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SOMACLONAL VARIATION IN *Dieffenbachia pecta var. tropic*

1. In vitro callus culture of *Dieffenbachia pecta var. tropic*

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ABSTRACT

Dieffenbachia pecta var. tropic auxiliary buds were used for callus induction and regeneration. Murashige and Skooge medium (1962) was used with different growth regulators namely : 2, 4-D (2, 4 - Dichlorophenoxy acetic acid), IAA (Indol - 3 - acetic acid), NAA (Naphthyl acetic acid), IBA (Indol-3- butyric acid), 6-BA (6-Benzylamino purine) and 2ip (Dimethyle-allylamino purine) to evaluate the hormonal combinations and concentrations responsible for callogenesis and morphogenesis of Dieffenbachia pecta var tropic.

The obtained results showed that 2, 4 - D (0.2 mg/L) combined with 5 mg/L./ of 2ip was the best hormonal combination and concentration for callus induction. On the other hand 2ip (10.0 mg / L.) with IAA (0.1 mg/L.) gave high response for regeneration.

Key words : *Tissue culture, Asexual propagation, Callus, Regeneration, Somaclonal variation.*

Introduction

Rapid asexual propagation using tissue culture has been reported for some ornamental plants such as *Hemerocallis sp.* (Heuser and Apps, 1976), Iris (Meyer *et al.*, 1975) and *Dieffenbachia maculata* (Paola, *et al.* 1986 and Voyiatzi and Voyiatis 1989). Tissue culture techniques has allowed the rates of multiplication of some plants to be enhanced to such an extent that micropropagation *in vitro* has

become economically advantageous (Murashige, 1974). It is evident that tissue culture is used to induce genetic variability or to recover pre-existing natural genetic variability to be used in breeding programs (Evans and Sharp, 1981). As regards auxin. it is known that cell and tissue cultures of monocotyledons, for instance cereals, require as a rule high concentrations of auxins (Dmitrieva, 1985) for callus induction. Voyiatzi and Voyiatzis (1989) reported that 2ip was more efficient in shoots induction of *Dieffenbachia maculata* than kinetin. More and Khalatkac (1988) reported that GA_3 and Kinetin as shoot initiators gave unconstant results in *Dieffenbachia*, while IBA induced early and good rooting. Organic nitrogen, L-glutamine is necessary when a callus is being initiated whereas, yeast extract increased cell growth in plant tissue culture (Gamborg and Shylack 1981). The development of a culture system for tissue culturing of *Dieffenbachia* would be increase the range of techniques, which could be applied in somatic approaches towards genetic modification specially in plant species that gave no seeds in some environments. The greatest advantage of cell culture for the geneticist is that, it makes large numbers of plantlets viable for screening for variant type. Further more, growth of cells under well known and defined conditions facilities direct selection and identification of variants (Essa *et al.*, 1990). Also, tissue culture is a novel source of genetic variation (Larkin and Scowcroft, 1981).

MATERIALS AND METHODS

I. Explants preparation :

The apical shoot of *Dieffenbachia pecta cv. tropic.* mother

plants were released (3 cm). Then, stems were treated with tetracycline (2ug / ml solution) in the place of cutting.

The treated mother plants grown in green house for 10 days until the auxiliary buds were 1 cm long to use it as explants for tissue culture. Explants surface sterilized with 20% clorox and two drops of tween $_{20}$ for 20 min. with shaking. Sterilized explants washed two times with s.d.H_2O. In aseptic conditions, explants were plated on MS medium (Murashige and Skooge, 1962) contained different hormonal combinations and concentrations.

II. *Callogenesis :*

Three auxins were used for callus induction in combination with two cytokinins. The three auxins namely : 2,4- Dichlorophenoxy acetic acid (2,4-D), Indol-3-acetic acid (IAA) and Indol-3- butyric acid (IBA) with concentrations of 0.1 and 0.2 mg/ L for each. The cytokinin 2ip was used by concentrations of 2 mg / L and 5mg / L. while, 6-BA was used by concentrations of 1 mg / L and 3 mg/ L. MS media were supplemented with Biotin (2mg / L.), Glutamine (100 mg / L.) Glycine (50 mg / L.) sucrose (3 %) and Agar (0.7 %) with pH 5.8 (Linsmaier and Skooge 1965). The media autoclaved at 121°C for 20 min. in conical flasks. Surface sterilized explants (20 buds) plated on each medium (One bud/ flask) and incubated at 25°C in dark for two weeks. Developed calli (two weeks old) incubated at 27°C and 16 hrs photoperiod for four weeks. The light intensity used in all periods of growth was 1000 Lx.

Calli fresh weight (CFW-gm) were calculated at the age of 5 weeks. Also, calli fresh weight increase percentage (CFWI%) were

calculated at the same age according to the formula:

III. Morphogenesis :

i. Shoot induction :

MS media supplemented with organic supplements, Glycin (50 mg / L), Glutamine (50 mg / L) Casein hydrolysate (300 mg / L.), Yeast extract (200 gm / L.), sucrose (3%) and Agar (0.7%). Shoot induction were stimulated by different concentrations of IAA and NAA (0.1 and 0.2 mg /L.) in Combinations of 2ip (5 and 10 mg/L.). Cultures incubated at 27°C, photoperiod 16 hrs and light intenisity 1000 Lx. Regenerated shoots per callus were calculated at the age of 6 weeks on shooting media.

ii. Root induction

MS medium without organic supplements was used for root induction. IAA and IBA were used as auxins (0.1 and 0.2 mg/L. for each) in combination with 2ip (2 and 5 mg/L.) Roots developed per plantlet were calculated.

IV. Statistical analysis :

Experiments were arranged in FACTORIAL design and data were analyzed statistically according to Snedecor and Cocheran (1979).

RESULTS AND DISCUSSION

a. Callogenesis

The data in Fig. (3) : Root formation in dieffenbachia. without growth regulators. 1 showed that calli of *dieffenbachia* induced successfully by 2,4-D. The efficiency of 2,4-D for callus induction was higher in the presence of 2ip (75 - 90%) while, in the presence of 6-BA calli induction ranged from 35% to 40%. These results are in agree with Lazar *et al.* (1988) and Mathios *et al* (1986) in wheat. On the other hand IAA showed moderate effect for callus induction in *Dieffenbachia* (40 - 60%) combined with 2ip while, its effect was low in combination with 6-BA (25 - 30%). These results were similar with those obtained by Lazar *et al.* (1983) on wheat callus induction.

In contrast, IBA when combined with either 2ip or 6-BA gave the lowest response for callus induction. The results mentioned before in table (1) showed that 2,4-D (0.2 mg / L.) with 2ip (5 mg /L.) was the best hormonal combination for Dieffenbachia callus induction (Fig. 1). Our previous results showed almost the same trend with *D. exotica* Voyiatzi, C. and Voyiatzis, D.G. (1989).

b. Callus Fresh weight Increase (CFWI) :

Calli fresh weight (CFW/ gm) at the age of 5 weeks showed that 2,4 -D (0.2 mg /l) with 2ip (5 mg /L.) gave the best hormonal combination for callus induction and CFW (7.06 gm) as shown in table 2 and fig. 1. Meanwhile, IBA with 6-BA or 2ip showed the lowest CFW while, IAA in combinations with both 6-BA and 2ip gave average CFW. Also, the Callus FWI% was higher in the presence of 2,4 -D than IAA and IBA when combined with 2ip or 6-BA (Table 3). The results of CFWI %. were significantly increased by using the different hormonal combination of 2,4 -D IAA, IBA, 6-BA and 2ip

Table (1) : Effect of growth hormones on dieffenbachia cullus induction as percentage

Auxins mg/L.		Cytokinins			
		2ip		6-BA	
		2 mg/L	5 mg/L	1 mg/L	3 mg/L
2,4-D					
	0.1	75 %	85 %	40 %	35 %
	0.2	80	90	40	40
IAA					
	0.1	50	50	25	30
	0.2	60	40	30	25
IBA					
	0.1	20	25	30	30
	0.2	18	20	30	25

The above data calculated as percentages from 20 buds per hormonal combination.

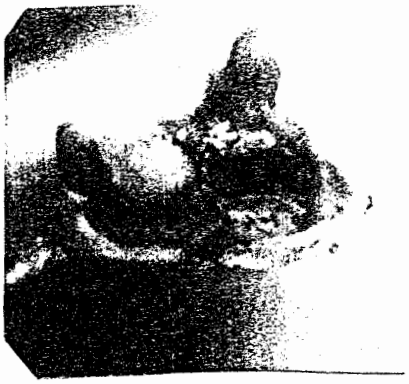
Table (2) : The effect of 6-BA and 2ip combined with 2,4-D, IAA and IBA in different concentrations on callus fresh weight (gm.) at the age of 5 weeks.

Auxins mg/L.		Cytokinins			
		2ip		6-BA	
		2 mg/L	5 mg/L	1 mg/L	3 mg/L
2,4-D					
	0.1	3.25 gm	5.10 gm	1.43 gm	2.44 gm
	0.2	4.14	7.06	2.17	3.31
IAA					
	0.1	2.36	1.94	2.37	2.28
	0.2	1.86	0.87	2.60	2.21
IBA					
	0.1	0.94	0.67	1.67	1.79
	0.2	0.84	0.73	1.03	1.09

L.S.D. = 0.37
0.05

0.2 mg/L. IAA with
5.0 mg/L. 2ip

0.2 mg/L. IAA with
3.0 mg/L. 6-BA



0.2 mg/L. 2,4 with
5.0 mg/L. 2ip

Fig. (1) : The effect of 2ip 6-BA, kin, 2,4-D and IAA on callus induction in *Dieffenbachia pecta var. Tropic*

(Fig. 1).

In fact, these variable results obtained from callus induction, CFW and CFWI % reflect different response to special phytohormones as reported by Hanzel *et al.* (1985), Bregitzer (1992) and Nasr *et al.* (1994).

c. Morphogenesis

i. Shoot induction :

Number of regenerated shoots / callus at the age of 6 weeks showed that IAA (0.1 mg /L.) with 2ip (10 mg /L) gave the highest number of shoots / callus (22.33). Whereas NAA (0.1 mg/L.) in combination with 2ip (10 mg /L.) gave 8.0 shoots / callus.

The data presented in table 4 clearly indicate that IAA combined with 2ip produced significantly more shoots than NAA combined with 2ip (Fig. 2). On the other hand 6 - BA in combination with IAA or NAA gave low response for shooting when compared with 2ip (Table - 4). Davis *et al.* (1977) found similar results in carnation.

ii. Root Formation

Roots development on MS medium without hormones after 2 weeks in mean 2.2 roots / shoot(Fig. 3). Formation of roots without hormonal supplementation was reported by Paola *et al* (1986) in *Dieffenbachia pecta cv. tropic*. It was noticed that the induction of roots without hormones gave natural roots in development and function . Roots developed on MS supplemented with IAA (0.2 mg/L)

Table (3) : The effect of 6-BA and 2ip in combination with 2,4-D, IAA and IBA on callus fresh weight increase percentage (CFWI %) at the age of 5 weeks.

Auxins mg/L.		Cytokinins			
		6-BA		6-BA	
		2 mg/L	5 mg/L	1 mg/L	3 mg/L
2,4-D					
	0.1	94.76%	96.66%	88.80%	93.03%
	0.2	96.13	97.93	93.08	94.86
IAA					
	0.1	94.49	92.78	93.24	92.98
	0.2	93.01	83.90	94.23	92.76
IBA					
	0.1	85.10	76.11	91.01	92.17
	0.2	80.95	79.45	85.43	85.32

Table (4) : Effect of 2ip and 6 - BA in combination with either IAA or NAA on regenerated shoots per cullus of Dieffenbachia.

Cytokinen		Auxins			
		IAA		NAA	
		0.1 mg/L	0.2 mg/L	0.1 mg/L	0.2 mg/L
2ip	5.0 mg / L.	13.33	10.33	3.66	3.66
	10 mg / L.	22.33	10.66	8.00	4.66
6-BA	3 mg / L.	4.11	3.24	4.36	5.13
	6 mg / L.	8.65	7.62	7.22	6.41

L.S.D. = 2.2
0.05

10 mg/L. 2ip with
0.1 mg NAA

5 mg/l. 2ip with
0.2 mg NAA

10 mg/L. 2ip with
0.2 mg NAA

Fig. (2) : The effect of 2ip and 6-BA, and NAA on regeneration of shoots in *Dieffenbachia pecta var. Tropic* .

With Hormones

Without hormones.

Adaptation

Fig. (3) : Root formation in dieffenbachia. without growth regulatos.

and 2ip (2 mg / L.) after 2 weeks in mean 4.6 roots. per one shoot.

Somaclonal variation in chromosomes will be study in a next research.

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الملخص العربى

استخدمت البراعم الجانبية لنبات الديفنباخيا لاستحداث تكوين كالوس وتكشفه إلى نباتات كامله

استخدمت بيئة موراشيج وسكووج ١٩٦٢ للزراعة النسيجية وأمدت بتوليفات بتركيزات مختلفه من الهرمونات وهى 6-BA, 2ip, 2,4-D, IAA, and IBA لتحديد أفضل هرمون بأفضل تركيز لكل مرحله من مراحل الزراعة النسيجية .

أظهرت النتائج أن 2,4 -D بتركيز ٢ر٠ مجم/لتر مع 2ip بتركيز ٥ مجم/لتر اعطيا أفضل هرمونات لتكوين كالوس (٩٠٪) اما ١٠ مجم/لتر من 2IP مع ١ر٠ مجم/لتر IAA كانا أفضل توليفة من الهرمونات فى مرحله التكشف (٣ر٢٢ نبات/كالوس) .

أيضا معدل زياده الوزن للكالوسات المتكونه تم تقديره فى عمر ٥ اسابيع للكالوس وأن اعلى نسبه زيادة فى وزن الكالوس عند ماإستخدم ٢ر٠ مجم/لتر من 2,4 - D مع ٥ مجم/لتر من الـ 2ip .

التباين النسيجى على مستوى الكروموسومات سوف يدرس فى بحث تالى .