhile, *Rhazya stricta* leaf aqueous extract (used as folkloric medicine for inflammatory conditions) caused mitotic index depression, induced chromosomal abnormalities, decreased total protein content and caused polymorphic DNA bands in *Allium cepa* root tip meristems[18]. However, chlomadion acetate (progesterone-like) induced chromosomal aberrations and sister chromatid exchanges in human lymphocytes *in vitro*[19]. The purpose of this study was to determine the genetic effect of clomid drug on *Vicia faba* plant using cytological and molecular genetics assays.

MATERIALS AND METHODS

MATERIALS

Biological material:

Vicia faba plant variety Giza 40 kindly provided by Crop Research Institute, Agricultural Research Center, Giza, Egypt was used for this study.

Tested durg clomid:

Drug which is produced by Patheon, France was tested on *Vicia faba* plant. The drug are presented in tablet form, each tablet containing 50 mg citrate clomiphene (chemical formula; $C_{26}H_{28}CIN$). The drug dosage used were; 1 table daily for 5 days, starting from the 5^{th} day of the menstrual cycle.

METHODS

1-Meiotic analysis

Vicia faba plants at the flowering stage were sprayed with four medical preparations of clomid drug which used as the recommended dose; 25, 50, 100 and 150 mg citrate clomiphene /100ml water. Control plants were sprayed with distilled water. Ten flower buds from ten different plants were gathered after 24, 48 hours and 15 days from spraying. For meiotic studies; the appropriate flower buds were collected and fixed in carnoy's solution (3 ethyl alcohol absolute: 1 glacial acetic acid) 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[20]. The data

recorded for different treatments were statistically analyzed using t-test to determine significant differences between these treatments.

2-Molecular analysis

a-SDS-PAGE protein analysis

SDS-protein was performed on vertical slab (20 cm x 20 cm x0.2 cm) using the gel electrophoresis apparatus (Manufactured by LABCONCO) according to Laemmli, 1970[21]. The fresh leaves were taken from *Vicia faba* plants after 15 days of spraying with four clomid concentrations and distilled water (control) were decocted 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 40 µl supernatant of SDS proteins were loaded in SDS-slab gel of 15% acrylamide containing 10%SDS . Gel was run at a current of 15 mA for 1 h. followed by 25 mA for 4-5 hours. Molecular weights of different bands were calibrated using the wide range protein marker ranged from 25-230 KDa according to Matta *et al*., 1981[22]. According to the electrophortic results, the treated *Vicia faba* plants with the highest clomid concentration (150 mg citrate clomiphene/100ml water) were selected to grow for seedling stage and then this treatment beside the control was planted to obtain the M_2 generation to conduct the RAPD-PCR analysis.

b-RAPD-PCR Analysis

****DNA extraction:***

Isolation of DNA from leaves in M_2 treated *Vicia faba* plants with the highest clomid concentration (150mg/100ml), the Protocol for DNA isolation from leaves was taken according to Doyle and Doyle,1990[23].

****Polymerase Chain Reaction (PCR):***

PCR reaction was conducted using Perkin Elmer (Germany) thermocycler. RAPD was carried out using ten random 10-*mer* primers (Operon Tech. Inc., USA) with the following sequences ($5'\rightarrow3'$) for RAPD analysis:

{OP-A03(AGTCAGCCAC); OP-A18(AGGTGACCGT);
OP-A20(GTTGCGATCC); OP-B10(CTGCTGGGAC);
OP-B14(TCCGCTCTGG); OP-C11(AAA GCTGC GG);
OP-C16(CACACTCCAG); OP-E18(GGACTGCAGA);
OP-G17(ACGACCGACA); OP-G18(GGCTCATGTG)}.

The reaction conditions were optimized and were mixtures consisted of the following: {dNTPs (2.5 mM)2.0 µl; Mg Cl_2 (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 ddH_2O-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^oC/4 min(1 cycle); 94^oC/1 min, 37^oC/1 min, 75^oC/2 min (38cycles); 72^oC/12min(1cycle), 4^oC(infinitive)}.

Agarose gel electrophoresis:

Agarose (1.2%) was used for resolving the RAPD-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 (20 cm X 20 cm). Bands were detected on UV-transilluminator and photographed by a Polaroid camera. Results were documented with Gel Doc 2000 (Bio RAD).

RESULTES AND DISCUSSION

1-Meiotic analysis

Results in Table (1) show that clomid significantly increased the percentage of abnormal PMC_s meiotic division at all treatments (p 0.01),where it ranged from 26.34% to 55.03% (5.06% in control).

Also, these abnormalities were increased as clomid concentration increased in each period duration except for 150 mg/100 ml at 48 h. However, the previous abnormalities increased as period duration increased from 24 h to 48 h, but it decreased at 15 days period as compared with 48 h at the three clomid concentrations (25, 50, 100 mg/100ml) as a result of recovery in this period. These results are in agreement with the results of many researchers[26,30].

Similarly, meiotic abnormalities percentage in the second division were lower than those recorded in the first division for all treatments as a result of recovery in this cell age, where it ranged from 15.91% - 52.69% and 33.47% - 59.02%, respectively (Table 1). Our results are in agreement with the results of many researchers, who found that meiotic abnormalities percentage in the second division were lower than those recorded in the first division after chemical treatments in mouse, faba bean and tomato plants[13,26,30].

Table (1): Percentages of abnormal PMCs in the 1st & 2nd meiotic divisions and total mean of meiotic abnormalities % in *Vicia faba* plants after (24,48) hours and 15 days from spraying with different concentration of clomid drug.

Time	Conc. mg/100 ml	Abnormal % in 1st division			Abnormal % in 2nd division			Total abnormal PMCs % in meiotic division		
		Divd cells No.	Abn cells No.	Abn. %	Divid. cells No.	Abn. cells No.	Abn. %	Divid cells No.	Abn. cells No.	Abn.PMC$_s$% ± S.E.
Control		268	18	6.72	206	6	2.91	474	24	5.06 ± 0.25
24 h.	25	245	82	33.47	294	60	20.41	539	142	26.34** ± 1,01
	50	282	108	38.30	257	72	28.02	539	180	32.39* ± 2.43
	100	306	146	47.71	292	118	40.41	598	264	44.14* ± 1.01
	150	346	162	46.82	256	112	43.75	602	274	45.51** ± 1.58
48 h.	25	364	152	41.76	225	78	34.67	589	230	39.04** ± 0.90
	50	311	144	46.30	249	100	40.16	560	244	43.57** ± 0.39
	100	240	136	56.67	353	186	52.69	593	322	54.30** ± 1.50
	150	246	132	53.66	196	92	46.94	442	224	50.67** ± 0.51
15 days	25	289	126	43.60	176	28	15.91	465	154	33.11** ± 0.54
	50	260	126	48.46	282	106	37.59	542	232	42.80** ± 0.73
	100	284	156	54.93	244	112	45.90	528	268	50.76** ± 1.25
	150	244	144	59.02	272	140	51.47	516	284	55.03** ± 1.46

PMC$_s$: Pollen mother cells. ** highly significant ($p < 0.01$)

On the other hand, results in Table (2) showed that the most meiotic abnormalities were present in metaphase and anaphase in both the first and the second meiotic divisions for all treatments. The induction of chromosomal abnormalities appear to be a common effect of many drugs in different biological systems[2-19]. Stickiness and disturbed chromosomes were the most dominant abnormalities in both the first and the second divisions, where they ranged from 18.00%-38.21%; 11.39%-20.77%; 5.08%-30,33%; 7.61%-13.33%, respectively. In addition, laggards, bridges, fragments, breaks and micronuclei occurred in meiotic division but with very low frequencies(Table 3).

Table (2): Abnormal meiotic phases % in *Vicia faba* plants after 24, 48 hours and 15 days from spraying with different concentrations of clomid drug.

Time	Conc. mg/l	First meiotic division			Second meiotic division		
		Abn.metaphase %	Abn.anaphase%	Abn.telo-phase %	Abn.metaphas %	Abn.anaphase %	Abn.telophase %
Control		3.73	2.99	-	0.97	1.94	-
24 h.	25	17.96	15.51	-	10.20	10.20	-
	50	17.73	17.73	2.84	17.90	10.12	-
	100	26.14	19.61	1.96	17.81	17.81	4.79
	150	20.23	23.70	2.89	22.44	18.75	2.56
48 h.	25	19.23	18.68	3.85	17.78	15.11	1.78
	50	21.22	21.22	3.86	17.66	19.28	3.22
	100	30.83	21.67	4.17	22.09	22.66	7.93
	150	30.08	19.51	4.07	19.39	19.39	8.16
15 days	25	21.45	15.92	6.23	9.09	6.82	-
	50	21.54	23.08	3.85	17.08	14.90	5.61
	100	26.76	22.54	5.63	17.21	19.67	9.02
	150	32.79	20.49	5.74	19.85	21.79	9.83

Table (3): Percentages of different meiotic abnormalities in the 1st and 2nd meiotic divisions in *Vicia faba* plants after 24, 48 hour and 15 days from spraying with different concentrations of clomid drug.

Time	Conc. mg/10 0ml	First meiotic division					Second meiotic division				
		Stickiness %	Disturbed ch.%	Laggards ch.%	Bridges %	Fragments & breaks%	Stickiness %	Disturbed ch.%	Laggards ch.%	Bridges %	Micronuclei%
Control		3.73	2.99	-	-	-	0.97	1.94	-	-	-
24 h.	25	180	14.66	0.82	-	-	12.24	7.61	0.51	-	-
	50	19.86	17.02	1.42	-	-	16.34	10.90	0.78	-	-
	100	32.72	13.71	1.31	-	-	26.71	11.64	2.05	-	-
	150	33.53	12.72	0.58	-	-	30.47	10.16	3.12	-	-
48 h.	25	24.18	14.55	3.03	-	-	18.67	13.33	1.78	-	0.89
	50	27.01	12.22	5.14	0.64	1.29	23.29	11.25	0.80	2.41	2.41
	100	35.00	17.50	2.50	1.67	-	30.81	11.72	3.93	2.83	3.40
	150	38.21	11.39	2.44	0.81	0.81	31.63	9.18	2.04	2.04	2.04
15 days	25	20.76	20.07	1.38	1.38	-	5.68	7.95	1.14	-	1.14
	50	21.54	20.77	4.62	1.54	-	18.43	12.77	1.42	2.13	2.84
	100	33.80	16.20	2.82	2.11	-	30.33	10.66	2.46	-	2.46
	150	37.70	18.85	0.82	1.64	-	31.62	9.56	1.47	2.94	5.88

Chromosomes stickiness % ranged between 18.00% - 38.21% and 5.68% - 31.63% in the first and the second meiotic divisions, respectively after clomid treatments showing that these abnormalities increased as clomid concentration increased in all treatments in both two meiotic divisions. Moreover, the pervious abnormalities recorded in the second division were lower than those recorded in the first division as a result of recovery in this cell age. Stickiness appeared in metaphase, anaphase and telophase in the two

meiotic divisions (Fig.1:A, B, C, D, I, L, R & S). Our results are in agreement with the results of many researchers[24-28], who suggested that the chromosomes stickiness may result from breakage and exchange between chromatin fibers on the surface of adjoining chromosomes.

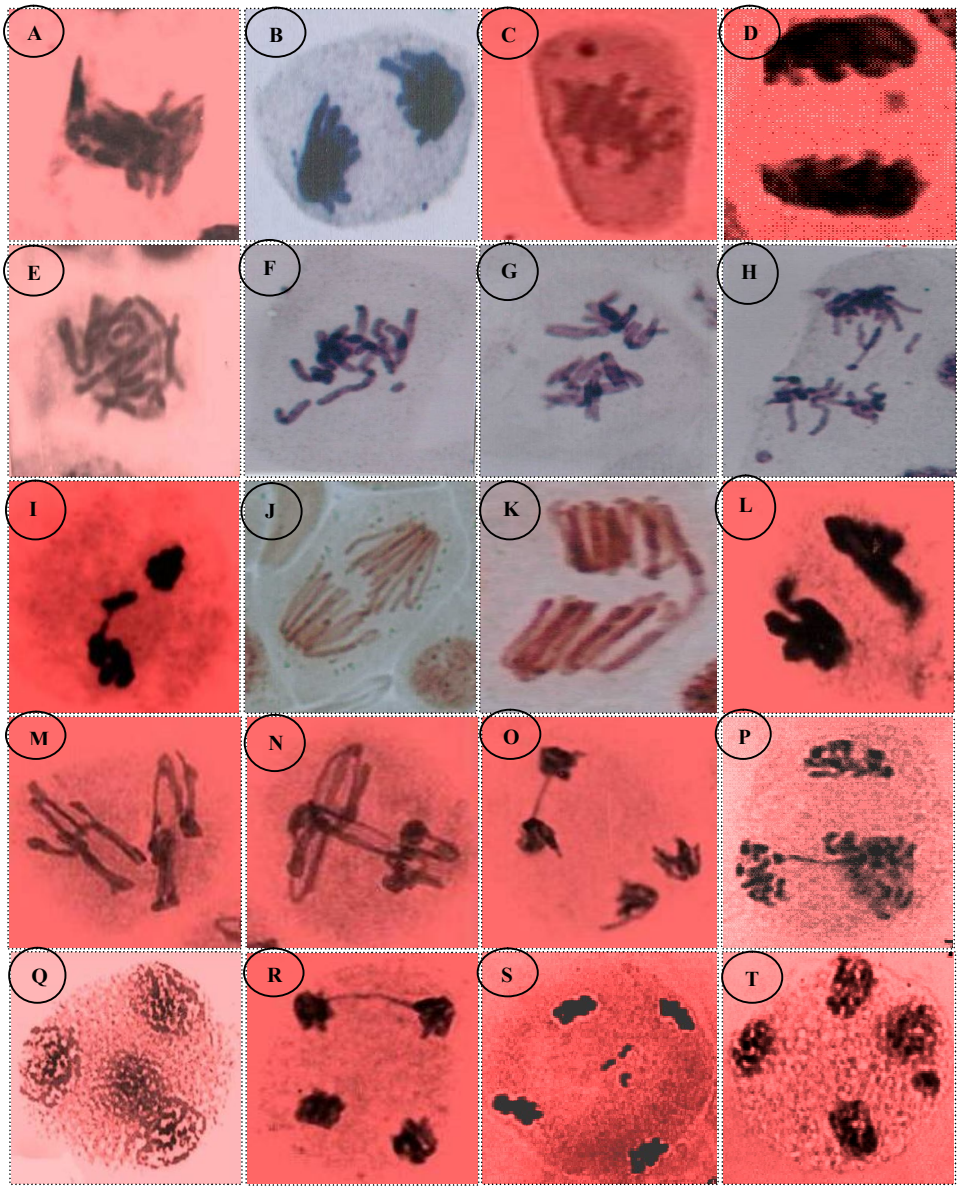
The second type of abnormalities is the disturbed chromosomes, which ranged from 11.39% - 20.77% and 7.61% - 13.33% in the first and the second meiotic divisions, respectively, after clomid treatments indicated the reduction of this abnormality in the second division than those recorded in the first division as a result of recovery in this cell age (Table 3).

This abnormality was shown in metaphase, anaphase in the two meiotic divisions and telophase only in the second division (Fig.1: E, F, G, H, K, M, N, P & Q). Disturbed chromosomes may be due to disturbance of spindle apparatus which allows the chromosomes to spread irregularly over the cell[29-31]. Laggards chromosomes was observed in all clomid treatments with low percentages ranging between 0.58% - 5.14% and 0.51% - 3.93% in the first and the second meiotic divisions, respectively. Whereas, laggards were present in metaphase in the two meiotic divisions (Fig.1: F & I) and could be attributed to the failure of spindle apparatus to organize and function in a normal way[32]. These laggards may be distributed randomly to either poles at anaphase or telophase in the first and the second divisions (Fig.1: H, I & S), and they may give micronuclei which were observed at telophase II at 48 h and 15 days periods for all clomid concentrations (Fig.1: T).

While bridges were shown at the obvious periods in some clomid concentrations, but fragments and breaks were observed only in 48 h period at clomid concentrations (50 & 150 mg/100ml). However, the last abnormalities were recorded with low percentages (Table 3).

Fragments and breaks appeared in metaphase I and anaphase I (Fig.1: C, D, F, & H). While, bridges were observed in anaphase I & II; metaphase II and telophase II (Fig.1: J, K, N, O,P & R). Bridges could be due to the breakage and reunion[32] or due to the general stickiness of chromosomes[30].

Finally, the induction of these chromosomal abnormalities were pointed to the cytotoxic effects potentiality of the applied concentrations of clomid drug.

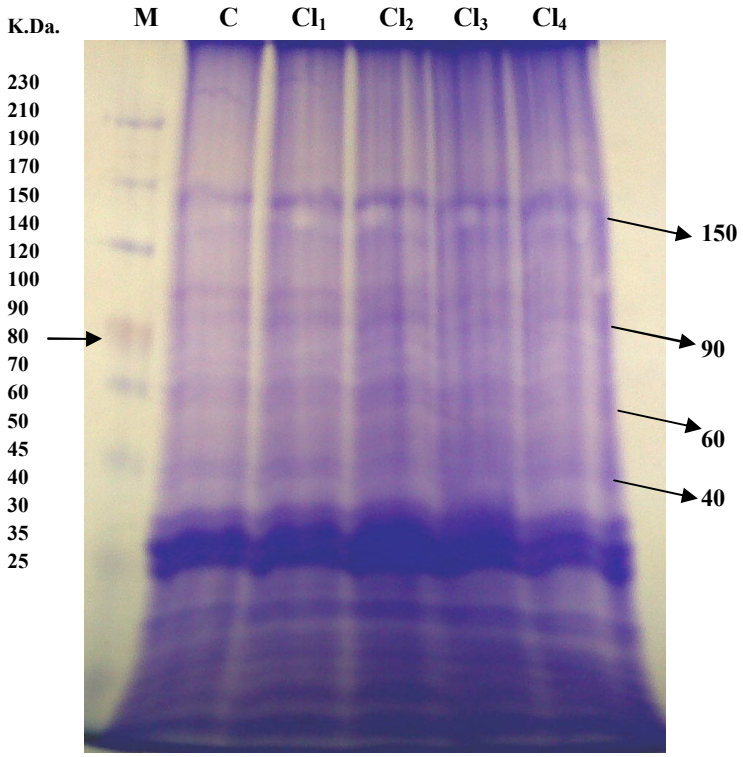


Figure(1):Different meiotic abnormalities produced after (24,48)h. and 15 days from spraying *Vicia faba* plants with four concentrations of clomid drug (A: stickiness at M_1, B: stickiness at A_1, C: stickiness and fragment at M_1, D: stickiness and fragment at A, E: disturbed at M_1, F: disturbed, fragment and laggard at M_1, G: disturbed at A_1, H: disturbed, fragment,break and laggard at A_1, I: stickiness and laggard and at A_1, J: bridge at A_1, K: disturbed and bridge at A_1, L: stickiness at M_2, M: disturbed at M_2, N: disturbed and bridge at M_2, O: bridge at A_2, P: disturbed and bridge at T_2, Q: disturbed at T_2, R: bridge at T_2, S: laggard at T_2, T: micronuclei at T_2). M_1,A_1,$T_{1::}$First(meta,ana, telo)phase; M_2.A_2,T_2:Second (meta,ana,telo)phase.

2-Molecular genetic studies

A-SDS-protein electrophoresis

Figure (2) shows SDS-PAGE banding patterns of SDS-proteins in *Vicia faba* leaves after 15 days from spraying plants with different concentrations of clomid drug. All clomid treatments increased intensity of four bands with molecular weights of: 40, 60, 90 and 150 KDa. Alteration in bands intensity could be attributed to change in the structure or performance of genes and thus they produce of changes in the gene expression of the regulatory genes used in the regulatory system of structural genes[7,18]. The increase in band(s) intensity could be attributed to gene(s) duplication which might result from cytological abnormalities induced by clomid drug. The presence of laggards chromosomes and bridges supported this conclusion which agreed with many researches[26-28].



Figure(2): SDS-PAGE banding patterns of water soluble proteins in *Vicia faba* leaves after 15 days from spraying plants with different concentrations of clomid drug.{ M: mrker ;C: control; Cl_1- Cl_4 : clomid concentrations (25, 50, 100, 150 mg/100ml water) }

B-RAPD-PCR-analysis:

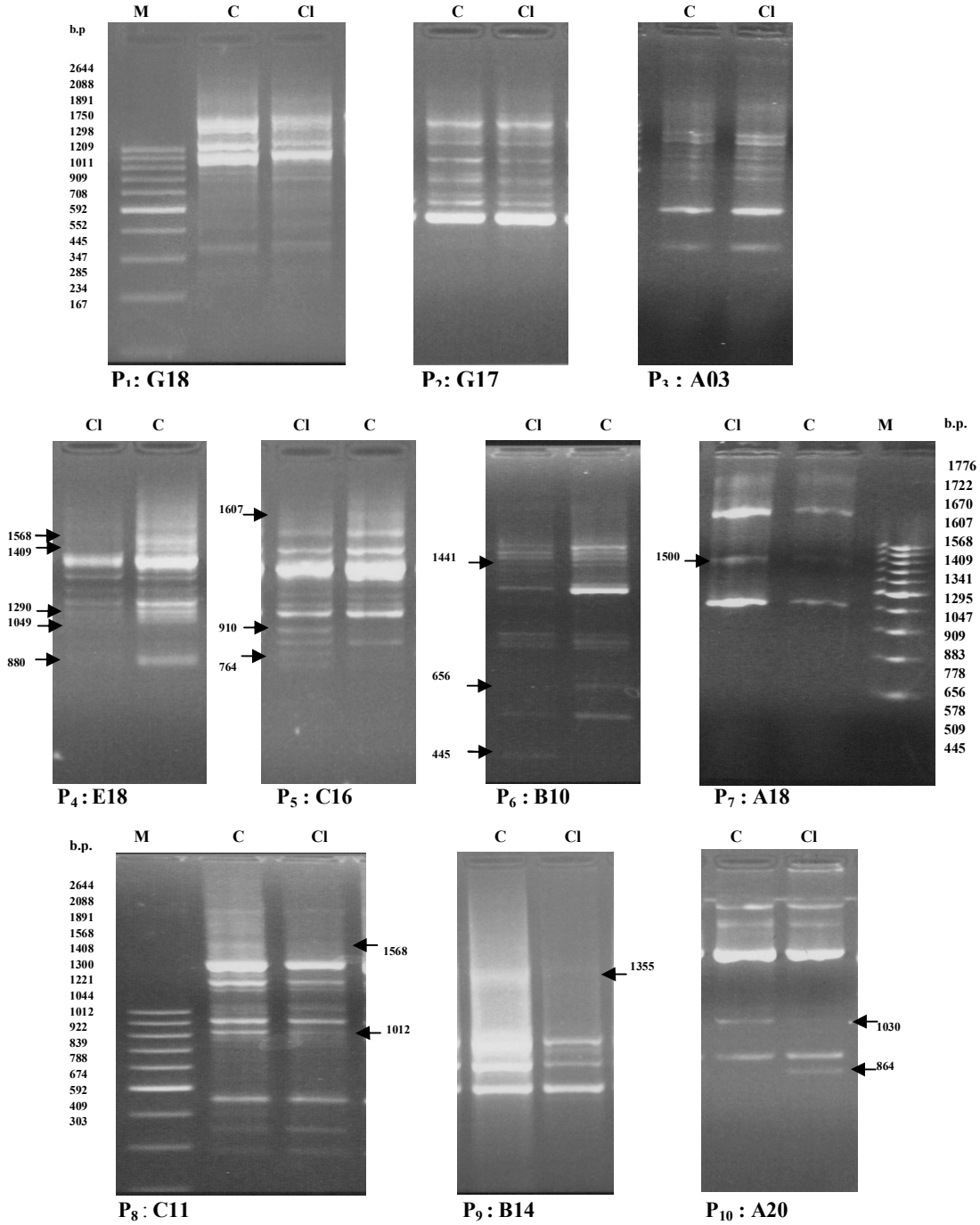
Figure (3) shows a RAPD profiles of genomic DNA from M_2 *Vicia faba* plants treated with the highest concentration of clomid drug (150 mg/100ml) using ten primers (OP-A03, OP- A18, OP-A20, OP-B10, OP-B14, OP-C11, OP-C16, OP-E18, OP-G17 & OP-G18). RAPD-PCR reaction by of these three primers (OP-A03, OP-G17 & OP-G18) didn't reveal any variation in DNA bands while the other seven primers exhibited variation in DNA bands, whereas clomid treatment altered the 17 DNA bands compared with the control (appearance of 5 new DNA bands and disappearance of 12 DNA bands). The polymorphic bands of the seven 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 7-primers in M_2 treated *Vicia faba* plants with 150mg/100ml clomid drug in compared with the respective control

Primer Code	Sequences $5^{l} \rightarrow 3^{l}$	Size of polym.bands(bp)	Control	Clomid treatment
OP-A18	**AGGTGACCGT**	**1500**	**0**	**1**
OP-B10	**CTGCTGGGAC**	**1441**	**1**	**0**
		656	**1**	**0**
		445	**0**	**1**
OP-C16	**CACACTCCAG**	**1607**	**1**	**0**
		910	**0**	**1**
		764	**0**	**1**
OP-E18	**GGACTGCAGA**	**1508**	**1**	**0**
		1409	**1**	**0**
		1290	**1**	**0**
		1049	**1**	**0**
		880	**1**	**0**
OP-C11	**AAAGCTGCGG**	**1508**	**1**	**0**
		1012	**1**	**0**
OP-B14	**TCCGCTCTGG**	**1355**	**1**	**0**
OP-A20	**GTTGCGATCC**	**1830**	**1**	**0**
		864	**0**	**1**

Clomid treatment (150mg/100ml) induced five new polymorphic bands with molecular sizes of: 1500 bp (OP-A18); 445 bp (OP-B10); 864 bp (OP-A20) and 910,764 bp (OP-C16). On the other hand, this clomid treatment caused disappearance of twelve polymorphic bands with molecular sizes of :1441, 656 bp (OP-B10); 1607 bp (OP-C16); 1508, 1409, 1290, 1049, 880 bp (OP-E18); 1508, 1012 bp (OP-C11); 1335 bp (OP-B14) and 1830 bp (OP-A20)

as compared with the control (Table 4, Fig. 3).



Figure(3): RAPD profiles of genomic DNA from M_2 *Vicia faba* plants treated with 150mg/100ml clomid drug by using ten primers. { M: marker ; C: control; Cl: clomid drug ;($P_1 \rightarrow P_{10}$) : primers }.

This observation gives good evidence to the ability of clomid drug (150 mg/100 ml) to induce insertion or mutations as a result of deletion compromises at least few nucleotides as revealed by the appearance or disappearance of many bands as compared with the control[18]. 150 mg/100 ml clomid treatment may act as intercalation agent or generates free radicals which interact with DNA to account for the observed variation. These are in agreement with many researches[33-37]. From cytological and molecular results, it could be concluded that clomid drug have a cytogenotoxic effects and should be recommend to take this drug under control.

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مجلة البحوث الإشعاعية والعلوم التطبيقية

مجلد ٥ عدد ٦ ص ص ١١٥٣ – ١١٦٩ (٢٠١٢)

التقديرات الخلوية الوراثية والجزيئية لتأثير عقار الكلوميد على نبات الفول

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تم إختبار السمية الخلوية الوراثية لأربع تركيزات لعقار الكلوميد (٢٥, ٥٠, ١٠٠, ١٥٠ ملليجم/١٠٠مل ماء مقطر) على نبات الفول حيث تم رش نباتات الفول (جيزة 40) في مرحلة الازهار بالتركيزات السابقة بالاضافة للرش بالماء للعينة الضابطة لدراسة سلوك الإنقسام الميوزي بعد 24, 48 ساعة و15 يوم من الرش ,كما تم تحليل بروتينات أوراق نباتات الفول بعد 15 يوم من الرش بالتفريد الكهربي للبروتين SDS-Protein Electrophoresis وبناءا على نتائج التفريد الكهربى للبروتين تم إختيار أعلى تركيز للكلوميد (١٥٠ملليجم/١٠٠ماء) لتقدير التغيير في ال DNA لأوراق نباتات الفول للجيل الثاني لتلك المعاملة وذلك بإجراء تفاعل البلمرة المتسلسل لبادئات عشوائية للتعدد المظهرى للمادة الوراثية DNA (RAPD - PCR reaction)وكانت النتائج كالتالي-:

١- أحدثت جميع المعاملات شذوذات ميوزية وكانت نسبتها عالية المعنوية والتى تزيد بزيادة تركير العقار، كما زادت نسبة تلك الشذوذات بأطالة فترة التعريض ، بينما انخفضت النسبة بعد فترة التعريض ١٥ يوم مقارنة بالفترة ٤٨ ساعة مما يدل على حدوث إستشفاء بتقدم عمر النبات .

٢- سجلت الشذوذات الميوزية نسبا أقل بالانقسام الميوزى الثاني عن مثيلتها بالانقسام الميوزي الأول مما يدل على حدوث إستشفاء بتقدم عمر الخلية.

٣- سجلت اغلب الشذوذات المختلفة في الطورين الإستوائي والإنفصالي في كلا الإنقسامين الميوزى الأول والثاني , وكانت اكثر الشذوذات شيوعا هي اللزوجة والتبعثر, كما ظهرت شذوذات اخرى في بعض المعاملات بنسب قليلة كالتلكؤ، الشظايا ,الكسور , القناطر و الأنوية الصغيرة التي ظهرت في بعض المعاملات بنسب قليلة.

٤ - أدت جميع معاملات الكلوميد لزيادة كثافة بعض الحزم البروتينة ذات وزن جزيئي 40, 60, 90 & 150 كيلودالتون مقارنة بالعينة الضابطة.

٥- إظهرت نتائج تفاعل البلمرة المتسلسل لنباتات الجيل الثاني للفول والمعاملة باعلى تركيز لعقار الكلوميد ١٥٠ ملليجم/١٠٠مل لتغير17حزمة DNA حيث أختفت 12 حزمة DNA بينما ظهرت 5 حزم DNA جديدة.

*ومن النتائج السابقة يتضح أن عقار الكلوميد يمتلك السمية الخلوية الوراثية ويجب الحذر من تناوله.