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Effect of growth regulators on *in vitro* propagation of *Ficus benjamina* cv. Exotica

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Abstract

Stem internodes with axillary buds were excised from 5-year old trees of *Ficus benjamina* cv. Exotica. The effect of 6-benzylaminopurine (BAP), gibberellic acid (GA_3), indole-3-acetic acid (IAA), naphthaleneacetic acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4-D) on shoot growth and proliferation *in vitro* was investigated. Multiple shoots were developed after 3 - 4 weeks from stem internodes with axillary buds incubated in Murashige and Skoog (MS) medium supplemented with phloroglucinol (PG) and BAP. Optimum shoot proliferation took place in the presence of 1.0 mg l^{-1} BAP. Shoots obtained could be elongated in a medium with 0.5 mg l^{-1} GA_3 prior to their rooting. The root initiation was successfully induced on MS medium either with IAA at 0.5 - 0.1 mg l^{-1} or in plant growth regulator-free medium. All rooted plantlets were subsequently transferred to a peat, humus and perlite mixture in a culture room with high humidity and covered with plastic bags. After one month the plantlets were established for growing in a greenhouse.

Introduction

Asexual propagation is an important commercial method of regenerating large quantities of genetically uniform plant materials (Davies *et al.* 1982). Many herbaceous angiosperms can now be propagated through tissue culture techniques (Murashige 1974), and during recent years the application of *in vitro* culture for tree breeding programs has also been investigated with success (Narayan and Jaiswal 1986).

Ficus species are tropical wood shrubs and trees that play a significant role as interior foliage plants (Lloyd 1990). Reports on the clonal propagation of various *Ficus* sp. (Muriithi *et al.* 1982, Pontikis and Melas 1986, Kristiansen 1991, 1992, Amo-Marco and Picazo 1992a) and plant regeneration (Debergh and de Wael 1977,

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Abbreviations: BM - basal medium; BAP - 6-benzylaminopurine; GA_3 - gibberellic acid; IAA - indole-3-acetic acid; NAA - α-naphthaleneacetic acid; 2,4-D - 2,4-dichlorophenoxyacetic acid.

Jaiswal and Narayan 1985, Jona and Gribaudo, 1987, Amo-Marco and Picazo 1992b) have already been published.

Ficus benjamina cv. Exotica is an ornamental tree propagated mainly by conventional vegetative multiplication from tip cuttings, and its culture requires a large space in the greenhouse. Nowadays, tissue culture techniques offer a viable alternative method for its rapid and mass propagation, and this species is now micropropagated and mainly used as stock plant for cutting production (Kristiansen 1991, 1992), because cuttings from micropropagated plants are more juvenile and with best growth rate.

We studied the effects of various plant growth regulators for the rapid *in vitro* propagation of *Ficus benjamina* cv. Exotica, and this paper reports a protocol which may provide a large-scale multiplication method through stem segments with axillary buds.

Materials and methods

Stem internodes (3 cm long) with an axillary bud were excised from actively growing 5-year-old plants of *Ficus benjamina* cv. Exotica. Leaves were clipped off and the explants were throughly washed for 10 min in running tap water with a domestic soap. Because of the initial high percentage of contamination in explants, the stem internodes were then surface desinfected for 15 min with a mixture of 50 % benomyl and 80 % zineb, each at 10 g l^{-1}. Afterwards, the explants were sterilized by dipping for 5 min in 80 % (v/v) ethanol and 20 min in commercial bleach diluted 30 % (v/v) with distilled water, with 0.1 % Tween-20 as a surfactant. All traces of chlorine were removed from the explants by four rinses with sterile distilled water. All operations were carried out in a laminar flow hood.

Two different types of explants were assayed: 1.5 - 2.0 cm long stem internodes with an axillary bud, and 0.3 - 0.5 cm long excised axillary buds.

The basal medium (BM) used was Murashige and Skoog's (1962) medium, supplemented with 100 mg l^{-1} myo-inositol, 5 mg l^{-1} thiamine-HCl and 30 g l^{-1} saccharose. To this medium cytokinins (BAP, kinetin), GA_3, and auxins (IAA, NAA, 2,4-D) of various concentrations were added according to the propagation phase. All propagation media were completed with 80 mg l^{-1} phloroglucinol (PG) according to the previous results obtained with the cv. Starlight (Amo-Marco and Picazo 1992a).

The pH of each medium was adjusted to 5.7 with KOH prior to adding 0.8 % (m/v) agar *Technical Oxoid № 3*, and all media were sterilized by autoclaving at 121 °C for 20 min. Gibberellic acid was dissolved in water, sterilized by filtration with *Millipore* filters (0.2 µm pore opening) and subsequently added to the autoclaved medium while still warm.

The explants were incubated in 55 × 75 mm glass flasks containing 30 ml medium and capped with aluminium foil. Cultures were maintained at irradiance of 120 µmol(PAR) m^{-2} s^{-1} (*Sylvania* cool-white fluorescent tubes, photoperiod of 16 h) and at temperature of 26 ± 2 °C during the light period.

The explants (5 per flask) were placed with the basal end down into the medium and 6 flasks were cultured for each treatment. Proliferatioin rate was defined as number of shoots > 5 mm formed and was calculated for each explant type.

The shoots proliferated on clusters were excised and subcultured for rooting. The number of days of emergence of first root was recorded when it was visible in the glass flasks. When the roots were 1.0 - 1.5 cm long, the plants were transferred to plastic pots, 8 - 10 cm in diameter, with 80 % peat, 16 % earth-worm humus and 4 % perlite. The pots were maintained for 4 - 5 weeks in a growth chamber at day/night temperature of 28 ± 2/26 ± 2 °C and 16 h irradiance of about 120 μmol(PAR) m^{-2} s^{-1} and were covered with a plastic bag to maintain high humidity. After one month, the plastic bags were removed and the plantlets were transferred to the greenhouse.

Results and discussion

Shoot multiplication: An important factor affecting morphogenic response is the type of explant used. Thus, Hutchinson (1982) and Yadav *et al.* (1990) observed that isolated buds are not the best type of explants for maintenance of apple or mulberry cultures, because most of them produce relatively few shoots. According to these authors, nodal explants with a bud showed better proliferation and elongation than shoot tip explants.

In our experiments with *Ficus benjamina* cv. Exotica, multiple shoots could be obtained from axillary buds attached to a stem internode after 30-d culture in media containing BAP without or in combination with 2,4-D or NAA (Table 1). However, the excised axillary buds failed to differentiate shoots in all media tested. The highest frequency of explants with developing shoots (85.7 %) that was also coupled with the greatest number of shoots per explant (5.3 shoots) was found in BM with BAP at concentration 1.0 mg l^{-1} (Table 1). When the BAP concentration was increased to 3.0 mg l^{-1} both parameters were significantly reduced. The highest number of shoot per explant was lower than that we observed for the cv. Starlight on medium with BAP and PG (Amo-Marco and Picazo 1992a).

A dose-response curve for BAP in apple was published by Lane (1978), and he found that 1.0 mg l^{-1} BAP was the optimal concentration, producing maximum number of shoots. A similar pattern of BAP effect was found in *Morus nigra* (Yadav *et al.* 1990), and by us with *Ficus benjamina* cv. Starlight (Amo-Marco and Picazo 1992a) and in the present work with the cv. Exotica.

A slight callusing was observed at the edges of explants in the presence of BAP alone after 10 - 15 d of culture, but this did not seem to have any effect on the differentiation of multiple shoots. These results were very similar to that obtained with the cv. Starlight (Amo-Marco and Picazo 1992a).

In media with 2,4-D alone (Table 1) callusing increased notably after 15 - 20 d and maximum callus growth was attained after 30 d of culture in the medium supplemented with 1.0 mg l^{-1} 2,4-D. Growth of axillary buds or differentiation of multiple shoots was not observed in this medium.

The presence of auxins in the medium with BAP was unfavourable for bud differentiation and promoted callus growth. Really, only BAP was required for shoot differentiation, with the optimum concentration 1.0 mg l^{-1} (Table 1).

Table 1. Effect of growth regulators on *in vitro* morphogenic responses of stem internode with axillary buds of *Ficus benjamina* cv. Exotica after 30 d of culture. Each treatment consisted of 30 explants.

Propagation medium [mg l^{-1}]	Explant survival [%]	Explant developing callus [%]	shoots [%]	roots [%]	Average No. of shoots [explant^{-1}]
BM[a] (hormone-free)	70.0 ± 8.3	0	0	0	0
BM + 2,4-D 0.5	63.3 ± 8.8	84.2[b] ± 8.3	0	0	0
BM + 2,4-D 1.0	80.0 ± 7.3	91.6[b] ± 5.6	0	0	0
BM + BAP 0.5	60.0 ± 8.9	72.2 ± 10.5	55.5 ± 11.7	0	4.1 ± 0.23
BM + BAP 1.0	70.0 ± 8.3	76.2 ± 9.3	85.7 ± 7.6	0	5.3 ± 0.27
BM + BAP 3.0	56.6 ± 9.0	58.8 ± 11.9	64.7 ± 11.6	0	3.9 ± 0.30
BM + BAP 1.0 + 2,4-D 0.1	63.3 ± 8.8	68.4 ± 10.6	36.8 ± 11.1	0	3.3 ± 0.35
BM + BAP 1.0 + 2,4-D 0.5	76.6 ± 7.7	60.8 ± 10.1	26.1 ± 9.1	0	2.8 ± 0.31
BM + BAP 1.0 + NAA 0.1	66.6 ± 8.7	65.0 ± 10.6	30.0 ± 10.2	0	3.3 ± 0.36
BM + BAP 1.0 + NAA 0.5	60.0 ± 8.9	66.6 ± 11.1	33.3 ± 11.1	0	3.0 ± 0.27

The results are expresed as frequencies ± SE, SE of percentage is calculated as follows:
$SE = (\sqrt{p(1-p)/n}) \times 100$,
where p = number of explants with response divided by n, n = number of explants cultured
a - all media were completed with 80 mg l^{-1} PG
b - extensive callus growth covering almost all the explant

Shoot elongation: The clusters of multiple shoots which developed from the initial axillary buds were subdivided into individual shoots with 2 - 4 leaves each and transferred to elongation medium with different GA_3 concentrations to yield shoots usable as cuttings *in vitro* or *in vivo*, before rooting. In media supplemented with 0.1 or 0.5 mg l^{-1} GA_3, internode length 2.1 to 2.5 cm was achieved with a high rooting percentage induced thereafter (Table 2).

Table 2. *In vitro* growth of subcultured *Ficus benjamina* cv. Exotica shoots evaluated after 10 d on elongation medium. Each treatment consisted of 30 explants (mean ± SE).

Elongation medium [mg l^{-1}]	Length of elongated shoots [cm]	Explants developing roots [%]	Number of roots [explant^{-1}]
BM + GA_3 0.1	2.1 ± 0.14	70.0 ± 8.3	2.2 ± 0.15
BM + GA_3 0.5	2.5 ± 0.16	73.3 ± 8.1	2.5 ± 0.18
BM + GA_3 1.0	3.4 ± 0.23	46.6 ± 9.1	2.0 ± 0.24
BM + GA_3 3.0	3.8 ± 0.19	16.6 ± 6.8	1.7 ± 0.19

Elongation was excessive in media suplemented with 1.0 and 3.0 mg l^{-1} GA_3, and explants were very weak. Furthermore, these shoots did not differentiate a good root system and their viability *ex vitro* was very low.

Explants 6 - 7 weeks old on undivided cluster in the propagation media with BAP alone showed internode length 2.0 cm which was comparable to that of explants in 0.1 mg l^{-1} GA_3. On the other hand, when shoots transferred to elongation medium are as undivided clusters they produced more vigorous shoots than when single shoots were used.

Shoot rooting: In the shoots cultivated on elongation medium a high percentage of rooting was induced after 10 d. In this medium, the best percentage of explants developing roots (73.3 ± 8.1) and the best root average per explant (2.5 ± 0.18) were obtained in medium with GA_3 at concentration 0.5 mg l^{-1} (Table 2). Nevertheless, the roots induced with GA_3 showed scarce growth.

The shoots developed on the cluster in the propagation medium were isolated and transferred to different rooting media with auxins (Table 3). The frequencies of rhizogenesis (% of rooted shoots) and root production (number of root per explant) were higher with IAA than with NAA. Maximum induction of roots (90 %) occurred on a medium containing 0.5 mg l^{-1} IAA and the shoots developed an average of 3.4 roots per explant within 7 - 10 d. Increasing this IAA concentration or the further addition of 0.5 mg l^{-1} GA_3 to the medium with IAA decreased rooting (Table 3).

Table 3. Effect of growth regulators on *in vitro* rooting of *Ficus benjamina* cv. Exotica shoots after 10 d in root-inducing medium. Each treatment consisted of 30 explants (means ± SE).

Rooting medium [mg l^{-1}]	Explants developing root [%]	Number of roots [explant^{-1}]	Basal callusing
BM (hormone free)	80.0 ± 7.3	3.0 ± 0.22	-
BM + IAA 0.1	83.3 ± 6.8	3.1 ± 0.26	+
BM + IAA 0.5	90.0 ± 5.5	3.4 ± 0.26	+
BM + IAA 1.0	73.3 ± 8.1	2.8 ± 0.20	+
BM + IAA 0.1 + GA_3 0.5	63.3 ± 8.8	2.6 ± 0.39	-
BM + NAA 0.5	30.0 ± 8.3	0.8 ± 0.31	++
BM + NAA 1.0	43.3 ± 9.0	0.9 ± 0.21	++

\- - no callus formed
\+ - little callus formed
++ - extensive callusing; roots thin and short

As reported for *Ficus benjamina* cv. Starlight (Amo-Macro and Picazo 1992a), the poorer rooting results were obtained with NAA. The percentage of rooting and the number of shoots per explant decreased notably in both cultivars of *F. benjamina* with this auxin. Moreover, the roots formed in medium with NAA were thin and basal callusing at the cut end of shoots in contact with the agar was induced in this medium.

The rooted plants were transferred to plastic pots covered with plastic bags to maintain high humidity. These pots contained a mixture of peat, humus and perlite. After 4 - 5 weeks of acclimation in a growth chamber the plastic bags were removed and the plantlets were successfully established under greenhouse conditions. At least 90 % of all rooted plants survived transplanting.

On the other hand, in the basal medium without plant growth regulators the percentage of shoots rooted and the average number of roots per explant were close to that obtained with 0.1 mg l^{-1} IAA (Table 3) and similar those obtained with the cv. Starlight (Amo-Marco and Picazo 1992a). Therefore a hormone-free medium could be used for the successful rooting of *F. benjamina in vitro*. Likewise, rooted plants of *F. lyrata* were also obtained on a medium without plant growth regulators (Jona and Gribaudo 1987).

Our propagation results concur with those reported for *F. religiosa* (Jaiswall and Narayan 1985) or *Morus nigra* (Yadav *et al.* 1990). In conclusion, the growth regulator BAP is essential to *in vitro* multiplication of *F. benjamina* cv. Exotica as well as the cv. Starlight and the auxins counteracts the effect of BAP. The effect of phloroglucinol in improving the rate of shoot multiplication in the cv. Exotica was smaller than that obtained with the cv. Starlight (Amo-Marco and Picazo 1992a).

References

Amo-Marco, J.B., Picazo, I.: *In vitro* propagation of *Ficus benjamina* cv. Starlight from axillary buds with BAP and phloroglucinol. - Gartenbauwissenschaft **57**: 29-32, 1992a.

Amo-Marco, J.B., Picazo, I.: Improvement of *in vitro* multiplication of *Ficus lyrata* from leaf explats and leaf callus by thidiazuron. - Fyton **53**: 51-55, 1992b.

Davies, F.T., Lazarte, J.E., Joiner, J.N.: Initiation and development of roots in juvenile and mature leaf bud cuttings of *Ficus pumila* L. - Amer. J. Bot. **69**: 804-811, 1982.

Debergh, P., de Wael, Y.: Mass propagation of *Ficus lyrata*. - Acta Hort. **78**: 361-364, 1977.

Hutchinson, J.F.: *In vitro* propagation of apple using organ culture. - In: Fujiwara, A. (ed.): Plant Tissue Culture. Pp. 729-730. Maruzuen, Tokyo 1982.

Jaiswal, V.S., Narayan, P.: Regeneration of plantlets from the callus of stem segments of adult plants of *Ficus religiosa* L. - Plant Cell Rep. **4**: 256-258, 1985.

Jona, R., Gribaudo, I.: Adventitious bud formation from leaf explants of *Ficus lyrata*. - HortScience **22**: 651-653, 1987.

Kristiansen, K.: Post propagation growth of cuttings from *in vitro* and *in vivo* propagated stock plants of *Ficus benjamina*. - Scientia Hort. **46**: 315-322, 1991.

Kristiansen, K.: Micropropagation of *Ficus benjamina* clones. - Plant Cell Tissue Organ Cult. **28**: 53-58, 1992.

Lane, W.D.: Regeneration of apple plants from shoot meristem tips. - Plant Sci. Lett. **16**: 337-342, 1978.

Lloyd, G.: The impact of tissue culture on *Ficus* spp. Propagation and production. - Proc. Int. Plant Propag. Soc. **40**: 163-165, 1990.

Murashige, T.: Plant propagation through tissue cultures. - Annu. Rev. Plant Physiol. **25**: 135-166, 1974.

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

Muriithi, L.M., Rangan, T.S., Waite, B.H.: *In vitro* propagation of *Ficus* through shoot tips culture. - HortScience **17**: 86-87, 1982.

Narayan, P., Jaiswal, V.S.: Differentiation of plantlets from leaf callus of *Ficus religiosa* L. - Indian J. exp. Biol. **24**: 193-194, 1986.

Pontikis, C.A., Melas, P.: Micropropagation of *Ficus carica* L. - HortScience **21**: 153, 1986.

Yadav, U., Lal, M., Jaiswal, S.: Micropropagation of *Morus nigra* L. from shoot tip and nodal explants of mature trees. - Scientia Hort. **44**: 61-67, 1990.

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