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Chromosomal changes linked with the effect of high dose of aluminum on faba bean (*Vicia faba* L.) root tips

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Abstract. In this work, we cytogenetically described the effects of different relatively high doses of aluminum (5, 15 and 25 mM $AlCl_3$) on mitotic activity and DNA integrity of faba bean (Misr 3 cultivar). Under Al stress, mitotic index (MI) decreased but total chromosomal abnormalities increased significantly compared to that of control. In addition, the detected chromosomal abnormalities in each mitotic phase increased significantly in comparison to that of control. All the used concentrations of Al enhanced micronuclei formation; no correlation could be detected between the size or number of micronuclei/cell and the applied conditions. Metaphase was the most sensitive stage to Al stress compared to the other stages of mitosis; C-metaphase was the common abnormalities and it increased strongly when the exposure time was more than 6 h. Under the influence of Al stress for 24 h, the appearance C-metaphase in high frequency decreased the frequency of appearance of other forms of abnormalities during metaphase or ana-telophase. The previous cytological events created alteration either at or between the primer binding sites which could be detected by RAPD and ISSR techniques. Application of ten RAPD primers resulted in amplification of 59 fragments including 20 monomorphic, seven unique and 32 polymorphic bands with polymorphism average of 60.09%. ISSR primers amplified 75 DNA fragments including 18 monomorphic, eight unique and 45 polymorphic bands with polymorphism average of 72.90%. These data indicated that faba bean cultivar suffered from harmful effect of Al on its genome when the duration of Al treatment was more than 6 hr. ISSR was better than RAPD to study genome stability of faba bean under abiotic stress agent.

Keywords: aluminum stress, chromosomal changes, molecular techniques, cytogenetic effect, *Vicia faba*.

INTRODUCTION

In several environments, soils are contained with metals in different concentrations. They resulted in beneficial or toxic effects on biological sys-

tems that inhabit their environments; aluminum (Al) is one of those widespread metals. In nature, the highest amount of Al is present in un-soluble form as aluminum silicate. While soluble Al form is present in a small amount, it finds a way to get from soil, water and air to biological systems (May and Nordstrom 1991) leading to positive effects on cultivated plants (Foy 1983; Rout et al. 2001). On the other side, transfer of Al from soil to plants in relatively high dose resulted in catastrophic effects and they vary depending on plant species especially under low pH (5.5) of the soil (Rout et al. 2001).

In root tips, Aluminum intervenes with cell division, increases cell wall rigidity, increases the rigidity DNA double helix and reduces DNA replication (Foy 1992). Authors were also reported that Al (0.2–1.0 mM) inhibits cell division due to severe inhibition of DNA synthesis within 16 –24 h (Minocha et al. 1992). While mitotic indices decreased, anaphase chromosome aberrations increased when faba bean root tips of faba ben exposed to different doses of Al (0.01–10 mM) for 12 h (Yi et al. 2010). Under Al stress, reduction in mitotic activity in several plant species was reported (Rout et al. 2001; Silva 2012). Several studies indicated that Al toxicity increased micronuclei formation, chromosomal abnormalities and sister chromatid exchanges (Lima et al. 2007; Yi et al. 2010).

Genotoxic effects of stress agents were associated with chromosome aberration, micronucleus formation and chromosomal recombination (Achary and Panda 2010). Genotoxicity and DNA destroy can be evaluated by cytogenetical or molecular techniques. RAPD and ISSR used as reliable molecular tools to detect DNA variation, damage and mutational events in cells of animals, microorganisms and plants (Liu et al. 2005, 2009; Salem and Hassanein 2017; Hassanein et al. 2018). They depend on the fact that the primer is joined with complementary DNA sequence on opposite DNA strands of the studied genome. Under the influence of stress agent, primer binding sites vary under the influence of induced mutation and DNA damage (Gupta and Sarin 2009; Achary and panda 2010) leading to amplification of different DNA fragments expressing different polymorphism values. The value of polymorphism gives a clear indication of the range to which the genome is affected by the factor in question. Then, chromosomal destroy can be revealed by cytogenetical or molecular techniques.

Vicia faba belongs to Fabaceae family and used as human food and animal feed. In addition, bean and other legumes are used to improve the fertility of soil through nitrogen fixation. Aluminum has clear genotoxic and cytotoxic effects on cells of faba bean root tips (Yi et al. 2010). Consequently, faba bean root tips were used as a model plant to study the cytogenetic effects of *Dipterygium glaucum* extracts (Altwaty et al. 2016), herbicides and other materials on mitotic activity (El-Rokiek et al. 2015; Shafeek et al. 2016; Prabhu et al. 2017). Exposing the faba bean plant to pollutants in various concentrations is considered as a prominent test to determine the genotoxicity (Foltête et al. 2011; Prabhu et al. 2017). Prominent study reported that application of Al in low concentration induced adaptive response that led to genomic protection from genotoxic effects of Al or other materials (Achary and Panda 2010).

Under the influence of Al stress, inhibition the growth of plant roots was described as the most common symptoms exhibited by higher plants (Rout et al. 2001; Hassanein et al. 2020a). While several cytogenetical studies were explained the effects of Al toxicity on root tips, clear view still needs further studies using model plants such as faba bean. In addition, molecular phenomenon that underlies cytogenetical events are still not fully understood. In this work, we described the effects of application of relatively high doses of Al for different exposure periods on mitotic activity and DNA integrity of faba bean root tips using cytogenetical and molecular approaches.

MATERIAL AND METHODS

Plant materials

Seeds of *Vicia faba* cultivar (Misr 3) were obtained from the Agriculture Research and Seeds Center in Qena, Egypt. Seeds were germinated in distilled H_2O for two to three days to get roots with 2-3 cm long. The obtained roots were treated in solutions containing different concentrations of $AlCl_3$ (5, 15, 25 mM) for different periods (0, 6, 12 and 24 h).

Cytogenetical analysis procedure

Ten root tips of Al treated seedlings were cut and placed 12 h in Carnoy's fixation solution containing ethanol and acetic acid, glacial (3:1 ratio). The cut seedling root tips were kept in 70% ethanol under dark condition at 4°C, hydrolyzed in 1 N HCl (Darlington and La Cour 1976) and subjected to the Feulgen squash technique. Total mitotic, mitotic indices, the frequency of mitotic stages and mitotic chromosomal abnormalities were determined according to the detailed formulas in Hassanein et al. (2020b).

Table 1. The applied RAPD and ISSR primers.

Primer type	Primer Sequence (5'---------3')	Primer type	Primer Sequence (5'---------3')
RAPD		**ISSR**	
OPA-02	TGCCGAGCTG	ISSR1	ACACACACACACACACCTG
OPA-05	AGGGGTCTTG	ISSR2	CACACACACACACACAAAGCT
OPA-07	GAAACGGGTG	ISSR3	ACACACACACACACACAAG
OPA-17	GACCGCTTGT	ISSR4	GAGAGAGAGAGAGAGACTG
OPat-08	TCCTCGTGGG	ISSR5	GAGAGAGAGAGAGAGACTC
OPaw-10	GGTGTTTGCC	ISSR7	CTCTCTCTCTCTA (CT)6A
OPD-1	ACCGCGAAGG	ISSR8	TCTTCTTCTTCTG
OPD-18	GAGAGCCAAC	ISSR9	TGTTGTTGTGC
OPJ-15	TGTAGCAGGG	ISSR10	GTGGTGGTGGC
OPP-13	GGAGTGCCTC		

Genomic DNA extraction

Roots of 2-3 seedlings were treated with 5 mM Al for different periods (0, 6, 12 and 24 h) and subjected for genome analysis to investigate how the genome of faba bean was influenced by Al toxicity. Genomic DNA extraction was extracted from root tips and analyzed using RAPD and ISSR techniques according to Porebski et al. (1997).

PCR conditions

A total of ten RAPD and nine ISSR primers (Table 1) were used to detect induced genetic variation in faba bean roots under the influence of 5 mM Al for different periods (6, 12 and 24 hr). Genomic DNA amplification was fulfilled in a DNA Thermal Cycler (Biometra TPersonal Combi, Biometra GmbH, Germany). Application of RAPD and ISSR primers were carried out in a 25 µl PCR mixture solution containing 12.5 µl of Go Taq® Green Master Mix (Promega, Madison, USA), 3 µl of primer 10 pmol, 6.5 µl of free nuclease water and 3 µl of 100 ng genomic DNA templates. Then, PCR run for DNA amplification was started using initial denaturation cycle at 94°C for 5 min. Then, 40 cycles were carried out using denaturation (94°C for 45 sec), annealing step (optimized for each primer), and elongation (72°C for 1 min) steps. Extension step was used to finalize the amplification process at 72°C for 7 min.

Reproducibility

Reproducibility was taken in consideration to minimize personal errors. In this concern, each primer was used three times under the same PCR conditions.

Detection of PCR products

The obtained amplification products of each PCR run were electrophoresed in a 1.5% or 2% agarose gel containing 0.5 µg/ml ethidium bromide in 1X TBE. Run was carried out in run buffer at 70 volts. Then, the amplified PCR products were visualized, photographed and analyzed.

Data Analysis

The experimental data were statistically analyzed by ANOVA. Data were compared using the least significant difference (LSD) test at 5% (*) and 1% (**) levels (Snedecor and Cochran 1980). Also, dendrograms were generated for cluster analysis according to Legendre and Legendre (1983) using the Community Analysis Package Software Program (CAP) Version 4.0 (Richard and Peter 2007).

RESULTS

Under the influence of all $AlCl_3$ treatments, the decrease in MI and increase of total abnormalities were found to be statistically highly significant compared to that of control (Table 2). Irrespective the concentration of Al, the value of MI was gradually decreased as the exposure time increased. Roots treated with the highest concentration of $AlCl_3$ (25 mM) for 24 hr showed the highest inhibition of cell division and it was associated with the highest total abnormalities. In addition, the increase in interphase with increase of the concentrations of $AlCl_3$ and exposure time was appeared at all Al treatments.

Table 2. Mitotic index (MI), % of total abnormalities, % of interphase and % of mitotic phases (prophase, metaphase and ana-telophase), include normal (Total) and abnormal (Abn) mitotic phases recorded for *Vicia faba* (Misr 3) root tips under the influence of different concentrations of $AlCl_3$ (5, 15 and 25 mM) and exposure times (6, 12 and 24 hr).

Treatment		Total mitosis	Mitotic Index (MI)	% Total abnormal	% Interphase		% Prophase		% Metaphase		% Ana-telophase	
Exposure time	$AlCl_3$ conc.				Total	Abn.	Total	Abn.	Total	Abn.	Total	Abn.
6hr.	0 mM	355	7.10±0.36	4.09±0.42	92.9	0.4	37.6	0.4	24.7	1.4	42.2	-
	5 mM	121	2.40±0.10**	83.93±6.41**	97.5	2.33	70.5	65.8	10.6	10.6	16.5	10.5
	15 mM	99	1.95±0.15**	76.67±6.65**	98.0	1.37	66.6	62.8	7.7	7.7	25.6	7.7
	25 mM	126	2.50±0.10**	65.15±11.05**	97.4	2.6	36.2	25.8	15.5	13.8	44.8	22.4
12hr.	5 mM	79	1.60±0.10**	92.60±1.30**	98.4	0.79	51.7	48.2	39.3	39.2	8.9	5.4
	15 mM	64	1.26±0.05**	89.30±10.70**	98.7	2.5	56.5	43.4	30.4	30.4	13.04	13.1
	25 mM	47	0.93±0.15**	95.70±0.10**	99.0	1.26	29.8	29.7	61.7	59.5	8.5	6.4
24hr.	5 mM	51	1.00±0.10**	88.13±8.15**	98.9	0.71	61.7	55.3	38.2	38.3	-	-
	15 mM	86	1.70±0.00**	80.20±9.30**	98.2	0.76	76	50	26.0	26.0	8.0	2.0
	25 mM	37	0.75±0.05**	97.90±2.10**	99.2	1.11	30.6	27.8	69.4	69.4	-	-

**. The mean difference is significant at the 0.05 level.

Table 3. Types and frequency of chromosomal abnormalities [C-metaphase (C-m), sticky (Stick), star, break, disturbed (Dist), and diagonal (Diag), bridge, free and C-anaphase (C-ana)] recorded for *Vicia faba* (Misr 3) root tips under the influence of different concentrations of $AlCl_3$ and exposure times (6, 12, and 24 hr).

Treatment		Interphase abnormalities		Metaphase abnormalities					Ana-telophase abnormalities							
Exposure time	$AlCl_3$ conc.	Micro	Bi nuclei	C-m	Stick	Star	Break	Dist	Stick	Bridg	Dist	Diag	free	break	Star	C-ana
6hr.	0 mM	-	-	-	1.3	-	-	-	-	-	-	-	-	-	-	-
	5 mM	1.76	0.37	4.7	-	2.4	2.4	1.2	1.2	2.4	3.5	1.3	2.4	1.2	-	-
	15 mM	0.79	0.28	2.6	-	1.3	-	3.8	-	-	1.3	-	-	1.3	2.6	1.3
	25 mM	0.30	0.13	3.4	-	1.7	3.4	5.2	6.9	-	10.3	-	1.7	3.4	-	-
12hr.	5 mM	1.09	-	39.3	-	-	-	-	-	-	-	-	-	-	1.8	3.6
	15 mM	2.53	-	26.1	-	-	-	4.3	8.7	4.3	-	4.2	-	-	-	-
	25 mM	0.31	0.04	57.4	-	-	2.1	-	-	-	-	-	-	-	2.1	-
24hr.	5 mM	2.39	0.41	38.3	-	-	-	-	-	-	-	-	-	-	-	-
	15 mM	1.22	0.45	18	-	-	-	-	-	-	-	-	-	-	2	-
	25 mM	1.05	0.06	66.7	-	2.8	-	-	-	-	-	-	-	-	-	-

The relative frequencies of different mitotic phases were affected by $AlCl_3$ treatments (Table 2). Variations in these frequencies appeared to be dependent on exposure time and concentrations of the applied $AlCl_3$ concentrations. Prophase frequency and prophase abnormalities (Fig. 1:4) usually increased as the concentration of $AlCl_3$ increased and the exposure time prolonged up to 15 mM $AlCl_3$. Prophase minimum values of 36.2, 29.8, and 30.6% were observed when root tips were subjected to 25 mM $AlCl_3$ for 6, 12 and 24 h, respectively. Metaphase frequency mostly increased as the concentration of $AlCl_3$ increased and exposure time was prolonged for more than 6 h; it was associated with increase in metaphase abnormalities. Maximum value of metaphase frequency (69.4%) was detected when plant root tips were subjected to 25 mM $AlCl_3$ for 24 hr. When plant root tips were subjected to $AlCl_3$ toxicity, the registered values of ana-telophase frequency were generally lower than that of control. Under the influence of 5 or 25 mM $AlCl_3$ for 24 h a complete inhibition in ana-telophase stage

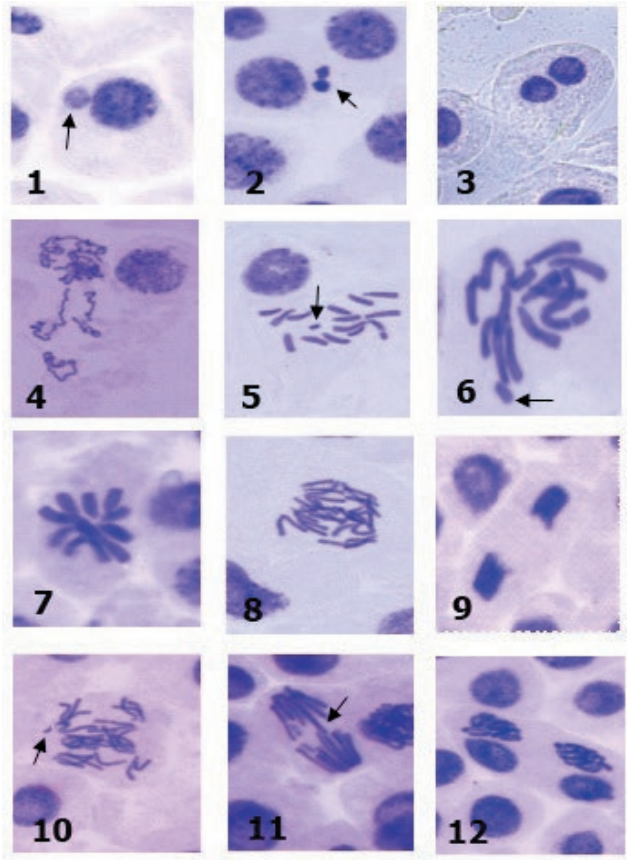

Figure 1. Chromosomal aberrations of Misr 3 cultivar root tips under the influence of $AlCl_3$ for different periods (6, 12, and 24 hr); (1, 2) micronuclei formation during interphase stage (arrows), (3) binucleated interphase, (4) irregular prophase, (5) C-metaphase with breaks (arrow), (6) disturbed metaphase, (7) star metaphase, (8) disturbed anaphase, (9) sticky anaphase, (10) breaks in anaphase (arrow), (11) bridge anaphase (arrow) and (12) star shape anaphase.

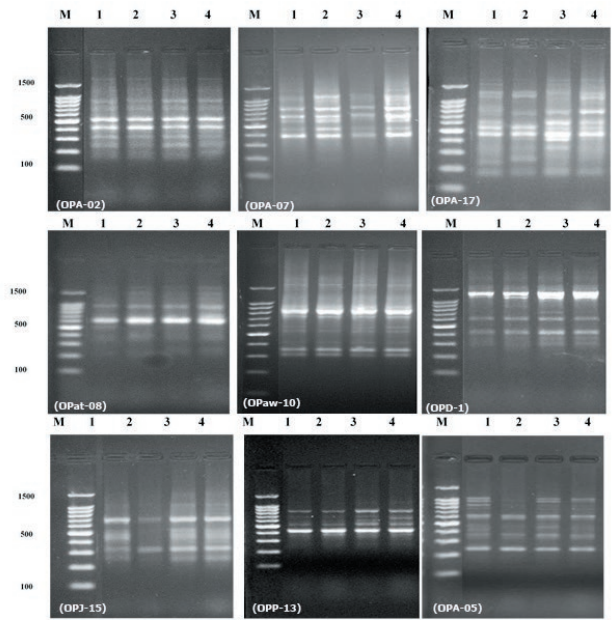

Figure 2. RAPD profile generated by 9 primers using roots of different *Vicia faba* Misr 3 cultivar subjected for different periods in 5 mM $AlCl_3$. Lanes1, 2, 3 and 4: 0.0, 6, 12 and 24 hr, respectively. M: DNA ladder.

was detected. The detected abnormalities in each mitotic phase were higher than that of control.

All the used concentrations of $AlCl_3$ enhanced micronuclei formation in the root tips (Table 3 and Fig. 1). The maximum value of micronuclei frequency (2.53%) was detected when root tips were subjected to 15 mM for 12 hr. Under all the applied conditions, one (Fig. 1: 1), or two (Fig. 1: 2) micronuclei/cell were detected. There was no correlation between the number of micronuclei/ cell and the applied concentration of $AlCl_3$ or the exposure time. Also, binucleated cells were detected under the applied conditions (Fig. 1: 3).

The type and frequency of chromosomal abnormalities that resulted from $AlCl_3$ treatments on faba bean (Misr 3) roots were included in Table 3 and illustrated in Fig.1. Metaphase was the most sensitive stage to Al stress compared to the other stages of mitosis, where the abnormalities were detected at all treatments (Table 3). C-metaphase (Fig. 1: 5) was the common abnormalities and their appearance was independent on Al concentration but it increased strongly when the exposure time was more than 6 h. In addition, during this stage, abnormalities including star shape (Fig. 1: 7), chromosomes breaks (Fig. 1: 5, 6) and disturbed configurations (Fig. 1: 6) were detected. Under the influence of the used high doses of Al for the longest period of exposure (24 h), MI was drastically lowered but total abnormalities were increased and it was associated with drastic increase in C-metaphase. The concentration of most of the chromosomal abnormalities of the metaphase stage in one form of alteration (C-metaphase) made the appearance of other forms of abnormalities rare during metaphase or telo-anaphase.

Ana-telophase chromosomal abnormalities including chromosomal bridges (Fig. 1: 11), chromosomal breaks (Fig. 1: 10), disturbed anaphase (Fig. 1: 8) and sticky anaphase (Fig. 1: 9) were detected. When plant root tips were subjected to the relatively high concentration of $AlCl_3$ (15 mM) for relatively long period (24 h), star shape chromosome was detected (Fig. 1: 12) but other types of abnormalities were not registered during anatelophase stage. The same results were obtained when plant root tips were treated with 25 mM $AlCl_3$ for 12 hr. Under different Al treatments, star chromosome was

Table 4. Ten RAPD primers, their sequences, annealing temperature, size of amplified fragments (bp), total number of amplified fragments, number of polymorphic bands and unique bands identified per primer used to access genome stability of *Vicia faba* Misr 3 cultivar under the influence of Al stress.

Primer	Sequence (5'→3')	Annealing temperature (°C)	Polymorphic bands	Monomorphic bands	Unique bands	Total bands	Size range (bp)	Polymorphism (%)
OPA-02	TGCCGAGCTG	32	4	2	1	7	141-753	71.43
OPA-05	AGGGGTCTTG	30	4	3	0	7	225-1102	57.14
OPA-07	GAAACGGGTG	30	3	2	0	5	497-1428	60.00
OPA-17	GACCGCTTGT	30	8	1	4	13	162-1811	92.31
OPat-08	TCCTCGTGGG	32	1	1	0	2	218-958	50.00
OPaw-10	GGTGTTTGCC	30	4	1	0	5	313-912	80.00
OPD-1	ACCGCGAAGG	32	2	3	1	6	236-1364	50.00
OPD-18	GAGAGCCAAC	30	0	4	0	4	292-1278	00.00
OPJ-15	TGTAGCAGGG	30	4	1	0	5	229-737	80.00
OPP-13	GGAGTGCCTC	32	2	2	1	5	345-876	60.00
Total			32	20	7	59		60.09

Table 5. Sequences and annealing temperature of nine ISSR primers were used to access genetic disorder of the root tip of *Vicia faba* (Misr 3 cultivar) as influenced by Al concentration and exposure time. Size of amplified fragments (bp), total number of amplified fragments, number of polymorphic bands, unique bands and specific bands identified per primer were included.

primer	Sequence (5'→3')	Annealing temperature (°C)	Polymorphic bands	Monomorphic bands	Unique bands	Total bands	Size range (bp)	Polymorphism (%)
ISSR1	ACACACACACACACACCTG	56	7	3	0	10	175-1287	70.00
ISSR2	CACACACACACACACAAAGCT	60	5	1	0	6	239-703	83.33
ISSR3	ACACACACACACACACAAG	58	6	1	2	9	206-870	88.89
ISSR4	GAGAGAGAGAGAGAGACTG	50	4	2	2	8	167-985	75.00
ISSR5	GAGAGAGAGAGAGAGACTC	50	2	6	2	10	188-565	40.00
ISSR7	CTCTCTCTCTCTA (CT)6A	38	6	1	1	8	804-1376	87.50
ISSR8	TCTTCTTCTTCTG	36	5	2	0	7	295-1489	71.42
ISSR9	TGTTGTTGTGC	32	7	0	0	7	169-828	100
ISSR10	GTGGTGGTGGC	38	3	6	1	10	457-1557	40.00
Total			45	18	8	75		72.90

the common type of chromosomal abnormalities during ana-telophase stage.

Root tips of Misr 3 cultivar were subjected to molecular analysis to reveal genome variation which in consistent with chromosomal abnormalities under Al toxicity (Table 4). In this concern, ten RAPD primers were used (Fig. 2). Misr 3 cultivar showed 32 polymorphic (54.24 %), 20 monomorphic bands (33.89 %) and seven unique bands (11.86 %) out of 59 fragments. The number of bands ranged from two using OPat-08 primer to 13 bands using OPA-17 primer. Percentage of polymorphism ranged from 0 % when OPD-18 was used to 92.31 % using OPA-17 with an average of 60.09 %. Primer OPA-17 gave the highest number of polymorphic RAPD markers. The average number of bands per polymorphic primers was 5.9 and the average number of polymorphic bands per polymorphic primers was 3.9. The highest number of bands was detected when OPA-17 primer was used. The size of the obtained fragments using all the RAPD primers ranged from 141 to 1811 bp.

Nine ISSR primers were used for amplification of genomic DNA of *V. faba* Misr 3 cultivar (Table 5 & Fig. 3). Under the application of these nine primers 75 amplified fragments were detected. Forty-five of them were polymorphic (60 %), 18 were monomorphic bands (24 %) and eight were unique bands (10.67 %). The number of bands per primers ranged from six using ISSR2 primer to ten bands using ISSR1, ISSR5 and ISSR10 primers. The size of the obtained fragments using all ISSR primers varied between 167-1557 bp. Primers ISSR1 or ISSR9

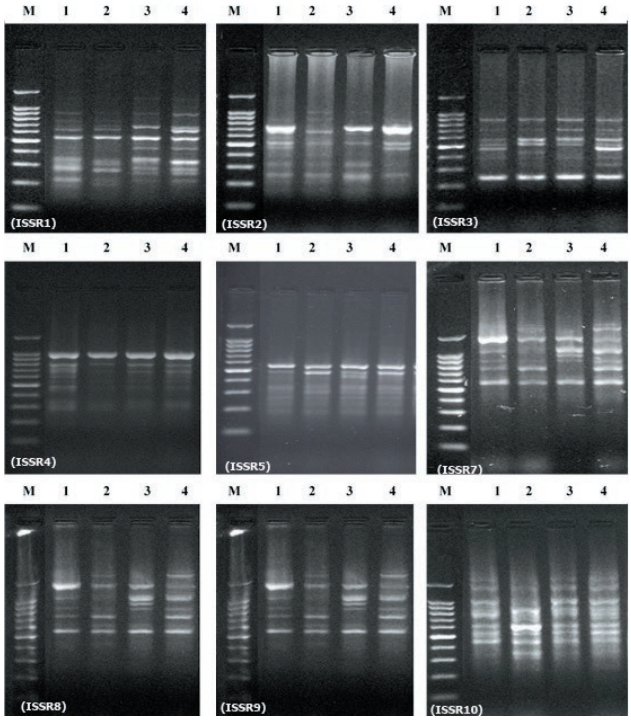

Figure 3. ISSR-PCR profiles generated by 9 primer using roots of different *V. faba* Misr 3 cultivar subjected for different periods in 5 mM $AlCl_3$. Lanes1, 2, 3 and 4 (0.0, 6, 12 and 24hr), respectively. M: DNA ladder.

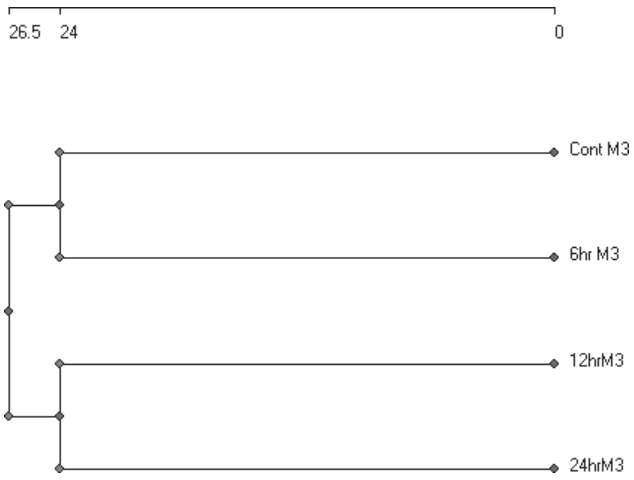

Figure 4. UPGMA based cluster tree of *Vicia faba* (Misr 3) cultivar exposed to 5 mM $AlCl_3$ for (0.0, 6, 12 and 24hr) based on (9 ISSR primers).

gave the highest number of polymorphic ISSR fragments. The average number of bands per polymorphic primers was 8.33 and the average number of polymorphic bands per polymorphic primers was 5.9. Percentage of polymorphism ranged from 40 % when ISSR5 and ISSR10 were used to 100 % using ISSR9 with an average of 72.90 %.

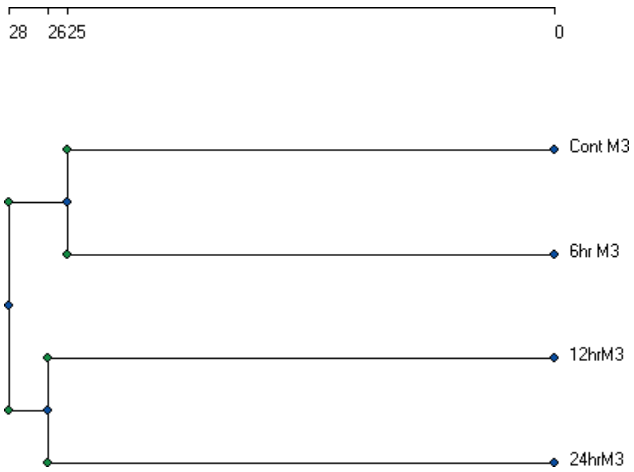

Figure 5. UPGMA based cluster tree of *Vicia faba* (Misr 3) cultivar exposed to 5 mM $AlCl_3$ for (0.0, 6, 12 and 24hr) based on (10 RAPD + 9 ISSR primers).

When results of the application of ISSR were compared with others of RAPD primers, valuable variations were detected. Total bands per nine primers of ISSR (75) were higher than that of ten RAPD (59) primers. In addition, the average polymorphism obtained from the application of ISSR primers (72.90 %) was higher than the average polymorphism obtained from applying of RAPD primers (60.09 %).

Cluster tree based upon UPGMA analysis of ten RAPD and nine ISSR primers of *V. faba* Misr3 cultivar resulted in two main clusters (Fig. 4 & 5). The first cluster included control and 6 h treated roots with $AlCl_3$. The second cluster included 12 and 24 h treated roots with $AlCl_3$. The obtained dendrogram as well as cytogenetical and polymorphism data showed that Misr 3 cultivar suffered from harmful effect of Al on the nuclear genome, especially when the duration of exposure time was more than 6 hr.

DISCUSSION

In this work, seedlings of faba bean were subjected to genotoxicity of high dose Al (5- 25 mM $AlCl_3$), the obtained nuclear genome variation was registered using cytogenetical and molecular approaches. Cytogenetical effects of Al on faba bean (Yi et al. 2010; Hassanein et al. 2020b) and other plant species (Li et al. 2015; Domingues et al. 2012; Jaskowiak et al. 2018) were studied. In this work, irrespective the concentration of Al,

the value of MI was gradually decreased as the exposure time increased as was also reported by other authors (Yi et al. 2010; Altwaty et al. 2016; Hassanein et al. 2020b).

Variations in frequencies of different mitotic phases were registered and indicated that Al induced cell cycle alterations in faba bean as was reported by Yi et al. (2010). Consequently, prophase frequency increased as the concentration of $AlCl_3$ increased and exposure time prolonged up to 15 mM $AlCl_3$. Also, metaphase frequency mostly increased as the concentration of $AlCl_3$ increased except plant roots subjected Al stress for 6 hr. Increase of prophase and metaphase frequencies associated with increase in their abnormalities. In addition, increase in prophase frequency associated with decrease in ana-telophase frequency under Al treatments and it showed complete inhibition when Al stresses were applied for 24 h.

Micronuclei were detected in the root tips exposed to different concentrations of $AlCl_3$ for different periods and ranged from small to large size. There was no correlation between the size or number of micronuclei/cell and the applied conditions. Binucleated cells are formed in Al stressed Misr 3 cultivar as the result of disturbed cytokinesis (Alberts et al. 2002) and cell plate formation (Gisselsson et al. 2001). Micronuclei frequency is a good indicator of the cytogenetic effects of tested chemicals such as $AlCl_3$ in faba bean and other plant species (Kanaya et al. 1994; Gecheff 1996; Grant and Owens 1998; Kundu and Ray 2017). Consequently, micronucleus assay was considered as the efficient, simplest and most effective tool to measure of chromosomal DNA damages and analyze the mutagenic effect of different substances (Auerbach 1962; El-Azab et al. 2018; Kursheed et al. 2018).

Under the used high doses of $AlCl_3$, metaphase was the most sensitive stage compared to the other stages of mitosis, where the abnormalities were detected at all treatments. In addition, C-metaphase was the common abnormalities, and their appearance was independent on Al concentration, but it increased strongly when the exposure time was more than 6 hr. In addition, metaphase abnormalities including chromosomal stickiness, star shape chromosomes, chromosomal breaks and disturbed configurations were detected. During metaphase, failure of broken chromosome to recombine correctly due to the stickiness of chromosomes and their inability to arrive to the poles led to the appearance of chromosomal breaks under Al or other stress agent (Agarwal and Ansari 2001). These abnormalities were also detected by other authors in faba bean (Yi et al. 2010; Hassanein et al. 2020b) and other plant species (De Campos and Viccini 2003; Mohanty et al. 2004).

Concentration abnormalities in one form (C-metaphase) during metaphase made the appearance of other forms of abnormalities rare during metaphase or telo-anaphase, especially under long exposure time (24 h). In addition, ana-telophase chromosomal abnormalities including chromosomal bridges, chromosomal breaks, disturbed anaphase, diagonal and star chromosomes, and C-anaphase were detected under Al treatments. The registered stickiness in faba bean and other plant species gave an indication about the direct destructive effect of a toxic agent on chromosomes (Renjana et al. 2013) or spindle disturbance (Gaulden 1987). Lagging chromosomes and chromosomal bridges were appeared which may be due to abnormal spindle fiber formation (Badr et al. 2013). Also, Rieger et al. (1991) believed that the inhibition of cytoskeletal proteins leads to the formation of lagging chromosomes.

Data of our study indicated that Al treatments in high dose induced chromosomal abnormalities and micronuclei formation. These two phenomenon may result from inhibition of DNA synthesis (Minocha et al. 1992), alteration of chemical or electrostatic properties of DNA (Unrau and Laster 1952), elimination of genetic material (Fernandes et al. 2007), induction of DNA fragmentation (Jaskowiak et al. 2018), formation of chromosomal bridges and chromosomal breaks (Ignacimuthu and Babu 1989), miss-repair of the broken DNAs or fused of telomeres (Souguir et al. 2018), stickiness of chromosomes (Badr et al. 2014). In addition to point mutation, the previous events created alteration either at or between the primer binding sites, which could be detected by RAPD and ISSR techniques (Liu et al. 2007; Gupta and Sarin 2009). During PCR program, binding between primers and complemented loci resulted amplification of DNA fragments with molecular weight of 100 to 2000 bp (Ng and Tan 2015). The amplified DNA fragments were dependent on the extent of chromosomal changes under the toxic effect of Al due to the previous events. These events created or abolished some primer binding sites leading to polymorphism. This means that the detected DNA polymorphism may be due to mismatching at the primer site, appearance of a new primer site and/or change the distance between two opposite primers. Using RAPD primers, Misr 3 cultivar showed 32 polymorphic out of 59 fragments primers (54.24 %), 20 monomorphic bands (33.89 %) and seven unique bands (11.86 %). Percentage of polymorphism ranged from 0 % when OPD-18 was used to 92.31 % using OPA-17 with an average of 60.09 %. Under the application of ISSR primers, polymorphism average was 72.90 %. The average polymorphism obtained from the application of ISSR primers

(72.90 %) was higher than that of RAPD primers (60.09 %). Then, ISSR is an ideal molecular marker to study natural or induced genetic variation of faba bean cultivars as well as other plants (Abdel-Razzak et al. 2012; Alghamdi et al. 2012; Wang et al. 2012; Salem and Hassanein 2017; Hassanein et al. 2018).

Cluster tree based on RAPD and ISSR primers indicated that Misr 3 cultivar suffered from harmful effect of Al on its genome when plant root tips were exposed to Al for more than 6 h where great cytogenetical events were happened and resulted in variation in primer binding sites leading to high polymorphism. In faba bean, Yi et al. (2010) reported that significant increase in frequencies of micronuclei formation and chromosome aberrations was induced when root tips were exposed to 0.01–10 mM Al for 12 hr. Consequently, Molecular markers, e.g. ISSR, could be used to confirm the cytogenetical data and supplemented us with valuable information about metal genotoxicity such as Al, especially when they were used in relatively high concentrations.

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