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ELECTROPHORETIC IDENTIFICATION FOR GENOTOXIC EFFECTS OF ETHYLE METANE SULPHONATE AND ENDOXAN IN ONION (*Allium cepa, L*). ROOT TIP CELLS

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ABSTRACT

Electrophoretic protein profiles were used in this study as a successful tool to estimate the possible mutagenic potentialities produced by the possible genotoxic effect Ethyle methane sulphonate ., Endoxan and its relation to the variations induced in mitotic index . This work aimed to study the relation between protein banding patterns and mitotic index in *Allium cepa*, L . root tip cells treated with three concentrations from each of EMS and Endoxan. The results appeared that there was a progressive decrease in protein synthesis of 16 out from 18 bands obtained from electrophoretic patterns .Protein biosynthesis was markedly affected by EMS than Endoxan as shown in bands intensity and mitotic index in EMS treatments compared with Endoxan. This indicated that the intensity of protein bands was related to mitotic index. Both chemical agents disappeared the band number five at high molecular weight region, which could be attributed to the deletion of their corresponding genes In contrast , the intensity of one band (band number 4 , which having a MW 72 KDa) was stable and did not affected by any of both chemical agents which were used in this study . Negative correlation value (-0.25) was obtained between the relative front and the molecular weight of bands. The results revealed positive correlation between mitotic index and protein intensity affected by EMS and Endoxan. The results indicated that EMS and Endoxan were markedly effective on the level of gene expression as shown from the intensity of bands obtained herein. Thus, electrophoretic technique of protein patterns have been used as a successeful tool to estimate the possible mutagenic potentialities induced by EMS and Endoxan via variations induced in protein intensity .

Keywords: Bands intensity, Endoxan, EMS, Mitotic index, Protein patterns

INTRODUCTION

Man today is concerned very much with the pollution of the environment. The field of environmental mutagenesis still needs more efforts in order to evaluate many thousands of pollutants that are released every day in our environment. Higher plants provide valuable genetic bioassay systems for screening and monitoring of genotoxic agents and have recognized as excellent monitors of mutagenic effects (Grant and Owens, 2002 and 2006). *Allium cepa* and *Vicia faba* chromosomal aberration bioassay is an efficient and reliable short-term bioassay for the rapid screening of chemicals for clastogencity (Ma,1982). Thus, higher plants, particularly *Allium cepa* and *Vicia faba* possess many advantages that make them ideal for use by geneticists in the field of environmental mutagenesis for screening and monitoring of genotoxic agents according to the International Program on Chemical Safety (IPCS) ; the World Health Organization and Enviromental protection Agency (EPA) , us . The advantages of using higher plant genetic

bioassays for testing, monitoring and screening chemicals or pollutants are; that higher plants are eukaryotes and have a chromosome structure similar to man and other animals. Plant cells also undergo mitosis and meiosis and can mutate in a manner similar to human and animal cells. Many authors investigated the potentialities of higher plant genetic systems for monitoring and screening chemical mutagens (Grantand Salamone 1994 and Kanaya *et al.* 1994) .

Many investigators had studied the side effect of the mutagenic agents and chemicals which used inhibitor cancer on heredity material of different plant cells. Chemicals had genotoxic effect on both mitotic and meiotic cells when treated with high doses. So that, mitotic index determine the percentage of divided cells with respect to the total number of cells. The divided cells are countered using all phases of mitosis. In this respect, it should be indicated that divided cells take a very short time and the cycle is completed very fast. Therefore, it is expected to count few numberof cells in a diving phase at a giving time. Since the mutagenic agents are known to affect cell division , thus , it was expected to count less number of dividing cells for treated root tips than the normal (Ivanova *et al* . 2005) .

Cell division needs to form mitotic spindle which consists of a complex network of proteins that segregates chromosomes in eukaryotes. The faithful inheritance of chromosomes is essential for the propagation of organisms. Central to this process in eukaryotes is the mitotic spindle, an elaborate array of microtubules and associated proteins that positions and segregates chromosomes during cell division. The fundamental nature of this dynamic structure is reflected by the significant number of components that shared by human and many simpler organisms such as *Saccharomyces cerevisiae* The proteins involved in spindle function not only encompass tubulin , motor proteins , and other microtubule – associated proteins , but also the microtubule – organizing centers , kinetochore complexes , chromatin – associated proteins , regulatory kinases and phosphatates , and the anaphase – promoting complex . The dependence of cell division on the mitotic spindle makes its disruption both a cause of diseases and a target for anticancer treatments (*Wang et al. 2007*).

Electrophoretic techniques of protein have been used as a successful tool to estimate the possible mutagenic potentialities produced to continuous and accumulative effects of chemicals and correlate with the produced variation in mitotic index (Ashton and Crafts,1972). Chromosomal damage produced by chemicals may be due to an action on DNA which affect on cell division via causing an inhibitory effect on DNA , RNA synthesis , protein synthesis and statistically effect the percentage of cell division (Bell *et al* . 1967) . Other investigations were carried out to indicate the relation between mitotic changes in nucleic acid and protein content as a result of treatment with pesticides and herbicides (Ebad *et al*.1993 and Soliman and Ghoneam 2004). In addition, many reports indicated various types of polypeptides in regulation of cellular processes. These polypeptides are classified as checkpoint proteins that may constitute a signaling complex (Kulberg and Morgan 1999). Hence, ultrastructure investigation of the sub-cellular

alteration induced as a result of chemical treatments provides changes in several compartments of the cell which processed basic biochemical systems. Organelles involved in protein synthesis including ribosomes, polysomes and endoplasmic reticulum are constantly submitted to exploration during growth under variable conditions (Zaki and Tawab, 2001). Indeed, protein synthesis has a remarkable influence on mitotic process and cell cycle control as shown in this study.

The work presented herein focuses on studying the relation between changes in mitotic index that occur at cellular level and associated its relation to with electrophoretic protein patterns. Also, the capacity of EMS and Endoxan on mitotic index and the resulting changes in protein electrophoretic profiles were investigated.

MATERIALS AND METHODS

Materials:

1-Plant materials:

Onion (*Allium cepa* , L .) root tip cells were used for bioassay . Onion bulbs were purchased from the local market in Mansoura city through April 2010 to be used in this study Adventitious. Root meristem raisedin water were treated with three different concentrations for each of EMS or Endoxan.

2- Mutagenic and anti carcinogenic agents:

- Ethyl methane sulfonate (EMS): It was used as amutagenic agent via subjected using germinated onion bulbs the following concentrations ; 200 , 300 and 400 ppm of EMS .
- Cyclophosphamide (Endoxan): It was used as anticancer agent via subjected germinated bulbs to the following concentrations ; 3.0 , 6.0 and 9.0 mg / ml OF Endoxan .

3- Protein extraction buffers

The total root tips proteins were extracted from onion roots using the extraction solution which containing 2% sodium dodecylsulfate (SDS), 6M (36.04g) urea and 1.5% β- mercaptoethanol (2-ME) (Abd El-Karim, 1999). The extraction buffer was prepared freshly for each electrophoresis run. The used solution for the extraction included 0.002% of tracking day (bromophenol blue).

4-Protein electrophoresis buffer:

The stock solution for total protein electrophoresis was prepared according to **Laemmli (1970)**.

5- Total protein staining:

Commassie blue (0.025%)	25 mg/100 ml
Methanol (MeOH)	50ml
Glacial acetic acid	5 ml
Trichloro acetic acid (TCA)	15gm
Distilled Water	200 ml

6- Gel destaining solution:

Methanol (MeOH)	240 ml
Glacial acetic acid	40 ml
Distilled Water	560 ml

7- 10% sodium dodecyl sulphate (SDS)

SDS	10 gm
Distilled water	100 ml

Methods:

Root tips proteins extraction :

The soluble root proteins were extracted from 0.5 g root material. One half gram from the roots was ground thoroughly in a per- chilled mortar and root was manually ground to a fine powder under liquid N_2 and mixed in a two ml buffer and centrifuged at 14000 rpm at 4°c for 15 min ., using Hettich EBA 12 R centrifuge . The clear supernatant was transferred into new tubes and stored at 20°c antifusage using (Abd El- Karim . *et al* ., 1999) .

Total protein banding patterns were separated electrophoretically using SDS discontinuous gel as slabs according to Stegemann *et al* . (1988).

Forty five µl of extracted proteins were applied and contast 20 ml Ambeer (mA) was adapted for about 6 hrs using CBS Scientific Co – Model : DSG – 200-02 . The gel was placed in staining solution using Brilliant blue – R250 as a protein chromocouple reagent for about two hours .Then the gel was transferred to the destining solution twice until background became clear. This part was conducted using the equibments of Researsh and Training Center of Rice (RTCC) , at Shaka , Kafr El – Sheikh Governorate , Agriculture Research Center .

Relative front (RF) : It was determined automatically by the software , for each standard band , acorrding to Warner and Onderdonk (2003) via measuring the distance from the top of the resolving gel till the dye front using the following formula ;

Rf = migration distance of the protein / migration distance of the dye front

RESULTS AND DISCUSSION

Electrophoretic protein profiles were successfully used by some authors to establish biochemical genetic finger prints of many plants (Ashtonand Crafts 1972) . Electrophoretic techniques of protein has been used in this study as a successful tool to estimate the possible mutagenic potentialities produced due to continuous and accumulative effects of chemicals and their correlation with the produced variations upon mitotic index .

As shown from the results presented in Figure (1) and illustrated in Table (1) that protein synthesis was progressive decrease in 16 bands (numbered ; 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17and18) out from 18 bands obtained from electrophoretic ptotein patterns. It was directly influnced by treatments of both EMS and Endoxan as well.

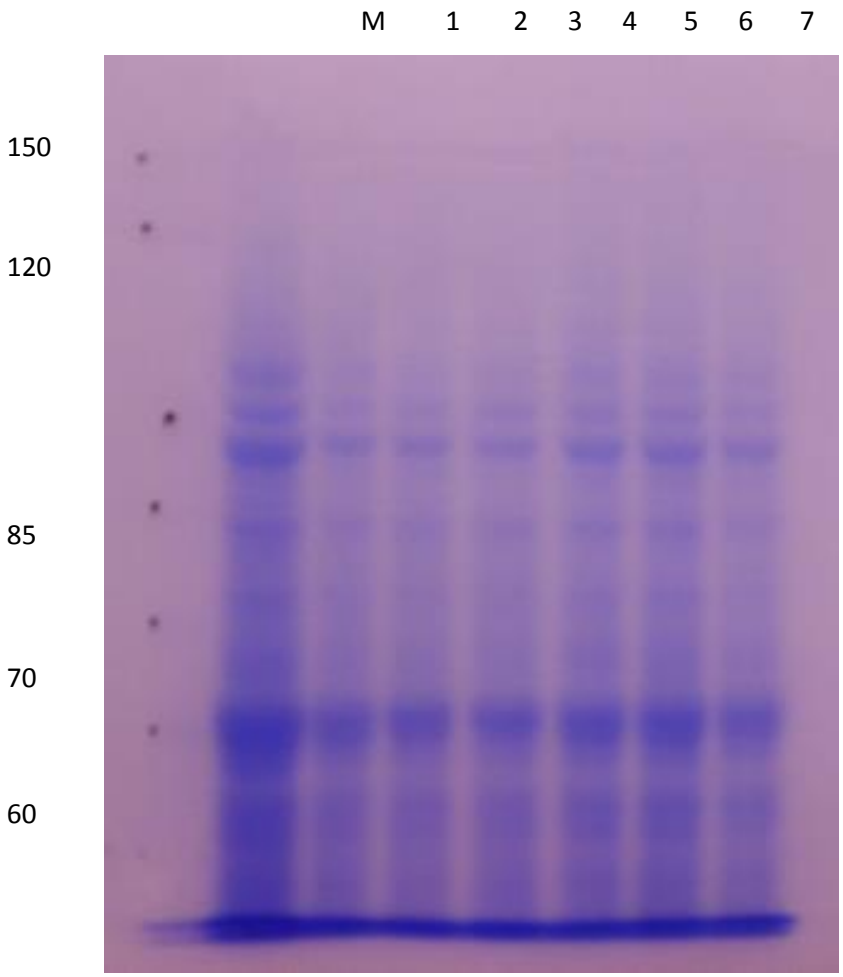

Figure1. Electrophotographs produced by SDS – PAGE analysis of protein patterns extracted *Allium cepa* L. root ips after treated with different concentrations of EMS and Endoxan.

Notes:

M: Marker ; 1: control ; 2, 3 and 4 : 200, 300 and 400 ppm of EMS ; 5,6 and 7 : 3,6 and 9 mg / ml of Endoxan ; respectively .

Table 1: SDS-PAGE bands of water – soluble proteins extracted from root tip cells of onion treated with EMS and Endoxan .

Bands No .	Control	EMS (ppm)			Mean	Endoxan (mg/ ml)			Mean	M.W (kDa)	RF
		200	300	400		3	6	9			
1	+++	+	+	+	1.00	++	++	++	2.00	88	0.396
2	++++	++	++	++	2.00	+++	+++	++	2.66	82	0.431
3	+++++	+++	+++	+++	3.00	++++	++++	+++	3.66	76	0.472
4	+	+	+	+	1.00	+	+	+	1.00	72	0.501
5	+	-	-	-	0.00	-	-	-	0.00	69	0.528
6	+++	++	++	++	2.00	++	++	++	2.00	66	0.554
7	++	+	+	+	1.00	++	++	+	1.66	59	0.625
8	++	+	+	+	1.00	++	++	+	1.66	57	0.648
9	++	+	+	+	1.00	++	++	+	1.66	54	0.68
10	++	+	+	+	1.00	++	++	+	1.66	52	0.709
11	+++++	+++	+++	+++	3.00	+++	+++	+++	3.00	48	0.759
12	+++++	+++	+++	+++	3.00	+++	+++	+++	3.00	47	0.777
13	+++	+	+	+	1.00	++	+	++	1.66	46	0.798
14	++++	++	++	++	2.00	++	++	++	2.00	43	0.848
15	++++	++	++	++	2.00	++	++	++	2.00	42	0.871
16	++++	++	++	++	2.00	++	++	++	2.00	41	0.9
17	++++	+	+	+	1.00	++	++	+	1.66	39	0.942
18	++++	+	+	+	1.00	++	++	+	1.66	38	0.958
Mean	3.86	1.86	2.06	1.86		2.53	2.46	2.00		**r= - 0.25**	
MI%	13.26	4.66	3.89	3.77	**r= 0.98**	10.10	9.78	9.66	**r= 0.99**		
MI% as of control	100	35.14	29.34	28.43	**r=0.99**	76.17	73.75	72.85	**r= 0.99**		

Where: MI: Mitotic index = $\frac{No.of\ divided\ cells}{Total\ No.of\ cells} \times 100.$

MW= Molecule weight; + = present ; - = absent ; R f = relative front ; r= correlation

Quantative decreases in total soluble protein are obvious as compared by the control . Protein biosynthesis was markedly affected by EMS than that by Endoxan treatments . Remarkable changes in electrophoretic protein profile induced by EMS was shown in bands numbered ; 1,2,7,8,9,10,17 and 18 . The intensity of these bands was faint

than that of those affected by Endoxan . This was corresponds with the data obtained herein in mitotic index. It was revealed that there was a progressive decrease in bands intensity and mitotic index in EMS treatments than in Endoxan. This result indicated that the intensity of protein bands was related to mitotic index, as well as , mitotic index was more affected by the genotoxicity of EMS which was higher than that of Endoxan because of lower mitotic index and faint intensity of bands in EMS than Endoxan . This result was agreed with that obtained by Hussein and Salam (1985), who stated that each band in the protein banding pattern of an organism reflects a separate transcriptional event . These changes in band intensity could be due to mutants induced at the regulatory system which modulates, attenuate transcription rate of a particular structural gene (Grist *et al*., 1992). This leads to the production of faint expressed protein bands (Barakat and Hassan ,1997) . In addition, the recorded changes in band intensity could be attributed to the cytological abnormalities and reduction in mitotic index induced by EMS and Endoxan treatments However Salam *et al*., (1993) found that the increase in band intensity could be interpreted on the base of gene duplication which was resulted from cytological abnormalities . Furthermore, electrophoretic analysis of the protein provides information concerning the activity of structural genes and their regulatory systems that control the biosynthetic pathways of that protein. The results showed that both chemical agents (EMS and Endoxan) caused many changes in protein banding patterns of *Allium cepa*, L. which was closely related to mitotic index. This indicated that there are some genes controlling mitotic cell division and they were more affected by EMS than Endoxan. Protein banding patterns reflected the expression of gene activity , as well as , any mutants may affect gene expression . The effects of both chemical agents were disappearance the band number five which induced at high molecular weight region . The disappearance of this band could be attributed to the deletion of their corresponding genes which related to fragmentation as shown. On the other hand, the intensity of one band (band number 4, which having a MW 72 KDa) was stable and did not affected by any of both chemical agents . The data cleared that relative front was negatively correlated with the weight of bands as shown from negative correlation (- 0.25) between both of them . The intensity of bands reflected genome activity as shown in EMS and Endoxan treatments if compared with untreated samples. The results indicated that there was a change in the level of gene expression and regulation as estimated by the bands intensity . The progressive decrease in protein synthesis of all treated root cells was directly more influenced by EMS than Endoxan . Severe decreases in total percentage of protein in all treated samples other than control may be due to the effect on one of three steps of protein synthesis machinery; on ribosome as to block either the translation reactionor polypeptidyl transferase reaction, as reported by Jimenez (1988) . or to block the mRNA synthesis as previously reported by (Perentesis *et al.,* 1992). However,mitotic index (MI) reflects the frequency of cell division and

is regarded as an important in evaluating the rate of cell division and root growth . The biochemical changesin protein intensity reflects the rate of mitotic index in onion root tips treated with EMS and Endoxan. Although, mitotic index values and protein intensity were progressively increased in Endoxan other than EMS treatments . This phenomenon was shown from positive correlation obtained between mitotic index and protein intensity affected by EMS and Endoxan which was equal ; + 0.98 and 0.99 , respectively .This indicated that the pattern of increase in protein intensity was closely related to the increase of division rate . These results agreed with that reported by Ghareeb (1998) , who found that biochemical analysis of M_2 plants of *Vicia faba* treated with topogard showed several changes in protein banding patterns as compared with the control pattern . Furthermore, El – Nahas (2000) mentioned that imazethapyr and its combination with urea has a great ability to induce changes in the protein banding patterns of *Vicia faba* seed storage protein as compared with untreated samples . In addition, Tomkins and Grant (1972) suggested that inhibitation of cell division may cause an inhibitory effect on DNA and RNA synthesis . The results obtained in this study indicated that there was a relation between changes in mitotic index with changes in protein intensity as a result of treatments with EMS and Endoxan. Thus, electrophoretic technique of protein have been used as a successful tool to estimate the possible mutagenic potentialities produced due to EMS and Endoxan via variations induced in protein intensity. This agreed with with the results obtained by Gorinstein *et al*. (1999), who confirmed that electrophoretic patterns of the protein fraction are directly related to the genetic background of the protein and used to certify the genetic makeup of wild type or newly derived.

The results also showed that EMS and Endoxan induced different mitotic changes on root tips of onion. Such changes vary from the reduction of mitotic index of meristematic cells, as well as, electrophoretic protein patterns. Inhibition of mitotic division in plants has been attributed to a number of factors (Deysson , 1968) .The inhibition of mitotic index may be due to the interference of the tested compound in the normal process of mitosis by reducing the number of the dividing cells (Ghareeb and George ,1997). Many other investigators have attributed the depression in mitotic index values to the inhibition of protein synthesis essential in the mitotic cycle among them, Kim and Bendixen (1987). Similar results were obtained by El-Nahas , (2000) , who found that the inhibition of cell division was accompanied with many changes in the protein banding patterns of *Vicia faba* M_2 seeds whose parents were previously treated with imazethapyr herbicide The inhibition of cell division could be due to the inhibition of DNA synthesis which is considered as one of the major perquisites for a cell to divide (Adam *et al*. 1990).

In conclusion, it can be concluded that EMS and Endoxan caused cytotoxicity in onion root tip cells via reducing the rate of cell division. Reduction in mitotic index which was more pronounced in this regard by EMS than that by Endoxan treatments There was a closely relation between mitotic index and electrophoretic protein patterns, as well.

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التوصيف الكهربى للسميه الوراثيه الناتجه من تأثير الإيثايل ميثان سلفونيت والإندوكسان فى خلايا القمم النامية لجذور البصل .

على ماهرمحمد العدل ، خليفه عبد المقصود زايد، كوثر سعد قش و ميرفت إبراهيم كمال
قسم الوراثة- كليه الزراعة – جامعه المنصورة .

إستخدم التحليل الكهربى للبروتين فى هذه الدراسه كأداة ناجحة لتقدير الطفرات المحتملة الحدوث والراجعة للتأثيرات السامه لكل من الإيثايل ميثان سلفونيت والإندوكسان وعلاقتها بالإختلافات الناتجة عن معدل الإنقسام الميتوزى للخلايا . يهدف هذا البحث إلى دراسة العلاقة بين أنماط حزم البروتين ومعدل الإنقسام الميتوزى فى خلايا القمم النامية لجذور البصل والمعاملة بثلاث تركيزات مختلفة من كل من الإيثايل ميثان سلفونيت والإندوكسان .

أظهرت النتائج وجود إنخفاض واضح فى معدل تخليق البروتين لعدد ١٦ حزمة من الـ ١٨ حزمة المتحصل عليها من الفصل الكهربى للبروتين . تأثير معدل تخليق البروتين بشكل واضح بمعاملات الإيثايل ميثان سلفونيت مقارنة بالإندوكسان . تعكس هذه النتائج أن كثافة حزم البروتين لها علاقة بمعدل الإنقسام الخلوى . نتج عن المعاملة بكلا المادتين المستخدمتين فى الدراسة إختفاء الحزمة البروتينية الخامسة عند المنطقة ذات الوزن الجزيئ المرتفع ، ويرجع ذلك إلى فقد الجينات المسئولة عن إنتاج بروتين هذه الحزمة . وعلى النقيض من ذلك ، فإن كثافة إحدى الحزم البروتينية وهى الحزمة الرابعة ذات الوزن الجزئ ٧٢ كانت ثابتة تماماً ولم تتأثر بأى من المواد الكيماوية المستخدمة فى هذه الدراسة . عكست النتائج وجود قيمة سالبة لمعامل الإرتباط– ٠.٢٥بين الحركة النسبية والوزن الجزيئ للحزمة البروتينية . كما أظهرت النتائج وجود إرتباط موجب بين معدل الإنقسام الخلوى وكثافة الحزمة البروتينية المتأثرة بكل من الإيثايل ميثان سلفونيت والإندوكسان . تضمنت النتائج المتحصل عليها أن الإيثايل ميثان سلفونيت والإندوكسان قد أثر بشكل واضح على معدل التعبير الجينى كما هو واضح فى كثافة الحزمة البروتينية المتحصل عليها فى هذه الدراسة . لذلك فإن تكنيك الفصل الكهربائى لأنماط حزم البروتين يمكن إستخدامه كطريقة ناجحة لتقدير التأثيرات الطفرية الممكنة والناتجة عن المعاملة بالمواد المطفرة مثل الإيثايل ميثان سلفونيت وتلك الناتجة عن المعاملة بالمواد المستخدمة فى علاج السرطان مثل الإندوكسان وذلك من خلال قياس معدل الإختلافات الناتجة عن كثافة حزم البروتين المفصوة كهربياً .

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