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SOMACLONAL VARIATION IN *Dieffenbachia pecta var. tropic* 2. CHROMOSOME ABERRATIONS IN *Dieffenbachia pecta* ROOT TIPS DERIVED FROM *in vitro* CALLUS CULTURE

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ABSTRACT

Chromosomal aberrations were studied in root tips of Dieffenbachia pecta plantlets derived from in vitro callus culture by both 6-BA (6-Benzyl amino purine) with IAA (Indol-3-acetic acid) and 2ip (Dimethyl-allylamino purine) with IAA. A high percentage of numerical variations were observed, these included tetraploid and octaploid (4. 87% for 6-BAA with IAA and 1.09% for 2ip with IAA). Also, aneuploid frequency was scored (0.56% for 6-BA with IAA and 0.876% for 2ip with IAA). Cells showing abnormal chromosome behaviour such as lagging and multipolar were found to be 1.1% for 6-BA with IAA and 2.01% for 2ip with IAA. Cells that showed structural variations in chromosomes such as chromatin bridges were 4% for 6-BA and IAA while it was 6.29% for 2ip and IAA. These chromosome abnormalities are the source of heritable somaclonal variations induced by in vitro callus cultures that can be used as genetic variability in Dieffenbachia breeding and improvement.

Key words : Somaclonal variations, Numerical and structural variations, Chromosomal aberrations.

INTRODUCTION

Some growth regulators lead to numerical and structural changes in chromosomes during *in vitro* callus culture (D, Amato 1977). These numerical variations seen occurring *in vitro* may be

euploidy as a result of endomitosis and endoduplication or aneuploidy arising from mitotic non disjunction. (D'Amato 1978).

Structural changes in chromosomes such as anaphase bridges and acentric fragments were observed during the cytological investigation in *Allium sativum* callus cells (NovaK 1974). Dicentric chromosomes were detected in wheat cultures (Kao *et al.*, 1970). Also, chromosome loss was observed in wheat plants regenerated from callus culture (Whelen, 1990).

In vitro callus culture induced polyploidy in potato (Pijnacher *et al.*, 1986), Barley (Singh 1986). Waara *et al.* (1992) resorted that potato plants regenerated from fusion of two dihaploid potato showed chromosomal aberrations such as deletions and translocations.

It has been reported that the use of auxins such as 2,4 - D, NAA and synthetic cytokinens (6-BA and Kinetin) increase the frequency of somatic mutations (D'Amato 1977), euploidy and aneuploidy (Thorpe, A.T. 1981 and Hughes 1986). Scowcroft (1985) evaluated the variability in plants regenerated *in vitro* culture of cells and tissues at the cytological, morphological and biochemical level.

Induction of cell division in differentiated tissues *in vitro* strongly increase the chance of mutations such as ploidy, aneuploidy, structural changes in chromosomes, differential - DNA replication and gene mutations in both nuclear and cytoplasmic-DNA (D'Amato, 1978). Benzion and Philips (1988) showed that frequency of deletions in chromosomes increased during tissue culturing in maize. Chromosome rings, bridges and laggard were observed during cytological investigation of regenerants from anther culture of four

Egyptian wheat cultivars (Ali *et al.*, 1995).

In the present study, the chromosomal aberrations induced *in vitro* callus culture of *Dieffenbachia pecta var. tropic* was investigated and scored. These somaclones in chromosomes induced by *in vitro* culture can be used as a new source of genetic variability in *Dieffenbachia* plant that gave no flowers in Egypt.

MATERIALS AND METHODS

1 - Plant material :

Plantlets regenerated from the callus induced by 6-BA and IAA on Murashige and Skooge (1962) medium (MS) were used for this study. The three phases of tissue culture (Callus induction, shoot regeneration and root formation) were done on MS medium supplemented with 6-BA (1 mg/l.) and 0.2 mg/l. of IAA for callus induction and 6 mg/l. of 6-BA in combination with 0.1 mg/l. of IAA for shoot regeneration while, 2 mg/l. of 6-BA and 0.2 mg/l. of IAA were used for root formation. On the other hand plantlets regenerated from one callus induced by 2 ip in combination with IAA on MS medium were used to compare effect of 6-BA and 2ip. For callus induction 5 mg/l. of 2 ip with 0.2 mg/l IAA were used. Shoot regeneration induced by 10 mg/l. of 2 ip and 0.1 mg/l. IAA while, root formation was on 5 mg/l. of 2ip and 0.2 mg/l. of IAA.

2 - Cytological analysis :

i. Numerical variations in chromosomes :

Rooted plantlets regenerated on MS media supplemented with

IAA in combination with 6-BA or 2 ip were treated with 0.3 mg/ml of colchicin for 3 hrs. Then, root tips of each plantlet (at the age of one week) were harvested together and dipped in fixative solution (3 : 2 absolute ethanol : glacial acetic acid) for 24 hrs. Fixed root tips were washed 3 times with tap water, then, stored in 75% ethanol at 4°C until use.

ii. Structural variations and abnormal behaviour of chromosomes:

Root tips of plantlets induced by 6-BA or 2 ip in combination with IAA were not treated by colchicin. The root tips at the age of one week of each plantlet were collected together and dipped directly in fixation solution for 24 hrs., Then, washed 3 times with tap water and kept in 75% ethanol at 4°C until use.

iii. Slide preparation and staining :

One root tip of each plantlet was boiled in 45% glacial acetic acid for 30 sec., then, transferred to 0.4% kcl/for 20 min. at 40°C. Chromosomes were stained with aceto-carmine and investigated. The Mitotic index was obtained from 4 well spread field (Contained ~ 500 cells).

RESULTS AND DISCUSSION

i. Mitotic Index (MI):

MI for control (plant root tips derived from cutting rooted without hormones) was scored as 7% while its was 24% for root tips regenerated *in vitro* by 6-BA in combination with IAA. On the other hand, MI of root tips regenerated *in vitro* by 2 ip with IAA was 27%. These results reflect the effect of of 6-BA and 2ip in combination

with IAA on cell division *in vitro* showing that 2ip induced high growth rate in *Dieffenbachia* cells rather than 6-BA (Dmitrieva, 1985).

ii. Numerical variations :

Dieffenbachia pecta is autotetraploid (Henny 1989) and its basic number is 7 chromosomes (Darlington and Tanake, 1945). Cytological investigation showed a wide range of chromosome numbers. Root tips induced by 6-BA and IAA varied in chromosome number from 21 to more than 64 chromosomes per cell (Table 1). Data in table 1 showed that cells containing 32 chromosomes (normal karyotype) ranged from 88.03% to 100% (Fig. 1-a), however, triploid cells (24 chromosomes) ranged from 1.67% to 2.52% (Table 1 and Fig. 1. b). Duplication of complete chromosome set (octaploid) have the highest percentage of chromosomal aberrations (3.15%- 10.57%) while, cells containing more than 64 chromosomes (Fig 1. c) showed the lowest frequency (O 8.4 - 1.40%). Also, data in table 1 showed that cells having 21 chromosomes ranged from 0.7% to 1.57% (Fig 1 - D). On the other hand, root tips regenerated *in vitro* by 2 ip and IAA showed low frequency of numerical variations in chromosomes compared with 6-BA and IAA. Data in table 2 showed that cells contained normal karyotype ranged from 97.05% to 100%. Also, triploid cells were 0.36% while, octaploid cells were 0.73% and 0.146% for cells contained 21 chromosomes. cells contained more 64 chromosomes not observed. The data in tables 1 and 2 suggested that *in vitro* callus culture induced numerical mutations in *Dieffenbachia* .

iii. Structural variations and abnormal behaviour of chromosomes:

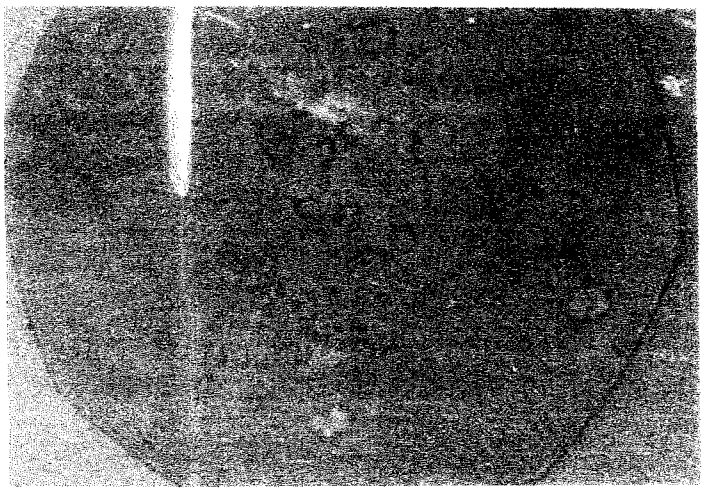
Table (1) : Chromosome analysis in *Dieffenbachia pecta* root tips regenerated from *in vitro* callus culture after treatment with 6-BA and IAA

sample (No)	Total examined cells	Chromosome number					% of normal karyotype
		32	24 Triploid	64 Octaploid	21	more than 64 Aneuploidy	
1	111	110	1	-	-	-	99.09%
2	100	100	-	-	-	-	100%
3	120	118	2	-	-	-	98.33%
4	116	115	-	-	1	-	99.14%
5	128	120	-	8	-	-	93.75%
6	127	121	-	4	2		95.28%
7	142	125	-	15	1	-	88.03%
8	119	108	3	7	-	1	90.76%
9	123	114	-	9	-	-	92.68%
10	143	130	-	11	-	2	90.91%
Σ	1229	1162	6.0	54.0	4.0	3.0	
%	--	94.54%	0.48%	4.39%	0.32%	0.24%	X = 94.54

Table (2) : Chromosome analysis in *Dieffenbachia pecta* root tips regenerated *in vitro callus* culture after treatment with 2ip and IAA.

sample (No)	Total examined cells	Chromosome number					% of normal karyotype
		32	24 Triploid	64 Octaploid	21	more than 64 Aneuploidy	
1	120	119	1	-	-	-	99.16%
2	112	111	-	1	-	-	99.10%
3	115	115	-	-	-	-	100%
4	109	107	-	2	-	-	98.1%
5	121	119	1	1	-	-	98.3%
6	117	117	-	-	-	-	100%
7	152	150	1	1	-	-	98.60%
8	113	112	-	1	-	-	99.10%
9	127	126	-	1	-	-	98.40%
10	142	140	1	1	-	-	98.50%
11	136	132	1	2	2	-	97.05%
Σ	1364	1348	5	10	2	-	98.82%
%	--	98.82%	0.36%	0.73%	0.146%	0.0%	

a



b

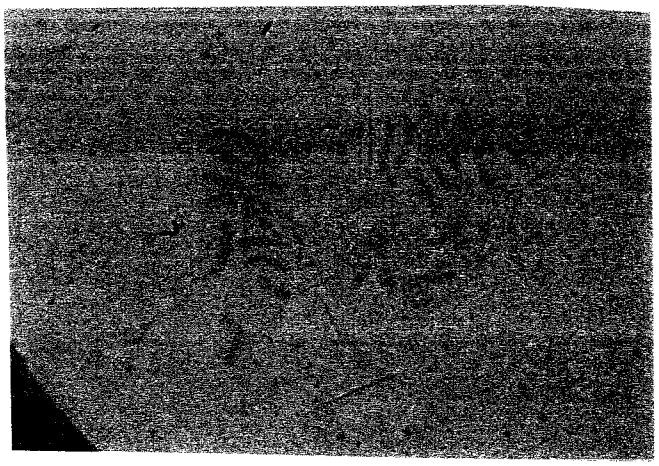
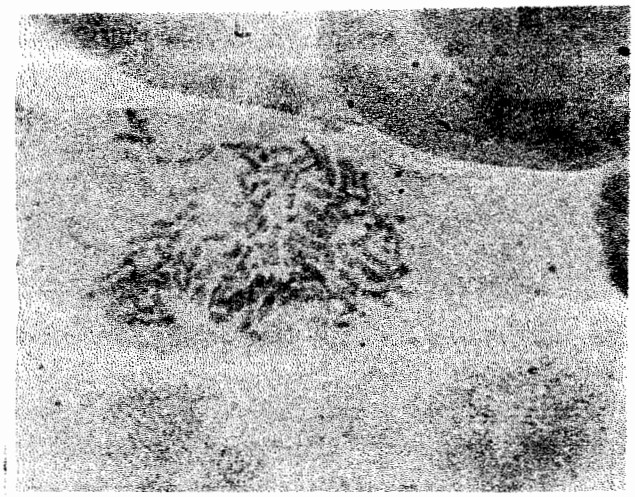


Fig. (1) : Numerical Variations in chromosomes of *D. Pecta var. tropic* root tips derived from *in vitro* callus culture :
a - Normal tetraploid (32 chromosomes).
b- Induced triploid (24 chromsomes).

c



d

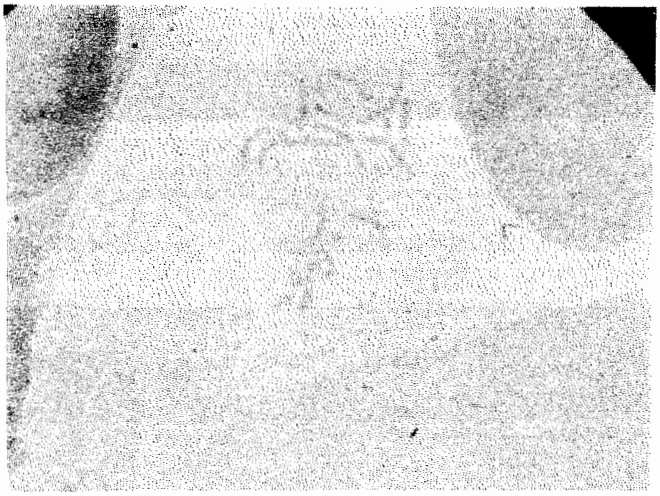
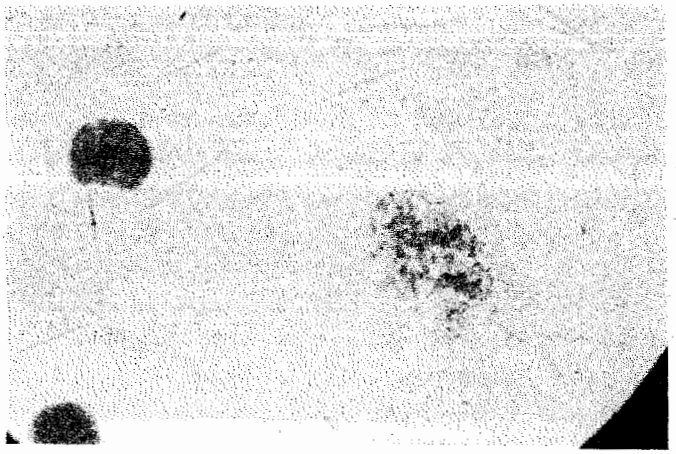
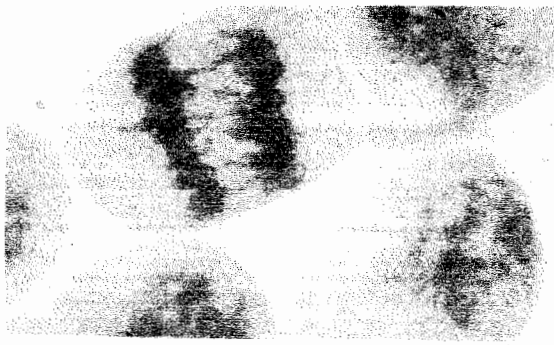


Fig. (1) : cont.
c. chromosome number is more than 64 chromosomes.
d. chromosome number is 21 chromosomes.

a



b



c

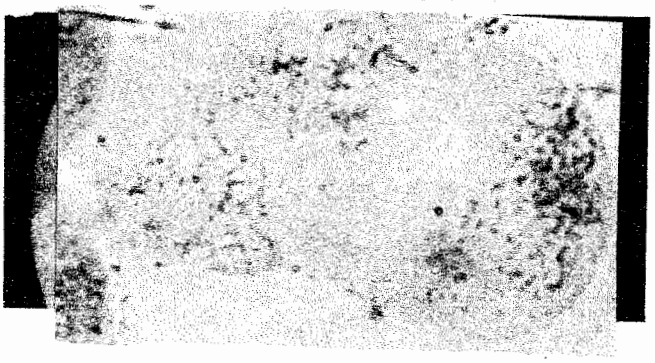


Fig. (2) : Structural variation and abnormal behaviour of chromosomes in root tips cells derived from *in vitro* callus culture of *D. Pecta* var. tropic.
a - Assynchronization .
b- Chromatin bridges and lagging.

Structural variations and abnormal behaviour of chromosomes were observed and scored as assynchronization (Fig. 2 - a), chromatin bridges (Fig. 2 -b), Lagging chromosomes (Fig. 2-b) and multipolar cells (Fig. 2 - c).

The cells containing structural variations and having abnormal behaviour of chromosomes were 5.1% for 6-BA and IAA while, 2ip and IAA showed high frequency of abnormal behaviour and structural variations (8.3%). It was concluded from these results, that *in vitro* callus culture of *Dieffenbachia pecta* induced chromosomal aberrations possibility by growth hormones or media components.

Chromosomal variations are considered a new source of genetic variability to use for *Dieffenbachia* improvement since this plant does not give flowers under Egyptian climate.

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التغيرات الكروموسومية فى خلايا القمم النامية لجذور نبات الديفينباخيا *Dieffenbachia pecta* الناتجة عن زراعة الأنسجة

د. عاطف أبو غالية

فى هذه الدراسة تم فحص كروموسومات خلايا القمم النامية الناتجة من مزارع أنسجة استخدم فيها ٦-بنزل أدنينين مع أندول حمض الخليك وكذلك جنور ناتجة عن استخدام ٢- أيزوبنتيل أدينوزين مع أندول حمض الخليك .

التغيرات الكروموسومية العددية كانت أعلى تكراراً والتى شملت تضاعفات وأظهرت الدراسة أن ٦- بنزيل ادنين مع أندول حمض الخليك كان أكثر تأثيراً من ٢- أيزوبنتيل أدينوزين مع إندول حمض الخليك فى استحداث التضاعف الكروموسومى الكامل أما التضاعف الكروموسومى غير الكامل كان أقل تكراراً فى جميع الحالات .

كما تم دراسة السلوك الكروموسومى أثناء الإنقسام الميتوزى وأظهرت النتائج أن السلوك الشاذ للكروموسومات ونسبة تكراره إختلف باختلاف الهرمون المستخدم وأن هرمون ٢- أيزوبنتيل ادينوزين مع أندول حمض الخليك كان أكثر تأثيراً من هرمون ٦- بنزل ادينين مع إندول حمض الخليك .

أما التغيرات الكروموسوميه التركيبية خاصة القناطر الكروماتينية فكانت نسبة تكرارها أعلا من تكرار الشذوذ فى السلوك الكروموسومى فى جميع الهرمونات المستخدمة .

واستنتج من الدراسة أن التغيرات الكروموسومة العددية والتركيبية الناتجة عن المزارع النسيجية يمكن اعتبارها مصدر من مصادر التباين فى نبات الديفينباخيا والتى يمكن استخدامها كمصدر تباين وراثى لتربية وتحسين نبات الديفينباخيا .