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Effects of Jasmonic and Salicylic Acids on Cell Division and Cell Cycle Progression

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THIS STUDY is concerned with the effect of both salicylic and jasmonic acids on mitotic cell division of *Allium cepa* (Lilliaceae*)* root tips and their ability to induce chromosomal aberrations. The study also deals with the use of image cytometry and SDS-PAGE techniques to estimate the changes in the cell cycle progression and seed protein banding pattern, respectively. Root tips of *Allium cepa* (Lilliaceae*)* treated for 3, 6 and 24 hr. At the cytological level both hormones caused reduction in mitotic index, particularly in roots treated with higher concentrations. A wide range of chromosomal abnormalities such as stickiness, c-metaphase, bridges, chromosome breaks, micronuclei and polyploidy cells were recorded in roots treated with both hormones. The effect of salicylic and jasmonic acids on DNA content corresponding to the proportion of cell cycle phases were calculated. Cytophotometric analysis showed decrease in the proportion of cells with 2C value (G1 phase) and increase in the fraction of cells in S and G2 phases as they compared with control. The lowest concentration of jasmonic acid in root treated for 24 hr and the lowest concentration of salicylic acid in root treated for 3 or 24 hr contradict this result since they increase the number of cells at G1 phase. The effect of both hormones seems to work on G2/M checkpoint at higher concentrations. At the biochemical level jasmonic acid induces changes in the electrophoretic profiles of seed protein of *Vicia faba* (Fabaceae*)*. These changes include the absence of some bands and the appearance of few novel bands as well as over expression of the others.

Keywords: *Allium*, *Vicia*, Mitotic index, Chromosomal aberrations, Growth regulators, Image cytometry, SDS-PAGE.

Plants need to produce a huge arsenal of chemical compounds to adapt and respond to environmental challenges. Among these compounds, the phytohormones are in prominent position. Plant hormones represent essential link of many plant processes, control a diverse array of plant responses affecting growth and development, either directly or indirectly. Plants respond to biotic and abiotic external stimuli using hormone signal transduction pathways that cause changes in hormone metabolism and in turn cause changes in their distribution within the plant (Davies, 2004).

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Recently, new classes of plant hormones gained much attention in agriculture, biomedical and industrial application. From these promising hormones are the salicylic and jasmonic acids (Tiryaki, 2004).

Salicylic acid (SA) has been recognized as growth regulator of phenolic nature while jasmonic acid is a naturally occurring lipid-derived compound. Jasmonic and salicylic acids play an important role in agriculture through control of different plant processes; such as root growth inhibition (Staswick *et al*., 1992), seed germination (Corbineau *et al.*,1988) and the senescence-promoting effect (Parthier, 1990).

During the past twenty years, jasmonates have attracted considerable attention. Jasmonic methyl ester plays a vital role as anticancer agents in the biomedical field. They were demonstrated to exhibit cytotoxic effects for a wide range of malignancies and various types of cancer (Flescher, 2005). Yeruva *et al*., (2008) evaluated the ability of methyl jasmonate and cis-jasmonate to inhibit growth in prostate cancer-cell lines, since they induce cell cycle arrest and apoptosis.

Genetic toxicology is a multidisciplinary field of research involved in detecting DNA damaging and their modes of action that lead to alternations of the genetic material (Uhi *et al*., 2003). There are many methods involved in determination of genotoxic action of chemicals. Among them are chromosomal aberration and cytometry assay.

In this study the classical *Allium* test and image cytometry were used togather and quantify data on the rate of cell division, the chromosomal aberrations and progression of the cell cycle following treatments with the plant hormones, salicylic and jasmonic acids. In addition, the effect of jasmonic acid on protein banding pattern in *Vicia faba* (Fabaceae*)* seeds was studied.

Material and Methods

Allium cepa (Lilliaceae*)* (var. Giza 6) and *Vicia faba* (Fabaceae*)*. (var. Giza 2) kindly supplied by the Agriculture Research Centre (ARC), Giza, Egypt, were used as experimental plants.

The chemical structure of salicylic and jasmonic acids are HOC6H4COOH and C12H18O3 respectively. Five concentrations of salicylic acid ranged between 1.25 and 20 mM were used that are compatible with many reviews (Rao, 2003). On the other hand, stock solution (0.1%) of jasmonic acid was prepared in ethanol as a solvent control (Sigma-Aldrich). Then four concentrations ranged from 0.075 to 0.0094 mM were prepared from the stock solution.

Cytological experiments

Young, healthy and uniformed *Allium cepa* bulbs were allowed to germinate in tap water. When the roots reached 2 – 3 cm long, they were treated with the test hormones for 3, 6 and 24 hr. Cytological preparations were carried out according

to Darlington and La Cour, (1976). Three replicates were prepared for each treatment and control. The preparations were examined microscopically and 90 fields were completely analyzed for each concentration. The photomicrographs were taken from the prepared slides. The frequencies of mitotic index (MI) as well as the frequencies of different mitotic abnormalities were determined. Mitotic index and total abnormalities were statistically analyzed using (*t*-test).

Cytophotometric measurements of DNA

The content of the DNA in root tip cells treated with different concentrations of both salicylic and jasmonic acids for 3 and 24 hr were estimated by the Image Analyzer System. The Feulgen staining preparations for chromosome aberration analysis were used in image cytometry. Cell image analysis was performed using Leica Qwin 500 image analyzer system. The amount of DNA in the nucleus, DNA ploidy and the frequencies of cells undergoing different phases of cell cycle were calculated from 500 cells of each treatment. These include cells with DNA amount less than the 2C value, cells with 2C DNA (G_0/G_1), cells with 3C-4C DNA (S-phase), cells with 4C DNA (G_2 phase) and cells with DNA more than 4C value. The nuclear-integrated optical density is the cytometric equivalent of its DNA content (Bocking *et al*., 1995).

Biochemical Studies

Characterization of protein profiles was carried out using one dimensional sodium dodecyle sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Polyacrylamide slab gel was prepared according to Laemmli, (1970). Seeds of *Vicia faba* (Fabaceae*)*. Plants previously treated with three concentrations of jasmonic acid for 24 hr were milled. For protein extraction, 0.2 ml of sample buffer (0.2M Tris-HCL pH 6.8 containing 2% SDS) was added to 0.02 g of seed meal and stored overnight at 4ºC. Centrifugation was performed at 9000 rpm for 6 min, then 60 µl supernatant for each concentration were denatured by heating at 90ºC for 3 min and loaded in 12% acrylamide slab gel containing 10% SDS. Run was performed at 15mA for 30 minutes followed by 25 mA till the tracing bromophenol blue dye reached the gel bottom. Protein bands were visualized by staining the gels with 0.25% Coomassie Brilliant Blue R-250. The gels were first photographed and documented for further analysis by appropriate software.

Results and Discussion

The cytological effects of both salicylic and jasmonic acids on meristematic cells of Allium cepa root tips are shown in Table 1 , 2. The Allium test is a very good plant bioassay for studying somatic mutation and DNA damage induced by chemicals. The mitotic index was determined as a parameter of mitotic activity and it is an acceptable measure of cytotoxicity for all living organisms (Smaka-Kinel *et al*., 1996).

TABLE 1. Frequency of different types of prophase, metaphase and ana-telophase abnormalities, mean MI/replicate and mean percentage of abnormal mitosis after treating *Allium cepa* root tips with different concentrations of salicylic acid for 3, 6 and 24 hr.

Concentration. mM	% of prophase abnormalities			% of metaphase abnormalities						% of ana-telophase abnormalities						Mean MI%/ replicate ± SE	Mean % of abnormal mitosis/ replicate ± SE
	Stick	Split	Irrg.	C-m (2n)	Star	Break	Stick	Dist.	Lag.	Lag.	Brid.	Multi.	Break	Stick	Dist.		
3 hours																	
Control		0.62	0.62					2.38	0.79						0.96	4.65±0.08	1.62 ± 0.44
1.25		0.97		10.29				17.65	16.18	3.75	1.60				5.35	3.79±0.09	14.25 ± 1.11
2.5		4.26	4.26	27.09	2.27	1.14	2.45	18.18	13.64	1.14	5.68		3.41	14.77	14.77	2.84±0.05	37.04 ± 1.51
5	2.70	8.11	10.81	28.06	1.61	1.61	15.16	18.06	12.92	4.72	18.88	1.87		18.88	16.98	2.54±0.22	59.02 ± 2.79*
10	15.63	12.50	18.75	30.0	1.72		35.52	18.97	10.34	1.86	28.27			26.85	8.33	1.94±0.11*	71.81 ± 2.11*
20	33.33	23.81	21.43	27.94	1.47		35.29	26.47	4.42	9.59	30.14		2.73	32.88	8.22	1.91±0.01*	86.89 ± 0.04*
6 hours																	
Control		1.17	1.18					4.26	0.95	0.56					1.12	4.01±0.06	2.78± 0.96
1.25	4.35	2.17		9.47				17.90	5.26	4.08	7.14	1.02			14.29	3.04±0.04	22.11± 1.42
2.5	13.64	4.54	2.27	12.99	1.30	3.90	6.88	26.88	5.19	13.20	13.21		0.94	6.33	20.09	2.55±0.03	48.46 ± 1.15
5	13.04	4.35	4.35	23.41		3.66	10.49	26.83	1.22	3.00	20.00		2.00	10.00	31.00	2.31±0.07	63.59± 1.44*
10	32.61	15.22	13.04	20.26	2.56	2.56	25.90	17.95	12.82	6.66	25.72			22.86	21.90	2.26±0.17*	75.55± 1.78**
20	23.08	23.08	49.99	7.25	1.45	2.90	54.58	30.43		2.60	28.57		2.60	40.90	10.39	1.77±0.09**	90.70± 0.95**
24 hours																	
Control		0.85						4.59	1.83		0.97				1.64	4.26±0.04	3.01± 0.87
1.25	3.39	3.39	3.39	9.54		1.77	1.84	16.39	7.96	3.00	8.00			2.00		2.96±0.07	22.51± 0.67
2.5	6.38	2.13	29.79	20.20	2.30	2.30	12.18	27.38	8.05	6.02	25.31	1.21		8.43	12.04	2.45±0.05*	57.60± 1.54*
5	14.81	7.41	35.19	23.08	1.54	1.54	21.54	20.00	7.68		20.73		2.44	12.20	14.63	2.19±0.03*	60.20± 0.66**
10	21.28	8.51	42.55	14.84	1.61	6.45	31.94	12.90	11.29		36.98			26.03	9.59	1.83±0.04**	74.73± 0.22**
20	13.89	2.78	44.44	12.68	7.04	8.45	46.61	16.76	4.23	10.14	15.94		7.25	28.99	23.19	1.96±0.05**	84.66± 0.25**

* Significant from control at 0.05 level (t-test). ** Significant from control at 0.01 level (t-test).

TABLE 2. Frequency of different types of prophase, metaphase and ana-telophase abnormalities, mean MI/replicate and mean percentage of abnormal mitosis after treating *Allium cepa* root tips with different concentrations of jasmonic acid for 3, 6 and 24 hr.

Conc. mM	% of prophase abnormalities			% of metaphase abnormalities							% of ana-telophase abnormalities						Mean MI% ± SE	Mean % of abnormal mitosis ± SE
	Stick	Split	Irrg.	C-m (2n)	C-m (4n)	Star	Break	Stick	Dist.	Lag.	Lag.	Brid.	Multi.	Break	Stick	Dist.		
3 hours																		
- ve Control (H2O)		0.63	0.63					0.87	0.87	1.74	0.62					0.62	5.08±0.29	1.83± 0.63
+ ve Control (alcohol)	2.32	4.65	23.26	12.70		5.41	2.70	13.51	6.22	2.70	3.33	10.00	3.33		13.33	11.68	2.03±0.01	38.57± 0.25*
0.0094		5.17	15.52	18.00	6.00				12.86	6.00	3.37	7.87	1.12	1.12		5.62	2.89±0.04	26.11± 1.01
0.01875	1.85		7.41	12.27	5.89			2.27	16.82	2.27	2.63	3.94	1.32	1.32	2.63	13.16	2.65±0.08	23.70± 1.16*
0.0375	2.70	10.81	35.14	20.64	10.64			2.13	13.40	6.38	6.82	6.82	3.55	2.27	2.27	16.05	2.15±0.12	46.51± 0.52**
0.075	8.51	14.78	36.17	14.99	16.67	5.00		20.00	6.67	6.67	3.78	5.66	3.77		16.98	11.32	1.84±0.04	57.33± 0.84**
6 hours																		
- ve Control (H2O)									1.12	1.12		0.69				0.69	4.53±0.04	1.13± 0.57
+ ve Control (alcohol)	17.02	4.25	12.77	16.25	2.33	2.33	2.33	12.33	22.10	2.33		5.03	2.63	2.63	19.57	17.89	1.97±0.07*	42.75± 1.50**
0.0094	3.45	5.17	6.90	13.64	6.81	2.27		3.64	23.64	2.27	1.23	2.47	2.47	2.47	4.94	3.70	2.42±0.05	25.14± 1.48
0.01875		3.03	3.03	11.96	7.84	3.93	5.88	5.88	15.50	1.96	1.43	12.86	1.43	1.43	5.71	8.56	2.21±0.09	33.12± 0.68*
0.0375		3.13	6.25	16.00	10.34	3.45	10.34	8.14	20.69	5.18	6.41	7.68	5.13	3.85	6.67	22.82	2.09±0.05*	51.79± 0.53**
0.075	8.33	8.33	33.34	15.00	5.00	2.50	2.50	27.50	17.50	5.00	2.13	7.00	4.26	4.26	8.50	10.02	1.53±0.05*	62.86± 1.32**
24 hours																		
- ve Control (H2O)																	4.36±0.10	0.30 ±0.31
+ ve Control (alcohol)	5.13	12.82	30.77	14.44	4.44			16.23	25.56	6.66		8.50	2.13	4.26	13.40	31.28	1.96±0.02**	59.26± 0.23**
0.0094			8.93	10.00		1.67	1.67	5.00	21.66	3.33	1.02	11.22			6.33	12.04	2.43±0.16	28.50± 0.20
0.01875	14.58	4.17	25.00	13.17	1.59	4.76	1.58	16.98	12.70	3.17		12.77	2.13	2.13	6.38	4.25	2.08±0.02*	43.04± 0.50**
0.0375	2.86		37.14	13.70	1.85	3.70	1.85	17.78	27.78	3.69	1.85	7.41	5.56		14.07	21.11	1.95±0.06**	55.24± 0.65**
0.075	4.88	10.68	43.90	10.81	11.59	2.70	8.11	27.03	29.92	2.70	4.26	6.38	2.13	2.13	31.91	8.51	1.65±0.01**	66.07± 0.80**

* Significant from control at 0.05 level (t-test). ** Significant from control at 0.01 level (t-test). +ve control: ethyl alcohol (0.1%).

One of the major effects of salicylic acid (SA) was shown on its influence on the activity of cell division. A decrease in mitotic index was observed after treatment with the different concentrations of salicylic acid. Such reduction increased with the increase of concentrations but there is no clear decrease in the mitotic index values as duration of treatment increased (Table 1).

Jasmonic acid (JA) showed a decrease in MI value in all periods of treatments as compared with negative control (water); but there is a slightly increase in MI values in all concentrations except the highest one as compared with roots treated with ethyl alcohol as positive control (Table 2). Ethyl alcohol by itself showed an inhibiting effect on the rate of cell division. In this respect JA dose not only antagonize the inhibiting action of the ethyl alcohol but slightly activates mitosis in most concentrations used. The mitodepressive effect of the hormones under study is in agreement with the effect of other hormones (Howell *et al*., 2007 and Kartal *et al*., 2009).

Both promotive and inhibitory effects of jasmonic acid on cell division have been reported. The inhibitory action of jasmonic acid is in accordance with the result obtained by Norastehnia *et al*., (2007). On the other hand, Advanci *et al.*, (2010) claim that jasmonic acid promotes cell division. These contradictory results have proven to be enigmatic. Similar effect on mitotic index of *Allium cepa* root tips was reported by the plant steroidal hormone, 24-epibrassinolide (BL) in which the low doses can increase the number of mitosis in plants while high BL concentrations are often inhibitory (Howell *et al.*, 2007).

The reduction in mitotic index can be attributed to a change in cell cycle progression. In the present investigation, the effect of different concentrations of both salicylic and jasmonic acids on DNA content corresponding to the proportion of cell cycle phases showed that there is a decrease in the proportion of cells with 2C value (G_1 phase) while there is an increase in the fraction of cells in S and G_2 phases as compared with the control (Table 3). Lowest concentration of jasmonic acid in root treated for 24 hr and the lowest concentration of salicylic acid in root treated for 3 or 24 hr contradict this result since they increase the number of cells at G_1 phase (Table 3). Similar results were obtained by Polit *et al*., (2003) who showed that treatment root tips of *Vicia faba* with the auxin "indole-3-acetic acid"; the cytokinin "benzyle-6- amino-purine" or mixture of both of them increased the number of G_2 cells; producing a characteristic profiles of nuclear DNA content. On the other hand, ethyl alcohol (+ve control) showed an increase in the number of cells at G_1 phase.

When the plant is under stress or exposed to any DNA damaging agents, the progression through the cell cycle is halted at checkpoints to allowing time for correction and repair (Friedberg *et al.*, 1995). These checkpoints are important in maintain the stability of chromosome complement. In this work cell cycle analysis showed that both salicylic and jasmonic acids can activate mechanisms at both G_1/S and/or G_2/M checkpoints as S-phase is markedly increased to buy

a time for repair pathways. These results are in accordance with work of Perennes *et al.*, 1999 in which SA efficiently blocked BY-2 tobacco synchronized cells in G_0/G_1 or G_2 phases and the work of Swiatek *et al.*, 2002 who showed that jasmonic acid freezes synchronized tobacco BY-2 cells in both the G_1 and G_2 stages of cell cycle causing inhibition of the cell cycle progression. Pauwels *et al*., 2008 showed that methyl jasmonate addition alters the transcriptome of *Arabidopsis* cultured cells, reprograms cell cycle gene expression by repressing M-phase genes and consequently cells become arrested in G_2. Also, Ofer *et al*., 2008 reported that jasmonates caused fragmentation and condensation of the DNA of *Trichomonas vaginalis* associated with apoptotic death, in addition cell cycle block at the G_2/M phase.

The division of the plant cells and progression through the successive phases of the cell cycle is driven by a common class of protein kinases (cycle dependent kinases CDKs) whose activity depends on the association with different classes of cyclins (Binarova *et al*., 1998). CDK-A or -B play a pivotal role in the G_1/S and G_2/M transition points (Harting and Beck, 2006). In plants mitotic progression is timely regulated by the accumulation and degradation of the mitotic cyclins A- and B-type (Weingartner *et al*., 2003). The inhibition in mitotic division obtained in this work may be explained according the work of Zhang and Turner, 2008 who showed that high concentrations of jasmonic acid reduced cell number by inhibiting cell division as a consequence of a G_2 arrest due to repression of *cycB1* and *cycB*2 gene expression. Several compounds have been reported to induce delay in the onset of mitosis; observed as reduction in the proportion of cells undergoing mitosis and blocking the mitotic cycle at interphase (Gul *et al*., 2006 and Enan, 2009).

Blocks of the cell cycle progression in both G_1 and G_2 phases after treatment with the two hormones may be also due inhibition of CDK suggesting that these hormones act as an inhibitor especially when higher concentrations are used. Binarova *et al*., 1998 reported that treatment *Vicia faba* cells with roscovitine - a drug that inhibited CDK, blocks cell cycle progression in both G_1 and G_2 phases.

Failure to stop at the DNA damage checkpoints may lead to chromosomal aberrations of a various kinds, including aneuploidy or polyploidy and increase the mutation rates. From the results obtained in this work using cytological and image cytometric analysis it is clear that the treated *Allium cepa* root tips showed aneuploid, polyploid cells and chromosomal abnormalities (Table 1, 2 & 3). A large percentage of polyploid cells (restitution cell) with DNA more than 4C were observed after treatment with 0.0094 mM of JA (Table 3). Polyploidy is type of ploidy status in which cells undergo iterative DNA replications without any subsequent mitosis and cytokinesis, in what is known as endoreduplication (Cortes and Pastor, 2003 & Sugimoto-Shirasu and Robert, 2003). The change in DNA content that is present in populations of cells reflects increase in the amount of chromosomal damage that not only causes changes in chromosome copy numbers but also change the shape and size of individual chromosomes (Smith, 1996).

TABLE 3. Effect of salicylic and jasmonic acids on the cell cycle parameters in *Allium cepa* root tip cells treated for 3 and 24 hr.

Treatments	Conc. mM	DNA < 2C ± SD	G1 phase ± SD	S-phase ± SD	G2 phase ± SD	DNA > 4C ± SD
3 hours						
Salicylic Acid	5	1.7± 0.176	36.5± 0.28	36.5± 0.26	19.1± 0.251	6.1± 0.959
	2.5	8.2±0.451	24.5±0.228	36.4±0.315	20.9±0.314	10.0±0.267
	1.3	9.0±0.133	55.0±0.27	30.6±0.25	3.6±0.183	1.8±0.11
Jasmonic acid	0.0375	2.3±0.116	34.9±0.289	35.7±0.245	17.1±0.269	10.1±0.377
	0.0188	0	21.6±0.242	45.0±0.302	22.5±0.276	10.8±1.498
	0.0094	1.7±0.092	14.4±0.269	28.8±0.259	30.5±0.244	24.6±0.624
	+ ve control	8.5±0.138	51.9±0.274	25.5±0.26	9.4±0.231	4.7±0.304
24 hours						
Salicylic acid	5	0.94%±0	24.30 ± 0.16	49.53 ± 0.30	19.63 ± 0.29	5.61 ± 0.40
	2.5	0.94%± 0	27.36 ± 0.23	43.40 ± 0.30	20.76 ± 0.25	7.55 ± 0.69
	1.3	8.33 ± 0.08	80.83 ± 0.26	10.83 ± 0.26	0.0	0.0
Jasmonic acid	0.0375	0	10.62 ± 0.25	38.05 ± 0.31	23.01± 0.29	28.32 ± 0.84
	0.0188	6.93 ± 0.10	23.76 ± 0.23	29.70 ± 0.31	25.74 ± 0.28	13.86 ± 0.76
	0.0094	5.13 ± 0.05	67.52 ± 0.23	20.51 ± 0.20	5.13 ± 0.32	1.71 ± 0.19
	+ ve control	0	8.91 ± 0.29	29.70 ± 0.28	20.79 ± 0.25	40.59 ± 2.26
control	control	16.67 ± 0.19	47.62 ± 0.28	23.81 ± 0.35	11.91 ± 0.34	0.0

The association between the inhibitory effects of the hormones under study with its action on the parameters of the cell cycle, it can be concluded that the reduction in mitotic activity may be regarded as a result of arrest of mitotic cycle at the G_1, G_2 phases and/or the prolonged duration of S-phase but not to inhibiting DNA synthesis cycle which directly related to decrease in mitotic index and appearance of chromosomal aberrations (Table 3). Many investigators attributed the inhibition in mitotic activity to blocking of mitotic cycle during interphase and accumulation of cells at G_1 or G_2 (Binarova *et al*., 1998 and Polit *et al*., 2003).

The result obtained in the present study showed that higher concentrations of salicylic and jasmonic acids lead to an arrest of metaphase stage. Incorrect attachment of the chromosomes to the spindle causes inactivation of anaphase promoting complex (APC) that inhibit the separation of sister chromatids and arrest the cell at metaphase stage and the anaphase is aborted or delayed. Failure of spindle checkpoint results in genetic instability and aneuploid cell formation (Hartil and Jones, 2001). In spite of the appearance of some metaphase arrest, the reduction in mitotic activity must be due mainly to the inhibitory action of these hormones on the onset of mitosis.

The inhibition in mitotic division can be attributed also, to increase in mitotic abnormalities and DNA damage (Enan, 2009*).* Sakr, *et al.,* 2009 showed that GA3 induced DNA damage and number of chromosomal aberration such as break, deletion in human lymphocyte culture. From results obtained in this work, it is clear that the tested hormones proved to induce a number of chromosomal aberrations in all mitotic phases. Their frequencies depend on both the concentrations used and duration of treatment. The mean percentage of abnormal mitosis demonstrated that salicylic acid induce higher percent of abnormalities than jasmonic acid. This implies higher DNA damage of salicylic acid than jasmonic acid. From cytogenetic analysis, it is clear that jasmonic acid was capable of decreasing the percentage of abnormalities induced by ethyl alcohol as the +ve control (Table 1).

The chromosomal aberrations induced by these hormones due to their action on DNA are well represented by production of bridges, nuclear buds, micronuclei as well as chromosome breaks. Chromosomal bridges at anaphase and telophase represent the most conspicuous abnormalities observed in all treatments (Table 1 , 2). Nucleoplasmic bridges and nuclear buds were observed at interphase stage after treatment roots with salicylic acid. Nucleoplasmic bridges originate from dicentric chromosomes that may be caused by mis-repair of double strand DNA breaks or telomere end fusions (Fenech, 2008 and 2009). They can be observed in cells completing nuclear division which are recognized by their binucleated appearance.

The nucleoplasmic bridges; nuclear buds and micronuclei are biomarkers that reflect genetic instability and are mechanistically related to one another. Generally when a break occurs in a chromosome, the broken pieces which lack the centromeres will lag as acentric fragment and latter appear as micronucleus or the broken ends may reunite with other broken ends of other chromosomes and led to the formation of dicentric chromosomes and appearance as chromosome bridge (Gömurgen, 2005). The present work showed both behaviors of the broken pieces in roots treated with the two tested hormones. Also, chromosome bridges may be due to the chromosomal stickiness and subsequent failure of anaphase separation (Gul *et al*., 2006 and Enan, 2009). Chromosomal stickiness appeared after treatments with higher concentrations of both the tested hormones.

Micronuclei are true mutagenic aspects which may lead to a loss of genetic material. A number of abnormal interphase cells with micronuclei were recorded in roots exposed to the different treatments with the two tested hormones. The micronuclei may be also resulted from lagging chromosome. Similar results were observed by the plant hormone homobrassinolide that induced a numbers of mitotic abnormalities in barley root (Kartal *et al.,* 2009).

Other chromosomal aberrations such as c-metaphase, star metaphase, disturbed mitotic phases and multipolar anaphase were induced by the tested hormones. C- metaphase may be due to the complete inhibition of the spindle formation by their action on the microtubules. C- metaphase was further produced by other hormones such as the hormonal herbicide avenoxan (Gul *et al.,* 2006). On the other hand, the induction of disturbed mitotic phases and multipolar anaphase by these hormones indicates that these hormones causes also partial inhibition of mitotic spindle fibers that causes the regular movement of daughter chromosomes to opposite poles at anaphase.

At the biochemical genetic level, jasmonic acid induces changes in the electrophoretic profiles of seed proteins of *Vicia faba* as compared with the control. The observed changes include appearance of new bands and disappearance of other bands (Fig.1, Table 4). Support for this view is the results obtained by Ananieva and Ananieva, 1997, where a set of specific proteins were marked and accumulated after treatment *Cucurbita pepo* with methyl jasmonate. These results also are agreement with the investigations carried by Chakraborty and Tongden, 2005 who showed that salicylic acid caused changes in protein banding pattern. Prasad and Zha 1992 showed that mutation caused variation in seed proteins of *Phaseolus vulgaris* that were associated with alternation in morphologically visible traits.

In conclusion, the result of the present study indicates to somewhat the hazardous of the hormones under investigation especially when applied in higher doses. They have a mitodepressive effect and induce a number of mitotic and chromosomal abnormalities. The effect of higher concentrations of both salicylic and jasmonic acids showed that there is a decrease in the proportion of cells with 2C value (G_1 phase) while there is an increase in the fraction of cells in G2 and S phases as compared with control. The reduction in mitotic activity may be attributed to blocking of mitotic cycle and accumulation of cells at G_1/S or G_2/M transition point. Similar results support this view obtained by both jasmonic and salicylic acids are used in the medical field. The inhibitory effect caused by jasmonic acid is of value in the treatment of cancer cells **(**Ofer *et al*., 2008). To achieve conclusive evidence, more attention must be taken to estimate and evaluate the mutagenicity of these hormones on the genetic material of human being using a number of mutagenic tests.

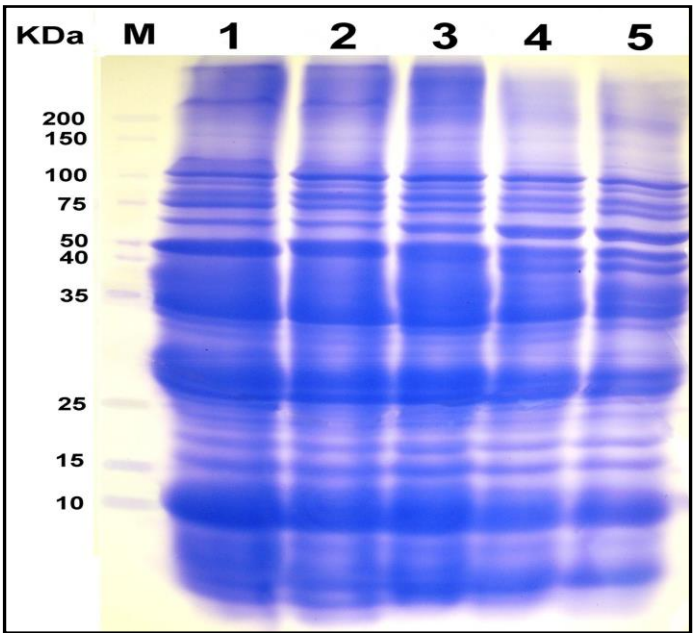

Fig.1. Electrophotograph of protein banding patterns of Vica faba seeds separated by SDS-PAGE after treatment with different concentrations of jasmonic acid for 24 hr.

M= Marker	1 = +ve control	2 = 0.0094mM
3 = 0.0187 mM	4= 0.0375mM	5= Control

TABLE 4. Effect of jasmonic acid on protein banding patterns of *Vicia faba* seed separated by SDS-PAGE.

Band No.	Mol. WT	Jasmonic acid				
		Lane 1 (+ ve control)	Lane 2 (0.0375 mM)	Lane 3 (0.0187 mM)	Lane 4 (0.0094 mM)	Lane 5 (- ve Control)
1	240	+	+	+	-	-
2	220	-	-	+	+	+
3	210	+	+	+	-	-
4	160	+	+	+	+	+
5	120	+	+	+	+	+
6	100	++	++	++	++	++
7	92	+	+	+	+	+
8	85	++	++	++	++	++
9	75	++	++	++	++	++
10	65	+	+	++	++	++
11	50	+++	+++	+++	++	++
12	42	-	-	-	++	++
13	40	-	-	-	+	-
14	38	-	-	+	+	+
15	36	++	++	++	++	++
16	34	+	+	+	+	+
17	32	+	+	+	+	-
18	30	+	+	+	+	+
19	28	++	++	++	++	++
20	26	++	++	++	++	++
21	24	+	+	+	+	+
22	22	+	+	+	+	+
23	20	+	+	+	+	+
24	15	++	++	++	++	++
25	12	+++	+++	+++	+++	+++
26	10	+	+	+	+	+
27	8	+	+	+	+	+
28	6	+	+	+	+	-
Total	--	24	24	26	26	23

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تأثير حمضى جاسمونيك وسالسيليك على انقسام الخلية ودورة الخلية

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تلعب الهرمونات النباتية ومنظمات النمو دوراً حيوياً فى مراحل تطور ونمو النبات ويعتبر حمضى الساليسيلك والجاسمونيك من الهرمونات النباتية التى تستخدم حديثا لهذا الغرض خاصة مع النباتـات المعرضة للاجهاد، لذلك تهدف هذه الرسالة الى دراسة التاثير السيتولوجى للمعاملات المختلفة من هذه الهرمونات على الإنقسام الميتوزى للقمم النامية لجذور نبات البصل ومعرفة مدى قدرتها على إحداث طفرات كروموسومية مختلفة. كما تهدف هذه الدراسة أيضاً إلى معرفة تأثير هذه الهرمونات على المراحل المختلفة لدورة الخلية من خلال قياس محتوى الحمض النووي DNA في الأنوية وذلك بإستخدام تحليل جهاز تحليل الصورة (Image cytometry) . بالإضافة إلى ذلك فقد تم دراسة تأثير حمض الجاسمونيك على طرز البروتين المفصولة كهربياً من بذور الفول وذلك باستخدام تقنية -SDS PAGE. عوملت القمم النامية لجذور نبات البصل بتركيزات مختلفة من الهرمونات موضع الدراسة وذلك لفترات زمنية مختلفة (٣، ٦ و ٢٤ ساعة) . استخدم الماء كعينة ضابطة مع جميع المعاملات السابقة ، بالإضافة إلى ذلك استخدم الكحول الإثيلي (٠,١ ٪) عينة ضابطة أخرى في حالة معاملة الجذور بحمض الجاسمونيك. أدت معاملة جذور البصل بهذه الهرمونات النباتية إلى تغير واضح فى نسبة الأطوار الميتوزية المختلفة و اعتمد هذا التغير على التركيز المستخدم وعلى فترة المعاملة. أظهرت نسب الأطوار المختلفة فى معظم المعاملات وجود إنخفاض ملحوظ في نسبة الطور التمهيدي وزيادة في نسبة الطور الإستوائي وذلك بزيادة التركيز بينما تأرجحت نسب الطور الإنفصالي- النهائي مما أدى إلى وجود علاقة عكسية بين الطور التمهيدي والطور الإستوائي بعد معاملة الجذور بحمضي الساليسيلك والجاسمونيك وذلك عند مقارنتها بقيم العينة الضابطة. كما أدت معاملة جذور البصل بالهرمونات إلى تأرجح نسبة الخلايا في الطور الإنفصالي – النهائي حول معدلها في العينة الضابطة. أوضحت نتائج هذه الدراسة أن حمض الساليسيلك قد أدى إلى انخفاض ملحوظ فى معدل الانقسام وذلك في جميع المعاملات وأن هذا الانخفاض يزداد بزيادة التركيز كما أظهر التحليل الإحصائى إنخفاضاً معنوياً عالياً و معنوياً خاصة عند معاملة الجذور بتركيزات مرتفعة. من ناحية أخرى لم تؤدي معاملة القمم النامية لجذور نبات البصل بحمض الجاسمونيك لمدة ٢٤ ساعة إلى زيادة إنخفاض معدل الإنقسام عما كانت علية في ٣ أو ٦ ساعات. على العكس من ذلك فقد أدت التركيزات المخففة منهما الى زيادة طفيفة فى معدل الأنقسام. أما بالنسبة لحمض الجاسمونيك فقد أدت معاملة الجذور بهذا الهرمون لفترات زمنية مختلفة إلى إنخفاض عام في معدل الإنقسام وذلك عند مقارنتها بالعينة الضابطة (الماء) في حين ظهرت زيادة طفيفة في معظم المعاملات عند مقارنتها بالعينة الضابطة (الكحول الإثيلي) مما يوضح أن حمض الجاسمونيك لم يقلل فقط من تأثير الكحول الإثيلي المثبط للإنقسام ولكنه أيضاً أدى إلى زيادة طفيفة في معدل الإنقسام.

أدت جميع المعاملات بحمض الساليسيلك و الجاسمونيك إلى ظهور أنواع عديدة من الشذوذات الكروموسومية وأوضحت النتائج أن النسبة الكلية لهذه الشذوذات تزداد تدريجياً بزيادة التركيز وفترة المعاملة وذلك عند مقارنتها بالعينة الضابطة (الماء). أوضحت التحاليل الإحصائية أن معظم المعاملات أدت إلى زيادة معنوية عالية أو معنوية في نسبة الانقسامات الشاذة. من ناحية أخرى أدت معاملة الجذور بحمض الجاسمونيك إلى حدوث إنخفاض ملحوظ في نسبة الشذوذات الكروموسومية وذلك عند مقارنتها بالعينة الضابطة (الكحول الإثيلي). من أهم أنواع الشذوذات التى ظهرت فى مراحل الانقسام المختلفة اللزوجة وخاصة فى الجذور التى عوملت بتركيزات مرتفعة. في الطور الاستوائى ظهر الطور الاستوائى الكولشيسينى والطور النجمي. أما فى الطور الانفصالى فقد ظهرت القناطر الصبغية نتيجة للزوجة أو نتيجة حدوث الكسور الكروموسومية وإعادة التحامها. هذا بالإضافة إلى ظهور عدد من الشذوذات الأخرى مثل الكروموسومات الحائرة أو المتلكئة والخلايا ثلاثية أو عديدة الأقطاب والتشتت الكروموسومى فى الطور الاستوائى أو الانفصالى- النهائى. ظهر أيضاً فى الطور البينى عدد من الخلايا ذات الأنوية الدقيقة أو الخلايا المتعددة الأنوية والخلايا المتضاعفة.

امتدت الدراسة أيضاً لتشمل تأثير كل الهرمونات الساليسلك والجاسمونيك على كمية الدنا فى الأنوية وكذلك على نسبة أطوار دورة الخلية. أدت المعاملة بالهرمونات النباتية الى تغير فى كمية الدنا وفى نسب أطوار الخلايا كما أدت ايضاً إلى تغير فى معامل الدنا DNA index مقارنة بالعينة الضابطة. أظهرت النتائج أن معظم المعاملات أدت إلى إنخفاض نسبة الخلايا في مرحلة G_1 وزيادة نسبة الخلايا وتراكمها في مرحلة G_2 ومرحلة تخليق حمض DNA وذلك عند مقارنتها بالعينة الضابطة. دلت قيمة معامل حمض DNA بعد المعاملة بكل من حمضي الساليسيلك و الجاسمونيك إلى ظهور متوسط للخلايا المطفرة aneuploidy. وأوضحت النتائج أن الانخفاض فى النشاط الميتوزى بعد معاملة الجذور بهذه الهرمونات يرجع غالباً الى تراكم الخلايا في مرحلة G_2 أو الى استطالة فترة تخليق الدنا S-phase وليس الى تثبيط الحمض النووي DNA.

أدت معاملة بذور الفـول بتركيــزات مختلفة من حمض الجاسمونيك لمـدة ٢٤ ساعة إلى تغير في عدد من الأشرطة البروتينية وذلك عند مقارنتها بالعينة الضابطة. كما أدت المعاملة أيضاً إلى تغير فى كثافة الأشرطة البروتينية وظهور أشرطة جديدة أو إختفاء أشرطة أخرى. وقد يكون هذا التغير فى الاشرطة البروتينية راجعاً إلى حدوث طفرات جينية أو إلى حدوث شذوذات كروموسومية التي قد تؤدي إلى فقد بعض أجزاء من المادة الوراثية مما يؤدي إلى حدوث تغيرات فى الجينات التركيبية أو فى الجينات المنظمة لعمل الجينات التركيبية وبالتالى يؤدى إلى ظهور بعض الحزم أو اختفاء اخرى.