INDUCTION OF POLYPLOIDY IN SUGAR BEET PLANTS BY DIFFERENT SPINDLE-FIBER INHIBITORS

Saleh, M. S. *; A.K. EL-Sayed**; O. M. Badawey* and Hayam, E. A. Ibrahim**
*Sugar Crops Research Institute, Department of Genetics and breeding (ARC).
** Department of Genetics, Faculty of Agriculture, University of Alexandria- (Saba-Bacha)

ABSTRACT

When it was discovered in 1937 that polyploidy can be induced in plants through the action of colchicine, many genetics and plant breeders held high hopes that a way had been opened for rapid development of novel and superior types that would revolutionize agriculture. Because polyploidy plant has larger cells than their diploid counterparts, and it seems reasonable to expect this larger cell size to contribute to larger plant size and higher yield. Two types of polyploidy sugar beet varieties were commercially produced sense this time anisoploids and triploid hybrids varieties. The present investigation was carried out in 2008 at El- Sabahia Agric. Res. Sta., Alexandria, Egypt to produce autotetrapliod beet plants,(2n = 4X = 36), which can be used in breeding program to produce sugar beet triploid varieties. Three spindle - fiber inhibitors (8-hydroxyquinoline, para-dichlorobenzene and colchicine). Three different concentrations beside control were used in this study to induce polyploidy in sugar beet plants. Data showed that the best treatment to induce polyploidy in sugar beet plant was colchicine at a level of concentration of 0.05 % which induced polyploidy percentage in root tips 84.8 % and 20.9 % in young leave cells.

INTRODUCTION

By the end of the 1930ₛ several sugar beet breeders and research workers had started to produce autotetrapliod beet (2n = 4X = 36), and hoping that these would result in substantial yield increases (Rasmusson and Levan, 1939 & Kloen. and Speckmann, 1953). Tetraploid beets tend to have larger leaves and roots, but are slower to mature; and the higher root yield is offset by lower sugar content (Poehlman, 1979).

First attempts by colchicine drug were done in 1939 by Muntzing and Runquist who treated 16 different plant species with colchicine in order to induce polyploidy. They observed that, the results in most case found to be negative, but in three species, *Linum usitatissimum, Solanum tuberosum* and *Galeopsis pubeacens*. Chromosome doubling was obtained. Rasmusson and Levan (1939) demonstrated new colchicine method for inducing tetraploid in sugar beets. Rasmusson (1953) started the successful colchicine method experiments in order to produce tetraploid sugar beet. Kloen and Speckmann, (1953) initiated a program for the development of tetraploid sugar beet by treating germinated seed with colchicines.

Sharma, (1990) reported that nearly of 400 chemicals suspected to be an eugenic to plants, ∽150 are agricultural chemicals, 60 are drugs, 60 are natural substances and 50–60 are industrial products. Nearly half of these agents were reported to affect spindle mechanism in mitosis and meiosis. About 60 affected phragmoplast function in root meristems. Many of the major events that occur during cellular segregation, such as respiration, spindle and phragmoplast functions and chromosome doubling, appear to be stressed by agricultural chemicals, drugs, natural and industrial products, leading to aneuploidy in both mitotic and meiotic cells. Sharma and Sharma (1980) demonstrated that the most important groups of compounds investigated for their action in studying chromosome structure, is the quinoline complex. Tjio and Levan (1950) maintained the importance of 8-hydroxyquinoline in

chromosome analysis.. Sentein (1970) demonstrated that quinoline in saturated solution (0, 46 M) progressively destroyed spindle and astral fibers. Saleh *et. al.* (2008) used 8-hydroxyquinoline to examine the chromosome number of twenty imported sugar beet varieties in root tip and young leaves.

Meyer (1945) was the first who demonstrat the importance of *p*-Dichlorobenzene (*p*DB) as a pro-treatment agent. It was also employed as a reagent for the production of polyploidy in plants, and because of its very low solubility in water it was used as saturated solution. Later workers (Dermen and Scott, 1950; Conagin, 1951) employed it effectively for chromosome counts. A detailed use of this compound has been made by Sharma and Roy (1955) in plants. Saleh (2008) examined different methods for chromosome counting in sugar beet plants to establish the good one in chromosome counting, He examined three chemical reagents (colchicine, 8-hydroxyquinoline and para-dichlorobenzine), at three concentrations, in both root tips and young leaf cells.

In the present study, three spindle - fiber inhibitors (8-hydroxyquinoline, para-dichlorobenzene and colchicine), at three different concentrations beside control were used to induce autotetrapliod (2n = 4X = 36) in sugar beet plants with the aim to recognize the best chemical at the proper concentration.

MATERIALS AND METHOD

1. Materials:

1.1. Sugar beet materials:

Sugar beet (*Beta vulgaris* L.) multigerm diploid breeding genotypes (C39) was obtained from Sugar Crops Res. Ins., Agric. Res. Center, Ministry of Agric., Egypt were employed in this work

1.2. Treatment:

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

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

Reagent	Concentration			
	Cont.	I	II	III
8- hydroxyquinoline	-	saturated solution (Oxy I)	½ saturated solution (Oxy II)	¼ saturated solution (Oxy III)
Para-dichlorobenzine	-	saturated solution (Para I)	½ saturated solution (Para II)	¼ saturated solution (Para III)
Colchicine	-	0.05 % (Colch I)	0.02% (Colch II)	0.01% (Colch III)

2. Methods:

2.1. Induction of polyploidy:

Seeds of sugar beet diploid breeding material (C39) were soaked in running tap water for 12 hours and then transferred into polyploidy treatment solution (8-hydroxyquinoline, or para-dichlorobenzene or colchicine) at the examined concentrations for another 12 hours before they were planted on moisture cotton in Petri dishes. Seeds were allowed to germinate in an incubator at 23 C °.

Germinated seeds were transferred into plastic pots contained sandy clay soil 1:1 in three replicates for each treatment and kept in incubator at the same degree until cotyledon leaves were appeared and then pots 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

2.2. Cytological study:

Root tips or young leaves of sugar beet materials were collected for chromosome examination from Petri dishes or plastic pots respectively.

Collected root tips and leaf samples taken for cytological investigation 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 studied materials was done by $1N$ HCL at 60 °C at three minutes for root tips and two minutes for young leaves.

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

2.3. Stomata examination analysis:

Stomata characters were studied control & treated plants to examine ploidy levels by the method described by (Saleh and Ghonema 2009). The stomata characters were focused on stomata density and stomata size (stomata length and width), and chloroplast number of the guard cell. They were examined in the lower epidermis of the treated plants. Leaf samples were collected from sugar beet plants after 45 days. The number and size of stomata was determined using the third leaf from the core. Approximately 10 leaf impressions from 10 individuals of each treatment were collected.

2.3.1. Stomata density:
Stomata density was calculated by counting the stomata in five microscopic fields per slide using a 10X objective and a 10X ocular.

2.3.2. Stomata size:
To determine stomata size, 100 stomata from each leaf impression were drawn using a camera lucida at 40X objective and a 10X ocular, and their length and width were then measured as the method described by De-Oliveira *et al.*, 2004.

2.3.3. Chloroplast number:
Chloroplast counts on the stomata guard cell of the lower epidermis were done at 100X objective and a 10X ocular by the method described by Deuter, 1970.

2.4. Statistical analysis:
The experimental design was Randomized Complete Block Design (CRD) with three replicates, and the data were analyzed according to Snedecor and Cochran, 1990.
Coefficient of variability (C.V.) was calculated for stomata characters.

RESULTS AND DISCUSSION
3.1. Cytological examinations
Root tips were examined through the investigation to recognize the effect of polyploidy treatment and its concentration on chromosome number, while, young leaves were

cytologically examined in plants after 45 days from planting to recognize the effects of polyploidy treatment and stability of polyploidy in plants. Figure (1) illustrated diploid and tetraploid sugar beet cells.

3.1.1 Effect of 8-hydroxyquinoline:

Data in (Table 2), shows that in root tip cells, averaged polyploidy cells was 71.5 %., while it was 33.3 % in young leave cells after treatment with concentration one of 8-hydroxyquinoline (Oxy I),. In the second concentration (Oxy II), polyploidy in root tip cells was 26.7 % and in young leave cells it was 12.5% of polyploidy. The third concentration (Oxy III) was not capable to induce polyploidy in root tip cells, while it was 7.1 % polyploidy cells in young leave cells. These results are in accordance with those obtained by Sentein, (1970), who demonstrated that quinoline in saturated solution (0.46 M) progressively destroyed spindle and astral fibers. Van Baarlen *et al.* (2000) used 8-hydroxyquinoline to examine the chromosome numbers in *(Taraxacum officinale* L.).

3.1.2. Effect of Para-dichlorobenzine:

Para-dichlorobenzine in concentration one (Para I) induced 71.3 % polyploidy cells in root tip cells while in young leave cells polyploidy was 30.9 %. In the second concentration (Para II) no polyploidy effect was found in root tip cells; while in young leave cells there was 6.3 % polyploidy cells. No polyploidy cells were found in root tip cells or in young leave cells in (Para III) treatment as shown in (Table 2). The results of para-dichlorobenzine treatment at all were similar with those obtained by Meyer (1945), firstly he reported that para-dichlorobenzine did not only causes spindle inhibition but also lead to clarification of chromosome constrictions due to the contraction and differential hydration of chromosome segments.

3.1.3. Effect of colchicines:

The effect of colchicine on sugar beet root tips and young leave cells is given in (Table, 2). Cytological examination revealed that the (Colch. I) was capable in inducing 84.8 % polyploidy in root tip cells, and 20.9 % in young leave cells. After (Colch.II), root tip cells showed 39.9 % polyploidy cells, while it was 11.2 % polyploidy cells in young leave. Third concentration (Colch.III), induced 4.1 % polyploidy in root tip cells and no polyploidy cells were showed in young leave cells. Many investigators and genetics used colchicine to induced polyploidy in sugar beet by different way and concentrations, (Rasmusson and Levan 1939; Muntzing and Runquist,1939).

3.2. Stomata characters:

As there have been a considerable number of reports upon the chromosome number and structure of sugar beet complement (e.g. Kakhidze, 1935; Adati & Mistuishi, 1962; De Jong & De Bock, 1978; and El-Maghawrey, 2001). All reports concerning chromosome number of sugar beet revealed that the chromosomes were 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).

For those reasons stomata characters (stomata density and stomata size) and chloroplast numbers were studied in the treated sugar beet plants compared with control plants at 45 days from plant age.

3.2.1. Stomata density:

Stomata numbers were measured in treated plants in five microscope fields to reflect stomata density. Figure (1) illustrated stomata density in diploid plants and tetraploid ones.

Table (3) presents means of stomata numbers after polyploidy treatments at 45 days from plant age. Data indicated that there were significant differences between the three studied chemicals between the examined concentrations. Significant differences were found between the interactions also. Stomata numbers varied in the examined chemicals with highest value (90.67) was found in (Para I), treatment, while the lowest one (25.07) was found in (Colch I). Figure (2 a & b) illustrated stomata density in sugar beet diploid, and tetraploid plants. The figures show the variability in cell size between diploid and tetraploid plants.

3.2.2. Stomata size

Stomata size (length and width) were measured using camera lucida in the sugar beet examined chemicals after 45 days from planting.

3.2.2.1. Stomata length:

Table (4) shows means of stomata length character after polyploidy treatment. The data indicated that there were significant differences between the three studied polyploidy chemicals significant differences were found also between concentrations and interaction.

Stomata length varied in the examined chemicals with lowest value of this character (40.83 μm), was found in (Para II) treatment while the highest value (62.5 μm) was found in (Colch II).

3.2.2.2. Stomata width

Table (5) presents means of stomata width character after polyploidy treatment at 45 days from planting. Significant differences were found between chemicals, concentrations and interaction. Lowest value in this character found to be (30.83 μm), in (Para III), treatment while, (Colch II) had the highest value in this character (45.83 μm)

4.3.3. Chloroplast number

Table (6) presents means of chloroplast numbers of the sugar beet plants at 45 days from plant age. Significant differences were found between chemicals, concentrations and interaction. Chloroplast number varied in the examined chemicals with the lowest value (7.8) was found in (Oxy III), while the highest value (14.3) was found in (Colch I). Figure (3) showed photomicrograph of chloroplast number in diploid and tetraploid sugar beet guard cells. Such a result is in accordance with those obtained by Rasmusson and Levan (1939), they reported that size of the stomata is easily demonstrated, at least between diploids and tetraploids. Boaventura et al. (1981) reported that higher levels of ploidy generally produce larger stomatal cells with a greater number of chloroplasts in some species of coffee. Manojlovic et al. (1995) illustrated that chloroplast number from 8 -12 in the guard cell of the diploid sugar beet plants. Yudanova et al. (2002) demonstrated that diploid sugar beet plants showed a broad variability in the number of chloroplasts in stoma guard cells, which is related to myxoploidy of cell populations in leaf apical meristems.

4.3.4. Coefficients of variation

Coefficient of variations was studied in stomata characters and chloroplast numbers to reflect the similarity degree in these characters. Table (7) presented means and coefficient of

variation (c.v. %) of stomata characters and chloroplast numbers after polyploidy treatments at 45 days from plant age. The data indicated that c.v. in stomata number character varied from 6.0 % in (Colch II) to 44.6 % in (Oxy II) treatment. In stomata length character, c.v. between 8.7 % in control to 18.0 % in (Colch I). The lowest value c.v. in stomata width character was 6.5 % in (Oxy III) and the highest value was 17.6 % in (Oxy I) treatment. In chloroplast character, the lowest value 9.3 % was found in control while highest value 20.2 % in (Oxy III). Stomata characters widely used in ploidy level determination in different kind of plant species. In some species of coffee with different numbers of chromosomes, higher levels of ploidy generally produce larger stomatal cells with a higher number of chloroplasts (Boaventura et al., 1981). Pringle and Murray (1992) studied pollen volume and viability, seed number per fruit, seed weight, fruit size, stomatal length and guard cell chloroplast number and measured in diploids and polyploids of the Tamarillo, *Cyphomandra betacea*. They found that pollen volume, stomatal length and guard cell chloroplast number were increased with higher ploidy.

Table (2): Polyploidy % in sugar beet root tip and young leave cells after treatment with the spindle inhibitors

Concentration	Polyploidy % in root tip cells		
	8-hydroxyquinoline	Para-dichlorobenzine	Colchicine
I	71.5	71.3	84.8
II	26.7	0	39.9
III	0.0	0	4.1
	Polyploidy % in young leave cells		
I	33.3	30.9	20.9
II	12.5	6.3	11.2
III	7.1	0	0.0

Table (3): Means of stomata number character after polyploidy treatments at 45 days from plant age

Treatment \ Concentration	0	I	II	III	Mean
Oxy	69.47b	60.67bc	49.53cd	39.40de	54.77b
Para	67.67b	90.67a	47.53cd	46.27cd	63.03a
Colch	64.17b	25.07e	35.07de	45.07cd	42.34c

Table (4): Means of stomata length (μm) after polyploidy treatments at 45 days from plant age

Treatment \ Concentration	0	I	II	III	Mean
Oxy	52.50bc	53.33b	46.67cd	51.67bc	51.04b
Para	51.67bc	56.50ab	40.83d	52.50bc	50.38b
Colch	55.0bc	60.83a	62.50a	56.67ab	58.75c

Table (5): Means of stomata width (μm) after polyploidy treatments at 45 days from plant age

Treatment \ Concentration.	0	I	II	III	Mean
Oxy	37.50bc	42.50ab	37.50bc	40.83ab	39.58a
Para	38.00bc	43.33a	34.17cd	30.83d	36.58b
Colch	40.00bc	41.67ab	45.83a	40.83ab	42.08a

Table (6): Means of chloroplast number character after polyploidy treatments at 45 days from plant age

Treatment \ Concentration	0	I	II	III	Mean
Oxy	8.53de	7.87e	7.97e	7.80e	8.04c
Para	8.13de	9.07cd	7.93cde	9.30cd	8.61b
Colch	8.53de	14.30a	9.63bc	10.47b	10.73a

Table (7): Stomata characters and chloroplast numbers after polyploidy treatments at 45 days from plant age ($\overline{X}$) means (c.v. %) coefficients of variation

| Conc. \ Characters	Stomata numbers		Stomata length (μm)		Stomata width (μm)		Chloroplast numbers	
	Mean	c.v.%	Mean	c.v.%	Mean	c.v.%	Mean	c.v.%
Oxy 0	69.5	10.7	52.5	11.3	37.5	14.3	8.5	13.3
Oxy I	60.7	21.8	53.3	12.3	42.5	17.6	7.9	14.0
Oxy II	49.5	44.6	46.7	11.9	37.5	13.0	8.0	14.5
Oxy III	39.4	30.4	51.7	15.2	40.8	6.5	7.8	20.2
Para 0	67.7	9.6	51.7	10.2	38.0	10.3	8.1	10.2
Para I	90.7	10.0	56.5	10.4	43.3	13.1	9.1	11.2
Para II	47.5	21.6	40.8	15.1	34.2	14.0	7.9	11.3
Para III	46.3	22.0	52.5	9.0	30.8	15.6	9.3	15.0
Colch 0	64.2	7.7	55.0	8.7	40.0	9.9	8.5	9.3
Colch I	25.1	11.7	60.8	18.0	41.7	14.8	14.3	17.3
Colch II	35.1	6.0	62.5	12.3	45.8	15.2	9.6	14.5
Colch III	45.1	6.1	56.7	9.8	40.8	16.0	10.5	19.4
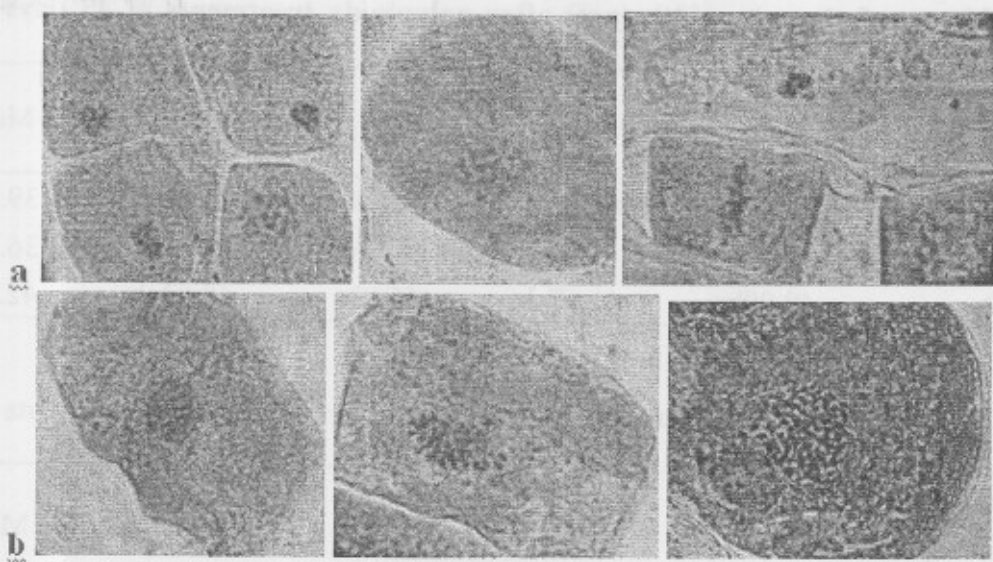
Figure: (1): Photomicrographs showing (a) diploid sugar beet cells (b) tetraploid sugar beet cells.
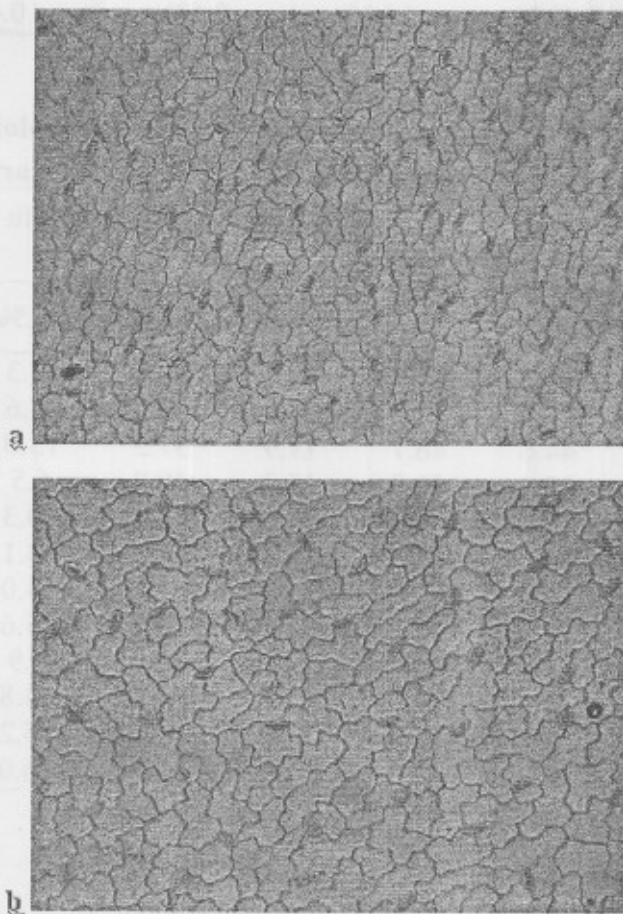

Figure (2): Photomicrographs showing (a) stomata density in diploid sugar beet plant and (b) in tetraploid plants.
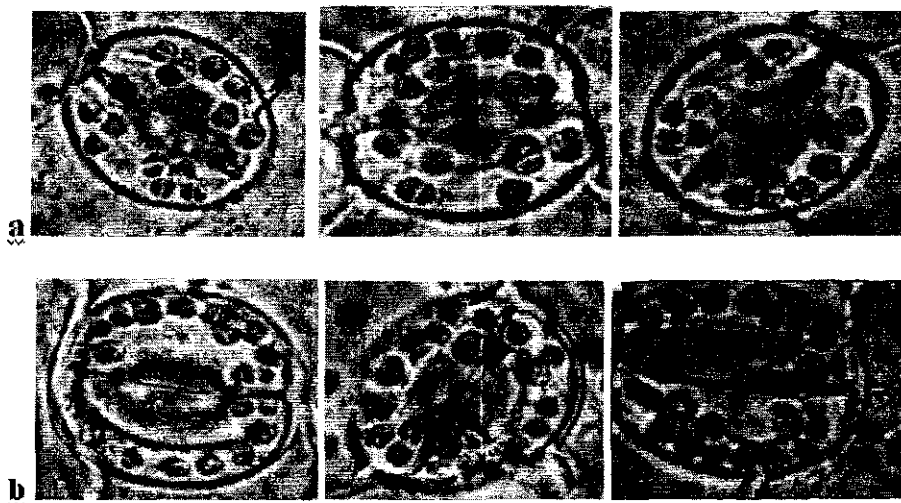
Figure (3): Photomicrographs showing chloroplast number in (a) diploid sugar beet cells (b) in tetraploid cells.

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الملخص العربى

إستخدام بعض المواد التى تحدث تثبيط لخيوط المغزل فى إستحداث تضاعف كروموسومى لنباتات بنجر السكر

مجدى سعد صالح* وأحمد السيد محمد خالد** أسامة مصطفى بدوى* وهيام عيد عبد القادر إبراهيم**

*قسم التربية والوراثة – معهد بحوث المحاصيل السكرية، ** قسم الوراثة – كلية الزراعة – جامعة الإسكندرية (سابا باشا)

أجرى هذا البحث فى محطة البحوث الزراعية بالصبحية بالإسكندرية فى ٢٠٠٨ والغرض الأساسى مــن هــذا البحث هوإستحداث تضاعف كروموسومى فى سلالة ثنائية العدد الكروموسومى من بنجر السكر وهى (C39) وقد تــم إستخدام ثلاثة من المواد المطفرة التى تحدث تثبيط لخيوط المغزل وهذه المواد هى :

١ – ٨ هيدروكسى كينولين   ٢ – باراداى كلوربنزين   ٣ – كولشيسين

وقد استخدمت هذه المواد الثلاث فى ثلاث تركيزات مختلفة بالإضافة إلى (الكنترول) لمعرفة التركيز الأمثل فى الحصول على أكبر نسبة من النباتات المتضاعفة وراثيا والتى لها ثبات عالى فى الإحتفاظ بهذه الصفة وهذه التركيزات هى:

١. التركيز الأول (محلول مشبع) وذلك فى حالة كل من (٨– هيدروكسى كينولين و باراداى كلـوربنزين) وتركيــز ٠,٠٥ % فى حالة الكولشيسين.

٢. التركيز الثانى (محلول نصف مشبع) فى حالة من (٨ هيدروكسى كينـولين و بــاراداى كلـوربنزين) وتركيــز ٠,٠٢ % فى حالة الكولشيسين.

٣. التركيز الثالث (محلول ربع مشبع) فى حالة كل من (٨ هيدروكسى كينولين و باراداى كلـوربنزين) وتركيــز ٠,٠١ % فى حالة الكولشيسين.

٤. الكنترول .

وقد تم فحص النباتات المعاملة سيتولوجيا سواء فى أطراف الجذور الحديثة أو فى الأوراق الصغيرة كمــا تــم دراسة صفات الثغور التنفسية سواء كثافة الثغور فى الحقل الميكرسكوبى أو طول وعرض الثغر أو عدد البلاسـتيدات الخضراء فى الخلية الحارسة الواحدة.

وقد أظهرت النتائج أن أفضل معاملة فى إحداث تضاعف كروموسـومى هــو التركيــز الأول ٠,٠٥ % مــن الكولشيسين ووجد أن نسبة التضاعف الكروموسومى فى الجذور الحديثة كانت ٨٤,٨ % أما فى الأوراق الحديثة فقـد كانت ٢٠,٩ %، وبالنسبة للتركيز ٠,٠٢ % من الكولشيسين فقد كانت نسبة التضاعف ٣٩,٩ – ١١,٢ % فى كل مــن الجذور والأوراق الحديثة على الترتيب، وعند إستخدام التركيز ٠,٠١ % فقد كانت نسبة التضاعف فى الجذور ٤,١ % ولم يؤدى هذا التركيز إلى إحداث تضاعف فى الأوراق الحديثة.