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AUTOPOLYPLOIDY IN SUGAR BEET GENOME: DIFFERENTIAL EFFECT OF CHEMICALS

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ABSTRACT

Three spindle fiber inhibitors (8-hydroxyquinoline, para-dichlorobenzene and colchicine) at four concentrations were used in sugar beet (*Beta vulgaris* L.) plants to induce polyploidy. The aberrations were recorded and recognized in root tip and young leave cells. The results obtained showed that different types of aberrations were observed. These types are fragmentation, ring shape, stickiness, gaps, bridge, cell in lyses and end to end association. Different ratios were recorded for the three tested compounds. However, colchicine was proven to induce the minimum ratio of aberrations and to be the effective compound in causing polyploidy compared with other compounds.

INTRODUCTION

Sugar beet as a newer crop in Egypt has been recently introduced for sugar production to reduce the gap between the national consumption and the total sugar production. The production of beet sugar in Egypt accounts for about 25% of the total sugar production. Breeding program of sugar beet started in Egypt during the last two decades of past century by several investigator's and breeders, Younan (1984); El-Manhaly *et.al.* (1987); Saleh (1993); Ghura (1995); El-Manhaly *et.al.* (2004); Ghonema (2005) and Saleh *et al.* (2008).

From a plant breeder's point of view, induction of polyploidy may originate new genetic combinations and providing breeders more variability. The induction of polyploidy to improve agronomic yields is a process that commonly used in plants of economic interest (Allard 1960). Polyploidy has been applied to different plant species by several investigators (Gupta and Roy, 1986), (Cruz *et al.*, (1993), (Ghandi and Patil, 1997) and (Romero-Aranda *et al.*, 1997). A better adaptability of individuals and increased organ and cell sizes are usually associated with polyploidy (Guerra, 1988).

By the end of the 1930_s several sugar beet breeders and research workers had started to produce autotetrapliod beet (2n = 4X = 36), and initially there were great hops that these would result in substantial yield increases (Rasmusson and Levan, 1939 & Kloen. and Speckmann, 1953). In Egypt, Saleh (2008), used three different spindle fiber inhibitor reagents (8-hydroxyquinoline,para-dichlorobenzene and colchicine) to produce autotetraploid sugar beet plants which can be used to improved the agronomic characters of sugar beet in sugar beet breeding program in Egypt.

The present investigation aims to induce autopolyploidy in sugar beet genotypes and to detect chromosomal aberrations in plant cells. To achieve such a purpose polygerm diploid genotype (C39) and three chemical

compounds i.e., 8-hydroxyquinoline, Para-dichlorobenzene and colchicine were chosen and employed.

MATERIALS AND METHODS

1. Materials:

1.1. Sugar beet materials:

Sugar beet (*Beta vulgaris* L.) polygerm diploid genotype (C39) was used in this investigation. This variety was obtained from Sugar Crops Research Institute, Agricultural Research Center (ARC), Ministry of Agriculture, Egypt.

1.2.Treatment:

Three reagents (8-hydroxyquinoline, para-dichlorobenzene and colchicine) were used in this study at a level of four concentrations as shown in (Table, 1).

Table (1): Chemical compounds used for induction of polyploidy

Reagent	Concentration			
	0	I	II	III
1- 8-hydroxyquinoline	-	saturated solution (Oxy I)	1/2 saturated solution(Oxy II)	1/4 saturated solution(Oxy III)
2- Para-dichlorobenzine	-	saturated solution (Para I)	1/2 saturated solution (Para II)	1/4 saturated solution(Para III)
3- Colchicine	-	0.05% (Colch I)	0.02% (Colch II)	0.01%(Colch III)

2. Methods:

2.1. 1st Experiment

Seeds of the examined sugar beet variety (C39) were soaked in running tap water for twelve hours, and then were transferred on filter paper moistened with the used chemical compound and allowed to germinate at 23ºC in incubator. Root tips were collected for chromosome examination after three days when a length of root tips of 1- 1.5 cm had reached.

2.2. 2nd Experiment

Sugar beet seeds were soaked in running tap water for twelve hours and then transferred for polyploidy treatment by planting on cotton moistened with the chemical compound in Petri dishes. Seeds were allowed to germinate in incubator. Germinated seeds were transferred into plastic pots contained sandy clay soil 1:1 in three replicates for each treatment and kept in incubator at 23ºC until cotyledon leaves were appeared and were transferred into open weather. Irrigation with polyploidy treatment was continued for three weeks after germination and then fresh water irrigation was started till the end of experiment. Leaf samples were collected for chromosome examination after 45 days.

2.3. Cytological examination

Root tip or young leave samples of **s**ugar beet material were collected for chromosome examination. Root tips and leaf samples were prepared for chromosome analysis by the method described by (Saleh, 2008).

2.3.1. Preparation of investigated materials

Root tip's and leaf samples were taken for cytological investigation collected and fixed by Carnoy solution (consists of 3 parts of absolute alcohol: one part of glacial acetic acid) for 24 hours at least, and then transferred to 70% ethyl alcohol and kept in a refrigerator until usage. Hydrolysis of the studying materials was done by 1*N* HCL at 60°C for three minutes (for root tips) and two minutes (for young leaves).

2.3.2. Staining technique:

Materials were stained by lacto-propionic orcein for at lest 15-20 minutes.

2.3.2.1. Preparation of Lacto-propionic orcein

a) Stock solution:

-Dissolve 1gm of synthetic orcein in 50 ml of a mixture of equal parts of lactic and propionic acid and Filtration was carried out at room temperature.

b) Working solution:

-Dilute the stock solution to 45 % with distilled water.

2.3.3. Metaphase index:

-Metaphase stages were examined and metaphase index was calculated according to the following formula:

$$\text{Metaphase index} = \frac{\text{No. of metaphase cells}}{\text{Total examined cells}} \times 100$$

RESULTS AND DISCUSSION

1. Cytological examination:

There have been a considerable number of reports upon the chromosome number and structure of sugar beet complement (e.g. Adati & Mistuishi, 1962; De Jong & De Bock, 1978; El-Maghawrey, 2001; Ghonema, 2005; Saleh, 2008 and Ghonema *et al.,* 2009). However, all reports revealed that the chromosomes are small in size; difficult to differentiate one from another; and the DNA content is lower than that of several plants (Arumuganathan & Earle, 1991; and Sangeeta *et al*., 2000).

Root tips and young leaves samples were cytologically examined to investigate the effect of treatment on chromosome number, behavior and aberrations. The effect of each compound caught be summarized as follow:

1.1. Effect of 8-hydroxyquinoline:

Metaphase index was calculated as shown in Table (2), it was (12.2, 11.7 and 11) in root tip cells and (13.3, 11.6 and 11.5) in young leave cells for concentrations (I, II, and III); respectively. Chromosomal aberrations in sugar beet root tip and young leave cells after treatment with the first concentration of 8-hydroxyquinoline (Oxy I) were presented in (Table, 2). The data show that the types of abnormalities in root tip cells after such treatment were 50.7% cell in lyses and 30.3% stickiness. In young leave cells the aberrations were 15.2% stickiness. In the second concentration, (Oxy II) the aberrations presented in root tip cells as ring shape 3.7%, stickiness 11.2%, gaps 4.7%, bridge 7.1% and cell in lyses 22.1%. While in young leave cells the

abnormalities was 37.7% stickiness. The data indicated that the third concentration (Oxy III) was not capable to induce chromosomal abnormalities in root tip cells, while the aberration was 33.5% stickiness in young leave cells, such aberration in young leave cells might be due to the long treatment period (three weeks). These results are in accordance with those obtained by Sentein, (1970) who demonstrated that quinoline in saturated solution (0.46 M) progressively destroys spindle and astral fibers. He reported that with less concentrated solutions monopolar mitoses and monopolar telophases (rosettes) were observed (1/8 saturated solution), then shortened bipolar mitoses (1/16 saturated). He noticed qualitative differences between quinoline and colchicine actions. Van-Baarlen *et al.* (2000) used 8-hydroxyquinoline to examine the chromosome numbers in *(Taraxacum officinale* L.), they first pre-treated in a 1% aqueous solution of the spindle inhibitor 8-hydroxyquinoline at 6–8°C.

Table (2): Chromosomal aberrations induced by 8-hydroxyquinoline treatment in sugar beet root tip and young leave cells

Concentration	Fragmen-tation	Ring shape	Sticki-ness	Gaps	Bridge	Cell in lysis	End to end associa-tion	Metaphase index
		Chromosomal aberrations in root tip cells %						
I	0.0	0.0	30.3	0.0	0.0	50.7	0.0	12.2
II	0.0	3.7	11.2	4.7	7.1	22.1	0.0	11.7
III	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.0
		Chromosomal aberrations in young leave cells %						
I	0.0	0.0	15.2	0.0	0.0	0.0	0.0	13.3
II	0.0	0.0	37.7	0.0	0.0	0.0	0.0	11.6
III	0.0	0.0	33.5	0.0	0.0	0.0	0.0	11.5

1.2. Effect of para-dichlorobenzine:

Metaphase index was calculated as shown in (Table, 3). Regarding para-dichlorobenzine, the obtained data can be noticed that at the first concentration (Para I) the type of aberration was 12.5% fragmentation, 4.5% ring shape, 4.2% stickiness, 4.9% gaps, 7.7% bridge, 8.3% cell in lyses and 11.3% end to end association in root tip cells. While in young leave cells the abnormalities was 20.1% Stickiness. Second concentration (Para II) was found to be negative in inducing chromosomal abnormalities in root tip cells; while in young leave cells there was 11.3% stickiness. No chromosomal aberrations was found in root tip cells after treatment with the 3^{ed} concentration, while in young leave cells 4.9% stickiness was found (Table, 3). The results of para-dichlorobenzine treatment are similar with those obtained by Meyer (1945), firstly he reported that para-dichlorobenzine not only causes spindle inhibition but also leads to clarification of chromosome constrictions due to the contraction and differential hydration of chromosome segments. De-Oliveira, *et al.,* (2004), pretreated the *Stevia rebaudiana* root tips with para-dichlorobenzene for 4 h at 16 -18°C and he obtained high percentages of C- metaphases.

Table (3): Chromosomal aberrations induced by para-dichlorobenzine treatment in sugar beet root tip and young leave cells

Concentration	Chromosomal aberrations in root tip cells %							
	Fragme-ntation	Ring shape	Sticki-ness	Gaps	Bridge	Cell in lysis	End to End associa-tion	Metaphase index
I	12.5	4.5	4.2	4.9	7.7	8.3	11.3	13.3
II	0	0	0	0	0	0	0	12.9
III	0	0	0	0	0	0	0	11.0
	Chromosomal aberrations in young leave cells %							
I	0	0	20.1	0	0	0	0	10.3
II	0	0	11.3	0	0	0	0	11.2
III	0	0	4.9	0	0	0	0	10.3

1.3. Effect of colchicine:

Metaphase index and the effect of colchicine on sugar beet root tips and young leaves are given in (Table, 4). Cytological examination revealed that the first concentration of colchicine was capable in inducing different types of chromosomal aberrations. These aberrations were not observed in the control group, there were 4.7% gaps, 13.3% bridge and 11.2% end to end association in root tip cells. In young leave cells chromosomal aberration was 17.7% stickiness. Chromosomal aberrations results after colchicine treatment at the second concentration was 4.5% ring shape, 7.9% stickiness, 8.9% gaps, 20.3% bridge and 6.3% end to end association. The abnormalities were 8.2% gaps, 7.7% bridge and 11.9% end to end association in young leave cells at the same colchicine concentration. Chromosomal aberrations in root tip cells induced by the third concentration of colchicine were 30.3% stickiness, 20.1% gaps, 7.4% bridge and 11.2% end to end association. In young leave cells the abnormalities after colchicine treatment in such concentration was 8.3% stickiness. These results are in agreement with those obtained by (Saleh, 2008).

Table (4): Chromosomal aberrations induced by colchicine treatment in sugar beet root tip and young leave cells

Concentration	Chromosomal aberrations in root tip cells %							
	Fragme-ntation	Ring shape	Sticki-ness	Gaps	Bridge	Cell in lysis	End to end associ-ation	Metaphase index
I	0.0	0.0	0.0	4.7	13.3	0.0	11.2	14.2
II	0.0	4.5	7.9	8.9	20.3	0.0	6.3	12.7
III	0.0	0.0	30.3	20.1	7.4	0.0	11.2	11.0
	Chromosomal aberrations in young leave cells %							
I	0.0	0.0	17.7	0.0	0.0	0.0	0.0	12.9
II	0.0	0.0	0.0	8.2	7.7	0.0	11.9	11.1
III	0.0	0.0	8.3	0.0	0.0	0.0	0.0	10.8

1.4. Efficiency of compounds:

The capability of the used compounds in causing polyploidy might according to the obtained results, be arranged in the following rank colchicine > 8-hydroxyquinoline > para-dichlorobenzene. On the other hand colchicine at the highest concentrations (0.05%) induced not only the minimum chromosomal aberrations but it was very effective causing polyploidy compared with other chemical compounds. Figure (1) shows autopolyploidy resulted from such a treatment.

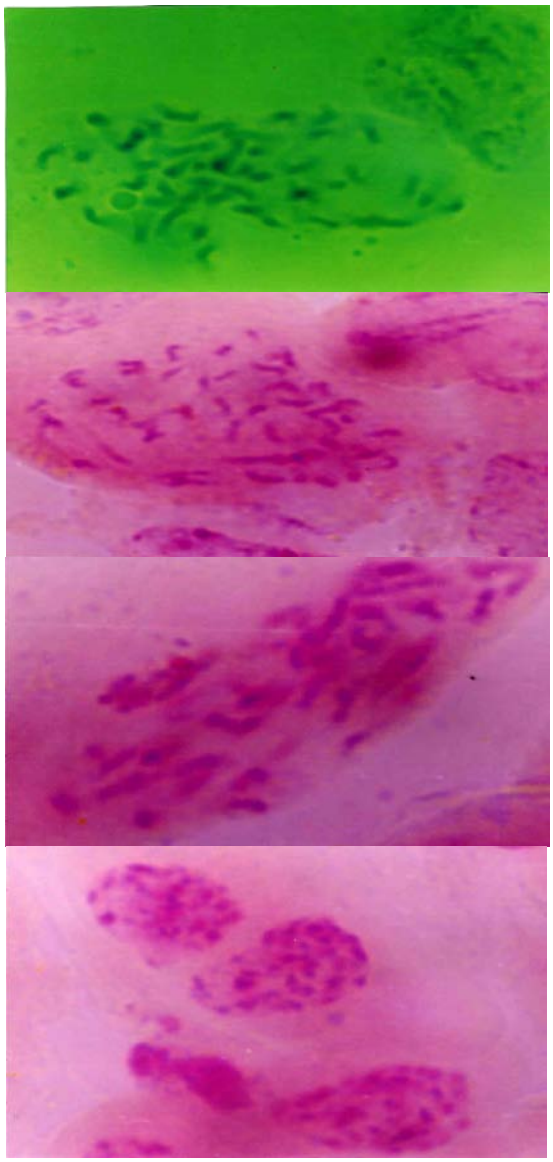

Figure (1): Photomicrograph of sugar beet root-tip cells showing metaphase stages after induction of polyploidy.

REFERENCES

Adati, S. and S. Mistuishi (1962). Karyotype analysis in the *Beta* species. Bull. Fac. Agric. Mie Univ. 25: 25-32.

Allard R.W. (1960). Principles of Plant Breeding. John Wiley & Sons, Inc., New York, 485 pp.

Arumuganathan K. and E.D. Earle (1991). Nuclear DNA content of some important plant species. Plant Mol Biol. Rep 9: 208-218.

Cruz ND, Boaventura MS, Conagin CHTM, Dutilh JHA, Forni- Martins ER, Medina DM, Mendes AJT, Pierozzi NI and Pinto-Maglio CAF (1993). Cinqüenta e Três Anos de Pesquisa em Citogenética Vegetal. Documents IAC, n. 27. 60 pp.

De-Jong, J.H. and T.S.M. De Bock (1978). Use of haploids of *Beta vulgaris* L. for the study of orcein and Giemsa stained chromosomes. Euphytica. 27: 41-47.

De-Oliveira, V. M.; Eliana R. Forni-Martins; Pedro M. Magalhaes and Marcos N. Alves, (2004). Chromosomal and morphological studies of diploid and polyploidy cytotypes of *Stevia rebaudiana* (Bertoni). Genetics and Molecular Biology. 27, 2: 215-222.

El-Maghawrey, A.M. (2001). Cytogenetical studies on sugar beet. M.Sc. Thesis. Fac. Agric. Alexandria University.

El-Manhaly, M.A.; N.Z Younan and M.A. Farage (1987). Sugar beet flowering and seed production in Egypt. Com. In Sci. Dev. Res., 19:45-61.

El-Manhaly, M.A.; M.S. Saleh; N.S.A. Ghura; M.M.M. Ahmed and B.A. Ali (2004). Identification of three Egyptian sugar beet genotypes and detection of DNA similarity. Proceedings of the 67th IIRB Congress, February 2004, Brussels (B). 169-186.

Ghandi S and Patil VP (1997). Colchicine-induced autotetraploidy in *Clitoria ternatea* L. Cytologia 62:13-18.

Ghonema, M. A. (2005). Genetical and cytological studies on bolting in sugar beet *Beta vulgaris* L. plant. Ph. D. Thesis, Faculty of Agriculture (Saba Basha), University of Alexandria Egypt.

Ghonema M. A.; M. S. Saleh and M. A. Seehy (2009). Cytogenetical Examination on Sugar Beet Bolting Phenomenon. International Conference on "World Perspectives for Sugar Crops as Food and Energy Suppliers" 1-4 March 2009, Luxor, Egypt, pp P. 3/1-15.

Ghura, Nabwya S.A. (1995). Studies on sugar beet. Evaluation of sugar beet monogerm lines and estimation of general and specific combining ability. Ph.D. Thesis, Faculty of Agriculture University of Alexandria Egypt.

Guerra M. (1988). Introduçmo à Citogenética Vegetal. Editora Guanabara, Rio de Janeiro, 142 pp.

Gupta S.K. and Roy S.K. (1986). Induced colchiploidy in *Nicandra physaloides.* Cytologia 51:319-324.

Kloen. D. and G. J. Speckmann (1953). The creation of tetraploid beets. Euphytica. 2 (3): 187 – 196.

Meyer, J. R. (1945). Stain Tech. 20: 121

Rasmusson, J., and Levan, Albert, (1939). Tetraploid sugar beets from colchicine treatments. Hereditas 25: 97-102.

Romero-Aranda R, Bondada BR, Syvertsen JP and Grosser JW (1997). Leaf characteristics and net gas exchange of diploid and autotetraploid citrus. Ann Bot 79:153-160.

Saleh, M.S. (1993). Genetical studies on sugar beet. M.Sc. Thesis, Faculty of Agriculture University of Alexandria Egypt.

Saleh, M. S, (2008). Determination of polidy levels in sugar beet plants I. Methods of chromosome counting in sugar beet plants. International Conference IS. Meeting the Challenges of Sugar Crops & Integrated Industries in Developing Countries, Al Arish, Egypt, pp 192-199.

Saleh, M. S.; El -Manhaly M. A.; Nabawya S. A. Ghura and M. A.Ghonema (2008). Genetic Profile of Three Promising Egyptian Sugarbeet (breeding materials), Genotypes. International Conference IS. Meeting the Challenges of Sugar Crops & Integrated Industries in Developing Countries, Al Arish, Egypt, pp 260-265.

Sangeeta, S.; H.M. Srivastava and S. Srivastava (2000). Cytological and karyotypic studies in four *Beta* species. Journal-of-Sugar-Beet-Research. 37: 135-142.

Sentein, P. (1970). Action de la quinoline sur les mitoses de segmentation des eufs d'urodèles: le blocage de la centrosphère. Chromosoma 32 (1): 97-134

Van-Baarlen, P.; P. J. van Dijk, R. F. Hoekstra, and J. H. de Jong, (2000). Meiotic recombination in sexual diploid and apomictic triploid dandelions *(Taraxacum officinale* L.) Genome 43: 827 – 835.

Younan, Z.N. (1984). Genetical studies on sugar beet. Ph. D. Thesis, Faculty of Agriculture University of Alexandria Egypt.

التضاعف الذاتي في نبات بنجر السكر:
مركبات كيميائيه ذات تأثير متفاوت

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إجراء التضاعف الكروموسومى لمواد التربية بصفة عامة من الأمور الهامة جدا فى برامج التربية، وبالنسبة لنبات بنجر السكر فإن إجراء التضاعف الكروموسومى يعتبر من الأهداف التى يسعى إليها العديد من الباحثين والمربين. وقد تم استخدام ثلاثة مواد مختلفة تعمل على تثبيط خيوط المغزل بغرض احداث تضاعف كروموسومى فى نباتات بنجر السكر وهذه المواد الثلاثة هى (8 هيدروكسى كينولين و الباراداى كلوروبنزين و الكولشيسين) وذلك بأربع تركيزات مختلفة لمعرفة المادة المناسبة والتركيز الأمثل لاحداث التضاعف الكروموسومى فى نبات بنجر السكر.

وقد اجري هذا البحث فى محطة البحوث الزراعية بالصبحية فى2008 وذلك بغرض معرفة تأثير هذه المواد الثلاثة تحت التجربة وتركيزاتها المختلفة على احداث تضاعفات و شذوذات كروموسومية فى نباتات بنجر السكر وذلك فى الخلايا النباتية المستخدمة فى الدراسة سواء (جذور حديثة أو أوراق حديثة) لمعرفة المادة المناسبة التى يمكن إستخدامها فى إجراء التضاعف الكروموسومى لنباتات بنجر السكر وكذلك التركيز الأمثل للمادة المستخدمة التى تستخدم بأمان أكثر من ناحية الشذوذات المختلفة.

وقد أظهرت النتائج المتحصل عليها أن الكولشيسين كان الأكثر فعاليه في احداث التضاعف عند التركيز الأعلى وكذلك الأقل في احداث الشذوذات الكروموسومية عند نفس التركيز.

قام بتحكيم البحث

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