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In Vitro Responses of *Dracaena fragrans* cv. Massangeana to Growth Regulators

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ABSTRAK

Kajian in vitro *ke atas* Dracaena fragrans *cv. Massangeana mendapati segmen batang muda berupaya membentuk pucuk diatas media pepejal Murashige dan Skoog (MS) yang mengandungi berbagai kombinasi dan paras BAP dan NAA. Peratus eksplan membentuk pucuk paling tinggi diperolehi pada medium yang ditambah dengan 3.0 mg/l BAP dan 0.1 mg/l NAA. Jumlah pucuk se eksplan paling tinggi berlaku pada medium yang mengandungi 2.0 mg/l BAP sahaja. Peratus pembentukan kalus dan min berat basah kalus dari segmen batang muda adalah paling tinggi pada medium MS yang dibekalkan dengan 1.0 mg/l 2,4-D. Akar adventitius terbentuk selepas pucuk dialih ke medium MS tanpa hormon. Pengakaran adalah 100% bagi pucuk yang dialih dari medium yang mengandungi 0-2.0 mg/l BAP dan kepekatan NAA yang rendah (0.1 mg/l).*

ABSTRACT

In vitro *studies on* Dracaena fragrans *cv. Massangeana revealed that young stem segments were capable of proliferating shoots on agar-solidified Murashige and Skoog (MS) basal medium containing different combinations and concentrations of BAP and NAA. Highest percentage of explants forming shoots was obtained on medium supplemented with 3.0 mg/l BAP and 0.1 mg/l NAA. The highest number of shoots per explant occurred on medium containing 2.0 mg/l BAP only. Highest percentage of callus formation and highest mean fresh weight of callus from young stem segments were achieved on MS medium supplemented with 1.0 mg/l 2,4-D. Adventitious rooting occurred after transferring excised shoots onto a hormone-free MS medium. Rooting was 100% for shoots derived from media with 0-2.0 mg/l BAP and a relatively low concentration of NAA (0.1 mg/l).*

INTRODUCTION

Dracaenas are woody monocotyledons belonging to the family Agavaceae. They are popular foliage ornamentals in tropical and temperate regions and are highly desirable as indoor plants and for outdoor landscaping. *Dracaena fragrans* cv. Massangeana, characterized by its sword-shaped dark green leaves with a yellow stripe running along the centre, is among the dracaena cultivars fast gaining the attention of commercial growers. However, propagation by conventional methods is slow and therefore production of clonal plants by tissue culture will be useful to meet the increasing demand for planting material. Establishment of a plant regeneration system through direct organogenesis or via callus is also a prerequisite to further *in vitro* genetic manipulation of the

cultivar. Tissue culture procedures have been described for *D. godseffiana* (Miller and Murashige 1976), *D. marginata* cv. Tricolor (Chua *et al.* 1981), *D. deremensis* cv. Warneckii (Debergh 1975), *D. congesta* and *D. invisa* (Debergh and Maene 1981) and green-foliage *D. fragrans* (Dragan 1989). There is no report on *D. fragrans* cv. Massangeana. The present paper aims to assess the *in vitro* response of *D. fragrans* cv. Massangeana to various growth regulators.

MATERIALS AND METHODS

Vigorously growing shoots taken from 1-2 year-old plants, with leaves removed, were washed under running tap water for 30 min. They were surface-sterilized for 20 min in 10% v/v Clorox with a few drops of Tween 20 emulsifier, and rinsed in five changes of sterile distilled water. The material was further immersed in 5% v/v Clorox for 2 min, rinsed five times with sterile distilled water and finally cut into segments 3-4 mm thick. Explants were cultured individually on 10 ml of Murashige and Skoog (MS) (1962) medium with 3.0% sucrose, 0.7% Bacto-agar and the appropriate combinations and concentrations of auxin and/or cytokinin. For shoot induction, 0-0.3 mg/l a-napthaleneacetic acid (NAA) in combination with 0-30 mg/l benzylaminopurine (BAP) was used. For the initiation and proliferation of callus, stem explants were cultured on MS medium containing 0.5-2.0 mg/l 2,4-dichlorophenoxy-acetic acid (2,4-D), and on MS medium containing 0.5 mg/l BAP in combination with 1.0 and 2.0 mg/l indolebutyric acid (IBA). Shoots which attained 5-15 mm in height were separated and cultured on a hormone-free MS medium to promote root formation and shoot elongation. All cultures were incubated under a 16-hour photoperiod using white fluorescent tubes (Philips, TLD 36w/54) at an irradiance of 65 uE m^{-2} s^{-1} and a temperature of 27 ± 1°C. The experiments were arranged in a completely randomized design with three replications for each treatment and four explants per replicate. Each experiment was repeated three times. *In vitro* regenerated plantlets were removed from culture vessels, washed thoroughly to remove traces of nutrient medium and planted in small pots containing a mixture of vermiculite and sand (1:1). For the first week of transfer the plantlets were covered with plastic perforated with small holes to maintain a high humidity. Plantlets were subsequently transferred to larger pots and placed outdoors.

RESULTS

Effect of BAP and NAA on Shoot Formation

Within 20 days of culture, adventitious buds were induced at the cut surface of stem segments placed on various combinations of BAP and NAA. In most explants, moderate callus formation preceded bud formation. On medium supplemented with a higher level of BAP (3.0 mg/l) but without NAA, adventitious buds were induced directly from the explants without an intervening callus. Shoots developed from the adventitious buds by week 7.

Table 1 summarizes the effect of BAP and NAA on shoot formation from stem explants of *D. fragrans* cv. Massangeana after 8 weeks in culture. Shoot formation occurred in all treatments including the control. In treatments containing 0.1 mg/l NAA the percentage of shoot formation increased with increasing levels of BAP, with 3.0 mg/l BAP producing the highest percentage of explants with shoots (88.8%). The highest number of shoots per explant (1.44 ± 0.10) occurred on medium containing 2.0 mg/l BAP but without NAA. Highest mean length of shoots (3.68 ± 1.47 mm) was also attained on the same medium (Table 1).

TABLE 1
Effect of BAP and NAA on shoot formation from stem segments of *Dracaena fragrans* cv. Massangeana at week 8

BAP (mg/l)	NAA (mg/l)	Explants with shoots (%)	Number of shoots per explant	Mean length of shoots (mm)
0	0	44.4	0.77 ± 0.29	1.94 ± 0.63
1.0	0	22.2	0.22 ± 0.10	1.99 ± 1.26
2.0	0	77.7	1.44 ± 0.10	3.68 ± 1.47
3.0	0	22.2	0.22 ± 0.10	0.33 ± 0.19
0	0.1	44.4	0.44 ± 0.10	1.21 ± 0.55
1.0	0.1	55.5	0.88 ± 0.22	2.47 ± 0.67
2.0	0.1	66.6	0.66 ± 0.19	2.22 ± 0.48
3.0	0.1	88.8	0.88 ± 0.11	1.44 ± 0.48
0	0.3	22.2	0.55 ± 0.10	0.33 ± 0.19
1.0	0.3	66.6	0.66 ± 0.01	1.77 ± 0.48
2.0	0.3	55.5	0.55 ± 0.22	1.10 ± 0.61
3.0	0.3	66.6	0.66 ± 0.01	1.66 ± 0.57

Values given are ± standard errors

TABLE 2
Callus induction on stem explants of *Dracaena fragrans* cv. Massangeana at week 6

Concentration (mg/l)	Explants with callus (%)	Mean fresh weight of callus (g)	Response
0.5 2,4-D	77.7	0.075a	fc
1.0 2,4-D	88.8	0.135a	hfn
2.0 2,4-D	44.4	0.082a	fcn
0.5 BAP + 1.0 IBA	77.7	0.095a	hcn
0.5 BAP + 2.0 IBA	55.5	0.046a	hn

Values with a similar letter in a column indicate no significant difference at 5% probability level.

Key to Table: h = hard, f = friable, c = chlorophyllous, n = non-chlorophyllous

Effect of 2,4-D and Combinations of BAP and IBA on Callus Initiation and Proliferation

Response of stem explants after six weeks of culture on media with different concentrations of 2,4-D, and combinations of BAP and IBA is shown in Table 2. Callus was initiated at the cut surface of explants by week 2 and was obtained in all treatments. Highest percentage of explants with callus and the highest mean fresh weight of callus

were obtained on medium with 1.0 mg/l 2,4-D. Mean fresh weight of callus produced did not differ significantly among the treatments. The callus produced on medium with 1.0 mg/l 2,4-D were either hard or friable and non-chlorophyllous. At a higher level of 2,4-D (2.0 mg/l) callus produced were friable, mostly yellowish, slow growing and gradually turned brown after the third week of culture. MS medium with 0.5 mg/l BAP and 1.0 mg/l IBA produced callus which were hard and which turned green after 6 weeks in culture. Shoot differentiation from callus was observed in both media containing 0.5 mg/l BAP with 1.0 mg/l IBA and 0.5 mg/l BAP with 2.0 mg/l IBA by the fifth week of culture.

Rooting

In vitro proliferated shoots excised and transferred to hormone-free MS medium rooted by week 2. Table 3 shows the effect of BAP and NAA concentrations (used previously to induce the shoots) on rooting. Shoots derived from media with 0.1 mg/l NAA and BAP 0-2.0 mg/l showed 100% rooting on transfer to hormone-free MS medium. Rooting was also 100% with shoots derived from a previously hormone-free medium (control). Shoots induced on media with 1.0-2.0 mg/l BAP and a higher NAA concentration (0.3 mg/l) showed a lower rooting response in the hormone-free MS medium. It is also evident from Table 3 that roots generally tend to be shorter as their number increases.

DISCUSSION

Shoot formation from stem explants of *D. fragrans* cv. Massangeana occurred in all combinations of BAP and NAA tested, including the control. An increasing trend in shoot formation was attained when 0.1 mg/l NAA was added to the increasing BAP levels, indicating a stimulatory effect of the auxin at a relatively low concentration. In media without NAA and in media supplemented with a higher level (0.3 mg/l) of NAA, shoot formation was variable. In all treatments except in a medium with a

TABLE 3
Rooting response of shoots on hormone-free MS medium

Previous treatment		% of shoots with roots	Mean number of roots per plantlet	Mean length of roots (mm)
BAP (mg/l)	NAA (mg/l)			
0	0	100.0	1.33 ± 0.33	15.17 ± 5.29
1.0	0	33.3	2.00 ± 0.01	9.00 ± 0.01
2.0	0	50.3	0.50 ± 0.40	17.50 ± 4.28
3.0	0	66.3	1.00 ± 0.10	26.50 ± 5.30
0	0.1	100.0	1.00 ± 0.10	16.67 ± 5.69
1.0	0.1	100.0	2.00 ± 0.57	11.33 ± 3.81
2.0	0.1	100.0	1.66 ± 0.01	19.50 ± 1.89
3.0	0.1	–	–	–
0	0.3	–	–	–
1.0	0.3	50.0	1.33 ± 0.66	18.33 ± 9.27
2.0	0.3	33.3	2.00 ± 0.01	10.50 ± 0.10
3.0	0.3	–	–	–

Values given are ± standard errors
- indicates no shoots were transferred

higher BAP concentration (3.0 mg/l) and without NAA, moderate callus formation preceded shoot formation. The production of shoot and callus even on the hormone-free medium strongly implies the presence of endogenous hormones within the stem tissues of *D. fragrans* cv. Massangeana. Ease of shoot and callus formation from stem explants in comparison to leaf and shoot tip explants has been reported in *D. deremensis* cv. Warneckii (Debergh 1975).

Dracaenas are among the few monocotyledons which possess a cambium in their stems (Zimmermann and Tomlinson 1970; Esau 1977). The occurrence of the cambium may have facilitated shoot and callus induction from stem explants of *D. fragrans* cv. Massangeana.

In the study on callusing ability of stem segments of *D. fragrans* cv. Massangeana, 2,4-D alone or combinations of BAP and IBA were effective in stimulating callus formation. Mean fresh weight of callus did not differ significantly among the treatments, but the percentage of callus formation varied. The gradual browning of callus observed on 2.0 mg/l 2,4-D indicated the deleterious effect of the auxin at this concentration. Other researchers have reported a similar effect of 2,4-D at 2.0 mg/l and above on other dracaena species and cultivars investigated (Debergh 1975; Chua *et al.* 1981; Dragan 1989). Debergh (1975) showed the addition of 1.0 or 2.0 mg/l kinetin to 2.0 mg/l 2,4-D could neutralize the effect of 2,4-D, resulting in a more organized type of callus.

Rooting of *in vitro* shoots of *D. fragrans* cv. Massangeana was achieved on a hormone-free MS medium. Successful rooting of other dracaena species required the inclusion of low levels of auxins in the rooting medium such as IBA (0.1-2.0 mg/l) or NAA (0.1-1.0 mg/l) (Debergh 1975; Chua *et al.* 1981; Dragan 1989), or using a low salt MS medium (Debergh and Maene 1989). This implies a species-specific rooting behaviour of dracaena species under *in vitro* conditions. It is also evident that a relatively low supplement of auxin (0.1 mg/l NAA) to BAP (0-2.0 mg/l) in the shoot induction and proliferation medium has a stimulatory effect on rooting of *in vitro* shoots of *D. fragrans* cv. Massangeana when placed on a hormone-free medium.

The present study demonstrates the totipotent capacity of stem segments of *D. fragrans* cv. Massangeana to regenerate plantlets *in vitro*.

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