GLASNIK INSTITUTA ZA BOTANIKU I BOTANIČKE BAŠTE UNIVERZITETA U BEOGRADU
BULLETIN DE L'INSTITUT ET DU JARDIN BOTANIQUES DE L'UNIVERSITE DE BEOGRAD
Tom XXIII, 1989. (1991), Beograd

UDC 581.14/.16 : 582.572.43
Original scientific paper

DRAGAN VINTERHALTER, BRANKA VINTERHALTER, DARINKA PETROVIĆ*

# IN VITRO PROPAGATION OF DRACAENA FRAGRANS KER., CORDYLINE TERMINALIS CV „KIWI" AND SANSEVIERIA TRIFASCIATA VAR. LAURENTII

Institute for Biological Research,
„Siniša Stanković", University of Belgrade, Belgrade
*Fruit and Grape Research Station,
RO INI „Agroekonomik–PKB", Belgrade

Vinterhalter, D., Vinterhalter, B., Petrović, D. (1989): *In vitro propagation of Dracaena fragrans Ker., Cordyline terminalis cv „Kiwi" and Sansevieria trifasciata var. laurentii.* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIII, 35–41.

The requirements for *in vitro* propagation of *Dracaena fragrans, Cordyline terminalis* and *Sansevieria trifasciata,* members of the Agavaceae family, have been comparatively investigated. Media employed for all three species contained mineral salts and organic ingredients of M u r a s h i g e and S k o o g (1962), differing only in hormonal composition. For all three species in the first propagation stage primary explants were induced to proliferate callus from which later shoots differentiated enabling shoot cultures to be established. Subsequent propagation stages coresponded to standard micropropagation including shoot multiplication, shoot elongation, rooting and finaly adaptation of rooted plantlets to *in vivo* conditions. Optimal hormonal balances for all propagation stages are presented and discussed in connection to specific requirements of certain species.

Key words: *in vitro,* propagation, callus, shoot cultures, *Dracaena fragrans* K e r., *Cordyline terminalis* cv Kiwi, *Sansevieria trifasciata* var. *laurentii.*

Ključne reči: *in vitro,* razmnožavanje, kalus, kulture izdanaka *Dracaena fragrans* K e r., *Cordyline terminalis* cv Kiwi, *Sansevieria trifasciata* var. *laurentii.*

## INTRODUCTION

*Dracaena, Cordyline* and *Sansevieria,* genera of the *Agavaceae* family, comprise many ornamental species. Some of them like *D. fragrans, C. terminalis* and *S. trifasciata* fall into the group of most popular indoor house plants.

Methods ofr the *in vitro* propagation of various *Agavaceae* species have already been elaborated. Thus, Chua et al. (1981), Debergh (1975, 1976), Miller and Murashige (1976), and Vinterhalter (1989), studied *in vitro* propagation of *Dracaena* species. Kunisaki (1975) Evaldsson and Welander (1985) studies *Cordyline* and Blazich and Nowitzki (1984) *in vitro* propagation of *Sansevieria.*

The main purpose of the study presented here was to investigate comparatively the specific requirements for the *in vitro* propagation of *D. fragrans, C. terminalis* and *S. trifasciata* as typical representatives of their genera.

## MATERIAL AND METHODS

Plants used in experiments were obtained from various Belgrade nurseries. Only healthy, good–looking plants grown previously in glasshouses were selected as mother–donor plants.

Primary explants for propagation of *Dracaena* and *Cordyline* were 3–5 m long segments of defoliated young stems. For *Sansevieria* primary explants were 4–5 x 6–8 mm rectangular fragments of mature leaves.

The surface sterilization procedure was same for all species. Explants were immersed for 20 minutes in 10% commercial bleach (4–5% sodium hypochlorite) to which a drop of liquid detergent was added per 60 ml of solution. Explants were then

*Tab. 1. – Hormonal combinations and concentrations recommended for in vitro propagation of (1) D. fragrans, (2) C. terminalis and (3) S. trifasciata*

| purpose of media | code | hormones $mgl^{-1}$ | | | | suitable for |
|---|---|---|---|---|---|---|
| | | BA | IBA | NAA | 2,4–D | |
| callus induction | A1 | – | – | – | 0.2–0.25 | 1,2,3 |
| | A2 | 0.5 | – | – | 0.2–0.25 | 1,2,3 |
| shoot differentiation and multiplication | B1 | 1.0 | – | 0.1 | – | 1 |
| | B2 | 1.0 | 0.1 | – | – | 3 |
| | B3 | 0.2 | – | – | – | 2 |
| shoot elongation | C1 | 0.1 | 2.0 | – | – | 1 |
| | C2 | 0.1 | – | 1.0 | – | 1 |
| | C3 | 0.05 | – | – | – | 2 |
| rooting | D1 | – | 0.5 | – | – | 1,3 |
| | D2 | – | – | 0.1 | – | 1,3 |
| | D3 | – | – | – | – | 2 |
| undifferentiated callus | E1 | 0.2 | – | – | 2.0 | 1 |

thoroughly rinsed with autoclaved distilled water, aseptically trimmed to the desired size and then transferred to suitable media.

Media contained mineral salts of Murashige and Skoog (1962), 2,0% sucrose, 0.62% agar, and in $mgl^{-1}$; inositol 100, glycine 2.0, pyridoxine–HCl 0.4, thiamine–HCl 0,5, and nicotinic acid 0.5. Hormones benzylaminopurine (BA), indolyl–3–butyric acid (IBA), naphthalene acetic acid (NAA) and 2,4–dichlorophenoxy acetic acid (2,4–D) were used for the control of growth.

Optimal media composition (hormone concentrations) for all species and stages of propagation are listed in Table 1. Media pH was adjusted to 5.6 prior to autoclaving which was performed at 114°C for 20 minutes.

Primary explants were cultured in 20 x 100 mm test tubes. Wide neck 100 ml Erlenmayer flasks were used for callus and shoot cultures. Rooting was perfomred in 20 x 200 mm test tubes.

The growth room was equipped with 20 W TT 4500°K white fluorescent lamps providing irradiance of 5.0–7.2 $Wm^{-2}$ at the surface of cultures. Photoperiod was adjusted to 16 hours light/8 hours darkness. Temperature in the growth room was 20 ± 2°C.

## RESULTS

### Callus induction and shoot differentiation

Primary explants of *Dracaena* produced callus after 6–8 weeks of culture on media A1 and A2. Callus was hard and yellowish in colour. In some cases shoots differentiated from callus while primary explants were still on media for callus induction. However, shoot differentiation was far better if callus was subcultured on media with increased concentration of BA (1.0–2.0 $mgl^{-1}$) and decreased concentrations of auxins. Optimal shoot differentiation was registered on B1 media. Callus remained undifferentiated on media E1 during 8 successive subcultures lasting 15 months. At the end of this period callus readily differentiated shoots upon transfer to B1 media.

Primary explants of *Cordyline* produced callus on media A1 and A2 same as explants of *Dracaena*. Callus proliferation was faster and occured within 3–4 weeks. Callus was soft and pale in colour. *Cordyline* callus was difficult to maintain in culture as it usually became necrotic in the first or second subculture, specially if A1 media was employed. Shoot buds which occasionally appeared on primary explants seemed to originate from preexisting lateral buds and not from callus. Truly, culture of *Cordyline* primary explants on media containing only BA (B3), induced development of lateral buds without the induction of callus. Thus, the later established *Cordyline* shoot cultures originated from lateral buds and not from callus tissue.

First signs of callus proliferation in *Sansevieria* were visible after 3–5 weeks of culture on media A1 and A2. Callus developped from leaf mesophyll and appeared only on cut surfaces. It was hard and yellowish in colour. Callus turned green prior to shoot differentiation. Shoot differentiation required a new medium in which BA concentration was increased and 2,4–D was replaced by IBA or NAA at low concentration. Optimal media for shoot differentiation was B2. Callus growth and differentiation were very slow. Explants cultured 4–6 weeks on media A1 and A2 differentiated only 2.0 shoots per primary explant upon transfer to media B2. Explants cultured for additional 4–6

weeks on media A1 and A2 before transfer to B2 produced 4.7–5.2 shoots per primary explant.

## Shoot multiplication

*Dracaena* shoots differentiated from callus multiplied on media containing 0.5–2.0 $mgl^{-1}$ BA and either 0.1–0.2 $mgl^{-1}$ NAA or 0.5–2.0 $mgl^{-1}$ IBA. Media supplemented only with BA did not support shoot multiplication and growth.

Optimal shoot multiplication was registered on media B1. Shoot multiplication on this media was accompanied by moderate callusing and most of the newly formed shoot buds appeared from surface callus layers via callus differentiation. The rest of the buds were adventitious, originating from basal shoot portions. True axillar buds appeared rarely. The increase of NAA concentration from 0.1 to 5.0 $mgl^{-1}$ (at constant BA concentration 1.0 $mgl^{-1}$) gradually shifted the proportion of newly formed shoot buds from those differentiated from callus towards adventitious and axillar buds.

High shoot multiplication rates were obtained only if cultures were trained to grow as clusters of shoots sprouting from a common base (shoot clusters). Single, isolated shoots exhibited very low rates of shoot multiplication.

*Cordyline* shoot cultures required only 0.05–0.2 $mgl^{-1}$ BA for shoot multiplication. Concentrations exceeding 0.2 $mgl^{-1}$ decreased shoot length. Up to now, *Cordyline* shoot cultures have been successfully cultured for more then 2 years on media which contained only BA. Presence of auxins in the media stimulated hypertrophy and callusing of the shoot base even at auxin concentrations as low as 0.1 $mgl^{-1}$.

True shoot cultures of *Sansevieria* were not obtained. Cultures consisitng of callus and differentiated shoots continued to proliferate new shoots via callus differentiation on media B2, but the shoots themselfs rarely produced adventitious or axillar shoot buds. The excision of the apical part of shoot stimulated activation of lateral shoot meristems but at a very slow rate. Slow growth of *Sansevieria* shoots could be interpreted as a kind of dormancy, probably induced by improper hormonal balance of the media.

## Shoot elongation

Shoot elongation of both *Dracaena* and *Cordyline* shoot cultures required low BA concentrations in the media which did not exceed 0.1 $mgl^{-1}$. Elongation of *Dracaena* shoots required also high auxin concentrations, 1.0 $mgl^{-1}$ NAA or 2.0 $mgl^{-1}$ IBA (media C1 and C2). Optimal shoot elongation of *Cordyline* was registered on medium (C3).

Media C1 for *Dracaena* and C3 for *Cordyline* enabled continuous culturing systems to be estableshed for these two species. Under continuous culturing system we consider a single media in which hormonal balance is adjusted in such a way that it supports both shoot multiplication and elongation.

Elongation of *Sansevieria* shoots was slow on a variety of hormonal balances which were tested. For this reason, optimal media for *Sansevieria* shoot elongation can nor be recomended.

### Rooting

Single isolated *Dracaena* shoots exhibited 100% rooting on media containing 0–2.0 $mgl^{-1}$ IBA. Optimal rooting media was D1. Root length depended on auxin concentration of the media and irradiation provided during rooting. Root length increased with decrease of auxin concentration or with the increase of irradiation. BA strongly inhibited rooting at concentrations exceeding 0.1 $mgl^{-1}$.

Single isolated shoots of *Cordyline* could also be rooted on media containing low auxin concentrations but rooting in presence of auxins always stimulated shoot base hypetrophy and callusing which was proportional to the concentration of auxin incorporated in the medium. Hormone–free medium (D3) was considered as optimal rooting medium for *Cordyline* because it did not support production of callus.

*Sansevieria* shoots exibited 90–100% rooting on media containing 0.5–1.0 $mgl^{-1}$ IBA or 0.1 $mgl^{-1}$ NAA. Roots formed on media containing NAA were short and thick.

Optimal time for planting all three species in soil substrates was when roots reached 0.5–2.0 cm in length.

### Adaptation and phenotypic stability

Rooted Dracaena plantlets easily adapted to *in vivo* conditions. In soil substrates based on peat (Bioflor, Jiffy pots) and with moderate watering adaptation was nearly 100% efficient. Rooted plantlets exhibited high resistance to fungal diseases.

Up to now more than 10000 *D. fragrans* plants have been produced by the procedure presented here. With exception of several off–type, variegated specimens all other plants were morphologicaly undistinguishable from the original mother–donor plant.

*Cordyline* plantlets required high air humidity for successful adaptation. At the same time plantlets were susceptible to fungal disease. Best results were obtained under mist where the efficienty of adaptation was over 75%. After adaptation plant continued to grow fast, developing the same leaf pattern as in mother–donor plant.

*Sansevieria* plantlets adapted to *in vivo* conditions only if watering was completely ommited until the first new leaves appeared. Adapted plantlets grew very slowly. Total number of successfully adapted plants exceeded 200. All propagated plants were green same as in conventional propagation by leaf cuttings. The use of leaf margin fragments as primary explants also resulted in production of green plants. These explants occasionally produced pale–yellow albino plants which gradualy withered in culture.

## DISCUSSION AND CONCLUSION

Comparative investigation of the methods for *in vitro* propagation of *D. fragrans*, *C. terminalis* and *S. trifasciata* presented here were focussed on the hormonal composition of the media, choice of primary explants and water requirement of plantlets transferred to soil substrates. Many external (light, photoperiod, temperature) and internal factors related to the composition of the media (mineral salts, pH, sucrose, agar) have not been considered in this investigations.

In all three species BA displayed the same kind of action: it stimulated shoot multipication, restricted shoot elongation and inhibited root initiation and growth.

Auxins, NAA and IBA, stimulated shoot elongation and root initiation. Low auxin concentrations in *Dracaena* and *Sansevieria* supported the stimulative effect of BA on shoot multiplication. In *Cordyline* auxins induced intensive callus proliferation in all propagation.

2,4–D was teh inevitable promoter for callus induction in *Dracaena* and *Sansevieria* while in *Cordyline* it could be replaced by IBA or NAA.

Among the three investigated species *Cordyline* exhibited the highest shoot multiplication rates. *Cordyline* shoot clusters cultured for 4 weeks on media supplemented with 0.2 $mgl^{-1}$ BA contained on the average 11.5 shoots per cluster, or 80 shoots per Erlenmayer flask. In *Dracaena*, shoot multiplication rate was lower while in *Sansevieria* it was very poor. In case of *Sansevieria* the dominant pattern of multiplication was shoot bud differentiation from callus and hardy any new shoot buds appeared by lateral or adventious branching.

Shoot elongation and rooting were well elaborated for *Dracaena* and *Cordyline; Sansevieria* shoots grew poorly, but the high percentage of rooting allows in vitro propagation of this species to be considered as successful.

From the standpoint of prospective commercial use of tissue culture propagation methods, both *Cordyline* and *Dracaena* fulfill all required criteria. In addition to more than satisfactory propagation parameters achieved in all stages of propagation, the obtained progeny did not differ from the mother–donor plant. More then 2000 *Dracaena* and over 100 *Cordyline* plants donated to nurseries for adaptation trials were finaly sold on the market.

In case of *Sansevieria* the propagation sheme presented here can not be recommended for commercial propagation. The reason for such judgement are low shoot multiplication rate, lack of a system for continuous culturing, and in general, slow growth of cultures in all stages of propagation. *Sansevieria* can easily be propagated from leaf cuttings and by division. The use of tissue culture propagation methods at this stage of knowledge except productivity offers no advantages in comparison to the conventional propagation methods. According to the method presented here, a leaf 50 cm long and 6 cm wide, under optimal conditions can provide 720 primary explants, giving a total of 2500 new plants.

Although taxonomy considers genera *Dracaena* and *Cordiline* to be closely related, investigations presented here revealed marked physiological differences, among which the outstanding feature of *Cordyline*, which does not require exogenous auxins for *in vitro* propagation.

REFERENCES

Blazich, F. A., Novitzky, R. T. (1984): *In vitro* propagation of *Sansevieria trifasciata.* – Hort. Sci. *19,* 122–123.

Xhua, B. U., Kunisaki, J. Y., Sagawa, Y. (1981): *In vitro* propagation of *Dracaena marginata* „Tricolor". – Hort. Sci. *16,* 494.

Debergh, P. (1975): Intensified vegetative multiplication of *Dracaena deremensis.* – Acta. Hortic. *54,* 83–91.

Debergh, P. (1976): An *in vitro* technique for the vegetative multiplication of chimaeral plants of *Dracaena* and *Cordyline.* – Acta. Hortic. *64,* 17–19.

Evaldsson, I. E., Welander, N. T. (1985): The effects of medium composition and *in vitro* propagation and *in vivo* growth of *Codyline* terminalis cv. Atoom. – J. Hort. Sci. *60,* 525–530.

Kunisaki, J. T. (1975): *In vitro* propagation of *Cordyline terminalis* L. Kunth. – Hort. Sci. *10,* 601–602.

Miller, ,L. R., Murashige, T. (1976): Tissue culture propagation of tropical foliage plants. *In vitro 12,* 797–813.

Murashige, T., Skoog, F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. – Physiol. Plant. *15,* 473–497.

Vinterhalter, D. (1989): *In vitro* propagation of green–foliaged *Dracaena fragrans* Ker. – Plant. Cell Tiss. Org. Culture. *17,* 13–19.

## Rezime

DRAGAN VINTERHALTER, BRANKA VINTERHALTER i DARINKA PETROVIĆ*

### IN VITRO RAZMNOŽAVANJE VRSTA *DRACAENA FRAGRANS* KER, *CORDYLINE TERMINALIS* CV KIWI, I *SANSEVIERIA TRIFASCIATA* VAR. *LAURENTII*

Institut za biološka istraživanja „Siniša Stanković", Beograd
*Zavod za voćarstvo i vinogradarstvo, RO INI „Agroekonomik–PKB", Beograd

Postupci za *in vitro* razmnožavanje vrsta *Dracaena fragrans, Cordyline terminalis* i *Sansevieria trifasciata,* članova familije *Agavaceae,* istraživani su komparativno. Hranljive podloge korišćene za sve tri vrste sadržale su mineralne soli i organske dodatke po Murashige i Skoog-u (1962) razlikujući se samo po sadržaju hormona. Za sve tri vrste u prvoj fazi razmnožavanja primarni eksplantati su indukovani da produkuju kaluse iz kojih su zatim pokrenute kulture izdanaka. U daljim fazama razmnožavanja je odgovaralo standardnoj mikropropagaciji uključujući multiplikaciju izdanaka, izduživanje izdanaka, oživljavanje i konačno adaptaciju ožiljenih biljaka u *in vivo* uslovima. Optimalni hormonski balansi za sve faze razmnožavanja prikazani su i diskutovani u odnosu na specifične zahteve pojedinih vrsta.