Proceed, Seventh Pl. Breed. Conf.
May 4-5, 2011 (Alexandria)

Egypt. J. Plant Breed (15) 2: 235-241
Special Issue (2011)

EFFECT OF THREE SPINDLE – FIBER INHIBITORS ON SUGAR BEET SEED GERMINATION

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ABSTRACT

The present investigation was carried out during 2008 at Sabahia Agricultural Research Station, Agriculture Research Center, Alexandria, Egypt. Three diploid polygerm sugar beet breeding materials (C790 – 68 H26, C92 and C39) were used in this study. Diploid sugar beet genotypes were exposed to three spindle-fiber inhibitor reagents; 8-hydroxyquinoline, para-dichlorobenzene and Colchicine, at three different concentrations beside control to induce sugar beet polyploidy plants which can be used later in breeding programs.

The obtained data revealed that the genotype (C39) had the best germination percentage between the three studied genotypes in all treatments, and Colchicine treatment gave the highest germination percentage compared with control group and the other two studied regents. The roots varied in color, length and thicken between different treatments and control group. The root showed red color, thicker and shorter emergence after treatments with 0.05 % Colchicine.

Germination percentages of (C39) genotype were measured in four period's (sex days between each period). The data indicated that control treatment finished a complete germination in first two periods (12 days), while, all different treatment and concentrations, germination continuous tile last period (24 days).

INTRODUCTION

Rasmusson and Levan (1939) demonstrated the new Colchicine method for inducing tetraploidy in sugar beets. The problem was attacked along three different lines; 1. seed treatment, 2. application of colchicine agar to seedlings and first year plants, and 3. treatment of shooting seed plants. The first methods gave an immediate effect, that was rather striking. But, if the treated plants were left for some time without renewed treatment, the effect disappeared very quickly, and the growing points returned to a purely diploid state. The same thing happened even if the growing point was treated several times. Kloen and Speckmann, (1953) initiated a program for the development of tetraploid sugar beet by treating germinating seed with colchicine.

Levan, (1942) reported that the first tetraploid sugar beets were produced in 1938, an entirely new factor was added to the variation system of this plant. Nothing was known about the effect of different chromosome numbers on the properties of the sugar beet beyond the fact that, other beet species with tetraploid and hexaploid chromosome numbers had been found in nature and had in certain tests shown a very high content of dry matter. Kloen and Speckmann (1956) recognized that tetraploid beets grow somewhat slower physiologically. About the time of the longest day they have one or two leave less than the diploids.

Sharma and Sharma (1980) demonstrated that the most important groups of compounds investigated for their action in studying chromosome structure, is the quinoline complex. Also

that Tjio and Levan (1950) was the first who stated the importance of 8-hydroxyquinoline in chromosome analysis.

The importance of *p*-Dichlorobenzene (*p*DB) as a pro-treatment agent was firstly by Meyer (1945). It is also employed as a reagent for the production of polyploidy in plants, and because of its very low solubility is used as saturated solution in water. Later workers (Dermen and Scott, 1950; Conagin, 1951) employed it effectively for chromosome counts. A detailed use of this compound in plants has been made by Sharma and Roy (1955).

The present study included three diploid polygerm sugar beet breeding materials (C790– 68 H26, C92 and C39). were treated by one of the three different spindle fiber inhibitor reagents (8-hydroxyquinoline, para-dichlorobenzene and colchicine) at four concentrations to produce polyploid sugar beet plants.

MATERIALS AND METHODS

1. Materials:

1.1. Sugar beet materials:

Three sugar beet (*Beta vulgaris* L.) diploid polygerm breeding genotypes (C790 – 68 H26, C92 and C39) obtained from Sugar Crops Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt, were employed in this work.

1.2. Treatment:

Three spindle-fiber inhibitor reagents (8-hydroxyquinoline, para-dichlorobenzene and colchicine) were used in this study at a level of three concentrations beside control (Table, 1).

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

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

2. Methods:

Three experiments were carried out in this study to induce auto tetraploidy in sugar beet plants which can be used in sugar beet breeding programs.

2.1. First experiment:

Seeds of the three sugar beet diploid breeding materials (C790 – 68 H26, C92 and C39) were soaked in running tap water for twelve hours. Then, the seed materials were transferred into polyploidy-treatment reagents for another twelve hours. Treated seed materials were planted over moistened filter paper in Petri dishes (each Petri dish contained 30 seeds) at three replicates, and allowed to germinate at 23 ° C in incubator. The germinated seeds were counted in each treatment and germination percentages were measured.

2.2. Second experiment:

Because of the highest germination percentage obtained from the first experiment. The genotype (C39), has been chosen. Seeds of the sugar beet genotype (C39) were soaked in running tap water for twelve hours and then transferred to polyploidy treatment reagents as descried before. Treated seeds were planted on moisture cotton in Petri dishes (each Petri dish contained 30 seeds) for three replicates. Seeds were allowed to germinate in incubator at 23 ° C, germination percentage was counted at four different periods from sowing. The interval was six daya.

2. 3. Third experiment:

Sugar beet seeds of the examined genotype were planted in earthen pots 25 cm. diameter in (sandy clay soil) in three replicates after treatment as previously described. Sugar beet polygerm seeds were placed on moistened cotton before they covered with soil. Fifty sugar beet seeds were planted in each pot. Seedlings number for each pot was counted in two dates (12 and 30 days) from sowing date.

3. Statistical analysis

The experimental design used Randomized Complete Block Design (RCBD) with three replicates, and the data were analyzed according to **(Snedecor and Cochran, 1990).**

RESULTS AND DISCUSSION

1. First experiment:

Table (2) presented the germination percentage of the three studied genotypes in the different treatments. The results obtained from this part of the study showed that germination percentages were varied significantly between the control group and treatment. The data showed that genotype (C39) had the best germination percentage between the three studied genotypes at all concentration, and colchicine treatment gave the good germination percentage compared with that of the control group and the other two studied regents. It can be observed that, such treatment found to be effective on root shape and size. The roots varied in color, length and thickness compared with that the control group. Figure (1) shows 8-Hydroxyquinoline treatment. It is obviously that root length and color were varied between them in the same age from planting (after 48 hours) and between the seed originated in control treatment. Figure (1) also showed the effect of para-dichlorobenzene treatment on root emergence and behavior, the roots had strong and tough shape. It is obviously that root emergence after colchicine treatments at concentration one resulted red color, thicker and shorter. While in concentration three of colchicine the roots were more tall and thickens in parts of roots. The results are in agreement with those reported by (Svirshcheuskaya and Svirshchevskaya, 1990), they demonstrated that leaf and root color, was useful for visual separation of the tetraploids from diploids in sugar beet tissue culture plants. They mentioned that tetraploid seedlings had pink primary roots.

2. Second experiment:

To confirm the results obtained second experiment was done on the (C39) genotype only which had the good germination percentage between the three studied materials at all treatments. Table (3) shows the germination percentages of (C39) genotype in period's (sex days between each period). The data indicated that control group finished complicate germination in first two periods (12 days), while, all different treatments and concentrations germination continuous till last period (24 days) as shown in (Table 3). Table (4) presented means of germination percentages of (C39) genotype after polyploidy treatment. Data showed that there were no significant differences between the three studied polyploidy treatment

reagents in germination percentage. The significant differences were found between control group and the first concentration of 8-hydroxyquinoline and between control group and third concentration of para-dichlorobenzene treatment. While, in colchicine treatment no significant differences were found between studied concentrations in germination percentages.

3. Third experiment:

Fifty seeds were sowing after treatment on moistened cotton in earthen pots 25 cm diameter. Seedling numbered at two dates after germination (after 12 & 30 days). Table (5) shows the average seedling number /50 seeds. Data indicated that average seedling number in the control group after twelve days was 63.3 while it was 85.0 after thirty days, and seedling numbers were increased with 8-hydroxyquinoline concentrations decreased. In para-dichlorobenzene treatment seedling numbers were doubled after thirty days in first and second concentration, while at the third concentration, seedling number not affected after thirty days. Seedling number in colchicine treatment affected by the time, there were a delay in seedling appearance. For example after treatment with the first concentration of colchicine treatment average seedling number was 1.3 after twelve days while it was 21.7 after thirty days. Figure (3) illustrates seedling originated from 50 seed in all studied treatments and concentrations. Seedling numbers were doubled after thirty days in the second colchicine concentration from 10.0 after twelve days to 22.3 after thirty days, respectively. In colchicine concentration three average seedling numbers were decreased from 60.0 to 50.7 after thirty days. Lowest number in seedling originated from 50 seeds was obtained after the first concentration in colchicine. This observation in contrast with the results found in the second experiment which include colchicine in concentration one had the best germination percentages in all studied regents and concentrations. The explanation of such observation is root emergence from seeds at early germination stages could be not affected by mortality effect of colchicine treatments, while in later germination stages abnormalities in root development affected in root shape as shown before, and shoot emergence on soil surface take more time if the root abnormalities not big enough to kill the plant. The results are in a harmony with those obtained by (Muntzing and Runquist, 1939). Who reported that seeds treated with colchicine had a more or less reduced germination, and many of the seedlings obtained presented the characteristic colchicine abnormalities.

Table (2): Means of germination percentage for three different genotypes (C790 – 68 H26, C92 and C39) after polyploidy treatment

Genotype	8-hydroxy quinoline				Para-dichlorobenzene				Colchicine				Mean
	Cont.	I	II	III	Cont	I	II	III	Cont.	I	II	III	
C790 – 68 H26	15.33	6.10	18.13	8.60	13.3	4.0	20.0	17.1	14.4	24.0	30.0	23.3	16.19
C92	10.0	40.0	12.5	28.0	13.7	22.2	19.0	26.3	13.7	31.3	35.3	64.2	26.35
C39	70.8	92.9	33.3	70.4	68.4	48.5	68.0	61.9	71.3	80.2	80.0	94.2	70.0

L.S.D. G = 1.373

Table (3): Germination percentage of (C39) after treatments for four periods (six days intervals)

Treatment / Period	8-hydroxy quinoline				Para-dichlorobenzene				Colchicine			
	Cont.	I	II	III	Cont.	I	II	III	Cont.	I	II	III
Period 1	73.3	14.5	14.5	28.9	71.3	30.0	28.9	24.4	77.4	41.1	43.3	41.0
Period 2	5.6	17.8	18.9	31.1	4.5	18.9	5.5	5.5	4.6	20.0	26.7	27.8
Period 3	-	10.0	19.9	8.9	-	31.0	21.1	14.4	-	10.0	7.8	6.7
Period 4	-	6.6	7.8	2.2	-	6.7	6.7	6.8	-	1.1	5.5	1.1
Total	78.9	48.9	61.1	71.1	75.8	86.6	62.2	51.1	82.0	72.2	83.3	76.6

Table (4): Means of germination percentages of (C39) after treatments

Concentration / Treatment	Cont.	I	II	III	Mean
Oxy	78.9a	48.9c	61.07abc	71.07abc	64.98a
Para	75.8a	86.63a	62.2abc	51.07bc	68.93a
Colch	82.0a	72.2abc	83.33a	76.63ab	78.54a

Table (5): Average seedling number /50 seeds germinated after treatments at 12 & 30 days

Period	8-hydroxy quinoline				Para-dichlorobenzene				Colchicine			
	Cont.	I	II	III	Cont.	I	II	III	Cont.	I	II	III
12 days	63.3	40.5	60.2	63.7	60.5	27.7	17.1	61.0	62.5	1.3	10.0	60.0
30 days	85.0	55.7	75.3	87.0	81.3	65.0	36.3	62.3	76.7	21.7	22.3	50.7

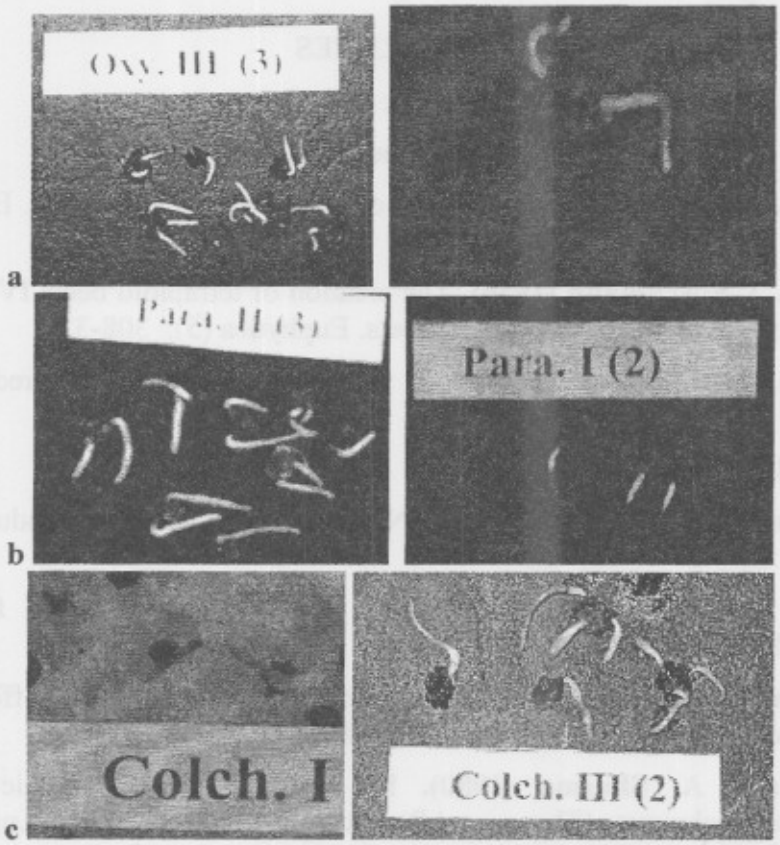

Figure (1): Effect of (a) 8-Hydroxyquinoline, (b) para-dicholorobenzene and (c) colchicine treatment on root emergence.

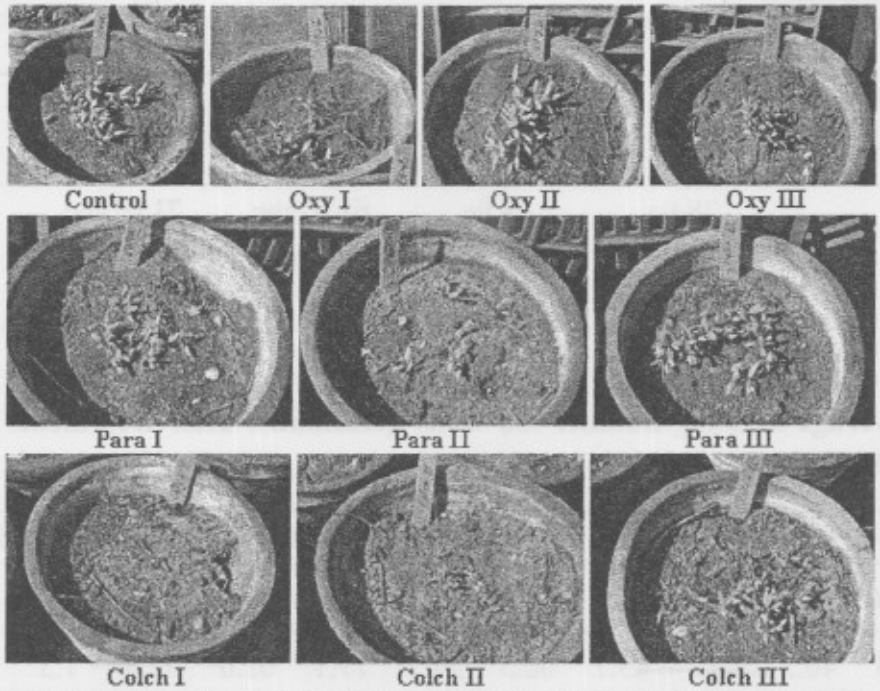

Figure (2): Seedling numbers originated from 50 seed after twelve days from planting in polyploidy treatments.

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الملخص العربي

دراسة تأثير ثلاثة من المواد التى تحدث تثبيط لخيوط المغزل على إنبات بذور بنجر السكر

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أجرى هذا البحث فى محطة البحوث الزراعية بالصبحية بالإسكندرية فى ٢٠٠٨ والغرض الأساسى من هذا البحث هومعرفة نسبة إنبات بذور بنجر السكر عند أستخام المواد المحدثة للتضاعف كروموسومى فى حالة بنجر السكر وقد تم إستخدام ثلاث تراكيب وراثية (C790 – 68 H26, C92 and C39) ثنائية العدد الكروموسومى من بنجر السكر فى هذا البحث، كما تم إستخدام ثلاثة من المواد المطفرة التى تحدث تثبيط لخيوط المغزل وهى :

- ٨ هيدروكسى كينولين - باراداى كلوربنزين - كولشيسين

وقد استخدمت هذه المواد الثلاث فى ثلاثة تركيزات تركيزات مختلفة بالإضافة إلى (الكنترول) لمعرفة التركيز الأمثل فى الحصول على أكبر نسبة للأنبات وبالتالى أكبر نسبة من النباتات المتضاعفة وراثيا والتى لها ثبات عالى فى الإحتفاظ بهذه الصفة.

وقد أظهرت النتائج أنه عند معاملة بذور التراكيب الوراثية الثلاثة تحت الإختبار وجد أن أفضل تركيب وراثى فى نسبة الإنبات هو التركيب الوراثى (C39) حيث كانت نسبة إنبات البذور المعاملة بالكولشيسين أعلى من متوسط نسبة إنبات البذور الغير معاملة (الكنترول). وعند إعادة إجراء التجربة مرة أخرى للتأكد من صحة هذه النتائج وجد أن البذور الغير معاملة قد أنهت الإنبات فى مدة زمنية ١٢ يوما بينما البذور المعاملة إستمرت فى الإنبات حتى ٢٤ يوما فى نفس ظروف الإنبات وعلى نفس درجة الحرارة ٢٣. كذلك وجد أن الجذور الناتجة من المعاملة أختلفت فيما بينها فى اللون والطول والسمك، حيث وجد أن الجذور الناتجة من معاملة البذور بالتركيز الأول من الكولشيسين (٠,٠٥%) تميزت بلون أحمر كما أنها كانت قصيرة فى الطول وسميكة بينما الجذور الناتجة عن التركيز (٠,٠١%) من الكولشيسين كانت أطول كما أن الجذور تميزت بالسمك فى بعض الأجزاء.