BIOLOGIA PLANTARUM 42 (3): 333-340, 1999

Effect of agar, MS medium strength, sucrose and polyamines on *in vitro* rooting of *Syzygium alternifolium*

P.S. SHA VALLI KHAN*, J.F. HAUSMAN* and K.R. RAO**

CRP-Centre Universitaire, CREBS,
*162A, Av. de la Faïencerie, L-1511 Luxembourg, Grand-Duchy of Luxembourg**
*Plant Tissue Culture Laboratory, Department of Botany, Sri Venkateswara University, Tirupati-517502, A.P., India***

Abstract

This paper describes the effect of agar, MS basal medium strength, sucrose and polyamines on the *in vitro* rooting of *Syzygium alternifolium* realized by a two step procedure involving root initiation (RI) and root elongation (RE). RI was carried out on solidified MS medium supplemented with 1.0 mg dm^{-3} indole-3-butyric acid (IBA) for 3 weeks, and RE following transfer to half-strength MS medium devoid of growth regulators for another 3 weeks. Agar and MS basal medium concentrations played important role on rooting response as well as on health of rooted shoots. Sucrose concentration was positively correlated with the rooting percentage, root number per shoot and root length. The combination of polyamines and 1.0 mg dm^{-3} IBA increases rooting percentage compared to media containing only 1.0 mg dm^{-3} IBA. Optimum rooting was attained with half-strength MS medium containing 1.0 mg dm^{-3} IBA, 2 % sucrose, 10 μM spermine and 0.8 % agar.

Additional key words: IBA, *Myrtaceae*, root elongation, root initiation.

Introduction

Adventitious root formation is the crucial aspect in the clonal propagation of different plant species both from cuttings and *in vitro*. In horticulture and forestry, adventitious rooting allows the cloning of superior genotypes and is an essential part of breeding

Received 6 November 1998, accepted 26 February 1999.
Abbreviations: BA - N^6-benzyladenine; IBA - indole-3-butyric acid; MS - Murashige and Skoog's (1962) medium; NAA - 1-naphthaleneacetic acid; PAR - photosynthetically active radiation; PUT - putrescine; RE - root elongation; RI - root initiation; SPD - spermidine; SPM - spermine.
Acknowledgments: Financial support provided by the Ministère des Affaires Etrangères, du Commerce Extérieur et de la Coopération Luxembourgeois, Luxembourg to PSVK in the form of a post doctoral fellowship is sincerely acknowledged. The authors are grateful to Dr. L. Hoffmann and Dr. D. Evers for their valuable suggestions.
Author for correspondence: Dr. J.F. Hausman, fax: (+352) 4666 44413, e-mail: hausman@crpcu.lu

programs (Altamura 1996). In micropropagation system, final culture stage prior to acclimatization is rooting. Survival of plants in field depends on the development of efficient vascular system between root and shoot and good root to shoot ratio.

Many species are difficult to propagate because of their inability to give an acceptable rooting percentage, either when cuttings or microcuttings are used. Rooting capacity is generally reduced in woody species as compared to herbaceous ones (Hackett 1988). It is well known that exogenously applied natural or synthetic auxin favours rooting, whereas little attention has been given to other aspects like concentration of agar, rooting medium strength and sucrose, which might also influence *in vitro* rooting. Polyamines such as putrescine, spermine and spermidine have been involved in the control of the induction phase of rooting in *Prunus avium* L. (Biondi *et al.* 1990), poplar (Hausman *et al.* 1994, 1995) and walnut (Heloir *et al.* 1996). Polyamines promoted early rooting and increased rooting percentage as well as root number per shoot in olive (Rugini *et al.* 1997), hazelnut (Rey *et al.* 1994) and poplar (Hausman *et al.* 1995, 1997). The examples given above are encouraging for extending the exogenous application of polyamines for *in vitro* rooting of other species.

Syzygium alternifolium (Wight.) Walp., a member of *Myrtaceae* family is a rare endemic fruit tree of medicinal and economical importance. The species distribution is restricted to several districts in the southern part of India. Natural regeneration of this species is poor, seed production is sporadic, the seeds cannot be stored for long periods and conventional vegetative propagation has not been successful. Plant tissue culture techniques are useful for the clonal multiplication, genetic improvement and conservation of this rare endemic species. In previous work, we have reported our results on *in vitro* shoot proliferation from juvenile nodal buds and application of auxin for the rooting of *in vitro* formed shoots of this species (Sha Valli Khan *et al.* 1997). The purpose of the present research is to study the effect of agar, MS basal medium strength, sucrose and polyamines on rooting ability of *in vitro* formed shoots of *S. alternifolium*.

Materials and methods

Shoot cultures of *S. alternifolium* were established from nodal explants of seedlings on modified Murashige and Skoog's (MS) medium supplemented with 4.0 or 0.5 mg dm^{-3} NAA (Sha Valli Khan *et al.* 1997). Shoot proliferation medium was supplemented with 2 % sucrose and pH was adjusted to 5.7 with KOH or HNO_3 prior to the addition of 0.8 % agar (*Roland*, Brussels, Belgium) and subsequently autoclaved for 20 min at 120 °C. Shoot cultures were proliferated in 500 cm^3 transparent polystyrol jars (*Polarcup*, Antwerpen, Belgium) containing 75 cm^3 of medium. The cultures were incubated in a culture room at a temperature of 25 ± 2 °C, 55 % relative humidity, and 16-h photoperiod with irradiance of 20 µmol m^{-2} s^{-1} (photosynthetically active radiation, PAR) provided by cool-white fluorescent lamps. Axillary shoot proliferation with a stable multiplication rate was maintained by subculturing every 6 weeks on fresh medium.

Rooting involved a two step procedure: root initiation (RI) and root elongation (RE). Root initiation (RI) was carried out with 1.0 mg dm^{-3} indole-3-butyric acid (IBA) for 3 weeks and root elongation (RE) following transfer to half-strength MS medium devoid of growth regulators for another 3 weeks. Single shoots resulting from 6 weeks old subculture of shoot multiplication were used for rooting experiments. The shoots were approximately 4 cm long and had 3 to 4 nodes and the shoot tip. The pH of the rooting medium was adjusted to 5.7 prior to autoclaving. All cultures were performed in 500 cm^3 transparent polystyrol jars containing 75 cm^3 of medium upon which 3 shoots were placed. Cultures of rooting were maintained under the same incubation conditions described for shoot proliferation cultures.

The effect of agar, MS basal medium, sucrose concentrations and polyamines were investigated in four sets of experiments at the stage of root initiation. In the first set, agar at four concentrations (from 0.6 to 1.2 %) was added to half-strength MS basal medium containing 2 % sucrose and 1.0 mg dm^{-3} IBA to determine the effect of agar concentration on rooting. Liquid cultures were also raised with the support of *Whatman No.1* filter paper bridge. In the second set of experiment, MS medium was reduced from full strength (1.0) to three quarters (0.75), half (0.50), quarter (0.25) and zero (0) strength to study the effect of MS basal medium concentration on rooting. All media were supplemented with 1.0 mg dm^{-3} IBA, 2 % sucrose and 0.8 % agar. In the third set of experiment, sucrose was incorporated at five concentrations (from 1 to 5 %) into half-strength MS medium containing 1.0 mg dm^{-3} IBA to determine the effect of sucrose concentration on rooting. The control treatment received no sucrose. All the media were solidified with 0.8 % agar. In the fourth set of experiments, polyamines such as putrescine (PUT), spermidine (SPD) and spermine (SPM) were added filter sterilized to half-strength MS medium at three concentrations (1, 5 or 10 μM) alone or in combination with 1.0 mg dm^{-3} IBA. The control treatment received no supplement of polyamines. All rooting media were supplemented with 2 % sucrose and 0.8 % agar.

All the rooting experiments had 12 replicates and each experiment was repeated three times. During each experiment, data collected were percentage of rooting, number of roots per shoot and root length after 3 weeks of root initiation. Mean values and standard error were used for statistical analysis.

Results and discussion

In the first set of experiments, root initiation was achieved from the bases of shoots after one week of incubation in liquid medium as well as on agar (from 0.6 to 1.2 %) solidified half strength MS medium containing 1.0 mg dm^{-3} IBA. However, there were differences in the overall rooting response and in the morphology of developed roots. Increasing the concentration of agar from 0.0 (liquid) to 0.8 % enhanced the rooting percentage, root number per rooted shoot and length of developed roots (Fig. 1*A,B,C*). Further increase in the concentration of agar (*i.e.*, 1.0 and 1.2 %) did not improve the response of rooting. The root morphology was better in agar solidified medium as compared to liquid medium. The developed roots are thin without laterals in liquid cultures, whereas on agar solidified medium roots are thick with laterals. The best

response of rooting as well as good quality of root system were obtained at 0.8 % agar. At this agar concentration, rooted shoots were green and healthy without any shoot tip necrosis. In contrast, rooted shoots showed shoot tip necrosis at 0.6 % agar. Necrosis was generally limited to the shoot tip, rarely exceeding the top node and therefore 2 nodes and axillary buds remained viable. These buds produced the axillary shoots that allowed survival of the *in vitro* formed shoots. Similarly 0.6 % agar did not support rooting and also led to the induction of shoot tip necrosis in *Cynara scolymus* (Debergh *et al.* 1981).

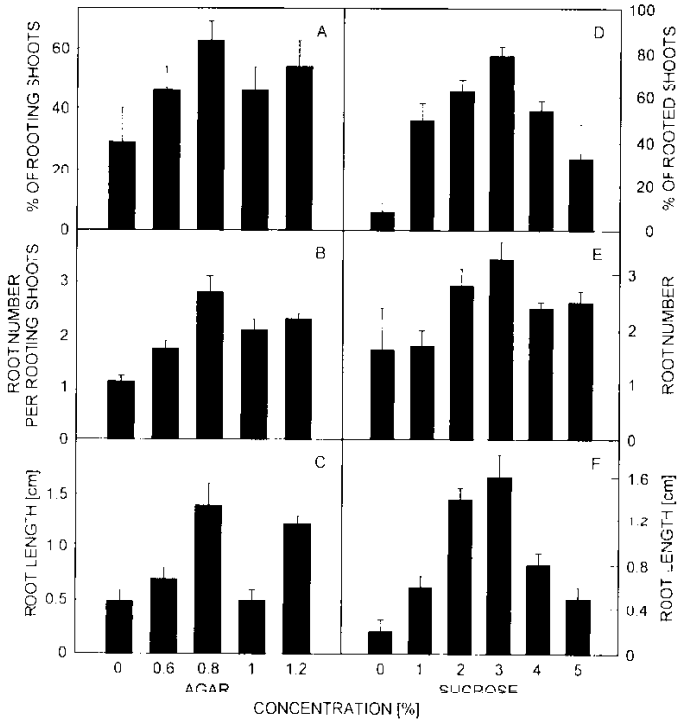

Fig. 1. Effect of agar (*A,B,C*) and sucrose (*D,E,F*) concentrations on the percentage of rooting (*A,D*), the root number (*B,E*), and the root length (*C,F*) at 3 weeks of root initiation (RI). Each bar represents the mean + SE of three replications (12 shoots for each replication).

No rooting occurred from the *in vitro* formed shoots if no basal medium (just agar and sucrose) was used and the leaves of the shoots showed chlorotic symptoms. This result clearly indicates the importance of MS salts for the response of rooting as well as for the general health of shoot. Reduction of the MS basal medium strength from full strength (1.0) to quarter strength (0.25) enhances both rooting percentage and number of roots per shoot (Table 1). The beneficial effect of reducing the MS basal medium concentration on *in vitro* rooting ability has been demonstrated in *Quercus sobur* L.

(Manzanera and Parados 1990) and *Wrightia tomentosa* (Purohit *et al.* 1994). The root growth was higher in the order of half-, quarter-, full-, and three quarters-strength MS basal medium (Table 1). The highest percentage of rooting and root number were obtained with quarter-strength MS medium. But, the rooted shoots showed chlorotic symptoms. On the contrary, half-strength MS basal medium resulted in a satisfactory rooting and the rooted shoots were green and healthy without any abnormal morphology.

Table 1. Effect of MS basal medium strength on rooting of *S. alternifolium* (after 3 weeks of root initiation). All media supplemented with 2 % sucrose, 0.8 % agar and 1.0 mg dm^{-3} IBA. Means ± SE for three replications (12 shoots for each replication).

MS medium strength	Rooting [%]	Number of roots [shoot $^{-1}$]	Root length [cm]
0	0	0	0
0.25	66.6 ± 4.1	3.0 ± 0.1	1.0 ± 0.1
0.50	62.5 ± 0.1	2.8 ± 0.3	1.4 + 0.3
0.75	45.8 ± 11.0	1.7 ± 0.4	0.8 ± 0.1
1.00	37.5 ± 12.5	1.3 + 0.1	1.0 ± 0.2

Sucrose concentration was positively correlated with the percentage of rooting, root number per rooted shoot and length of developed roots (Fig. 1*D,E,F*). The rooting percentage, root number and length increased with the rising concentration of sucrose from 1 to 3 %. Higher concentrations of sucrose (4 and 5 %) inhibited overall rooting response. Similar observations have been made in *Persea americana* (Plego-Alfaro 1988). Rooting medium lacking sucrose yielded very low percentage of rooting and developed roots also showed poor growth. From the literature it is quite clear that root initiation is a energy consuming process, which requires a source of carbon (Haissig 1982, Zimmerman 1983). The present study also proved that adventitious root formation is strongly dependent on sucrose supply. Pospíšilová *et al.* (1992) suggested incorporation of sucrose at lower concentrations into the rooting medium to enhance the autotrophic nature of rooted shoots, which ultimately increase the chances of survival of plantlets. In *S. alternifolium*, the best response of shoot proliferation was achieved on modified MS medium supplemented with 2 % of sucrose and 4.0 mg dm^{-3} and 0.5 mg dm^{-3} NAA (Sha Valli Khan *et al.* 1997). In contrast, the best rooting response was obtained at 3 % sucrose concentration. To improve photosynthesis ability and transplantation success, sucrose at 2 % concentration was considered as optimal for further studies at which a satisfactory rooting was achieved.

The rooting percentage, number of roots per rooted shoot and root length varied according to the type and concentration of polyamines as well as their combination with 1.0 mg dm^{-3} IBA (Fig. 2*A,B,C*). When polyamines (1, 5 or 10 μM) were added together with 1.0 mg dm^{-3} IBA rooting occurred at higher percentage. This result is due to the synergistic effect of polyamines and IBA as reported for *Prunus avium* (Biondi *et al.* 1990), poplar (Hausman *et al.* 1994, 1995) and walnut (Heloir *et al.* 1996). The highest percentage of rooting and root number were obtained in the presence of

1.0 mg dm^{-3} IBA and 10 μM spermine. During 3 weeks of root initiation period, polyamines alone or in combination with 1.0 mg dm^{-3} IBA did not enhance root elongation as compared to the shoots rooted in medium containing only 1.0 mg dm^{-3} IBA. Polyamines do not affect root elongation at a concentration able to increase

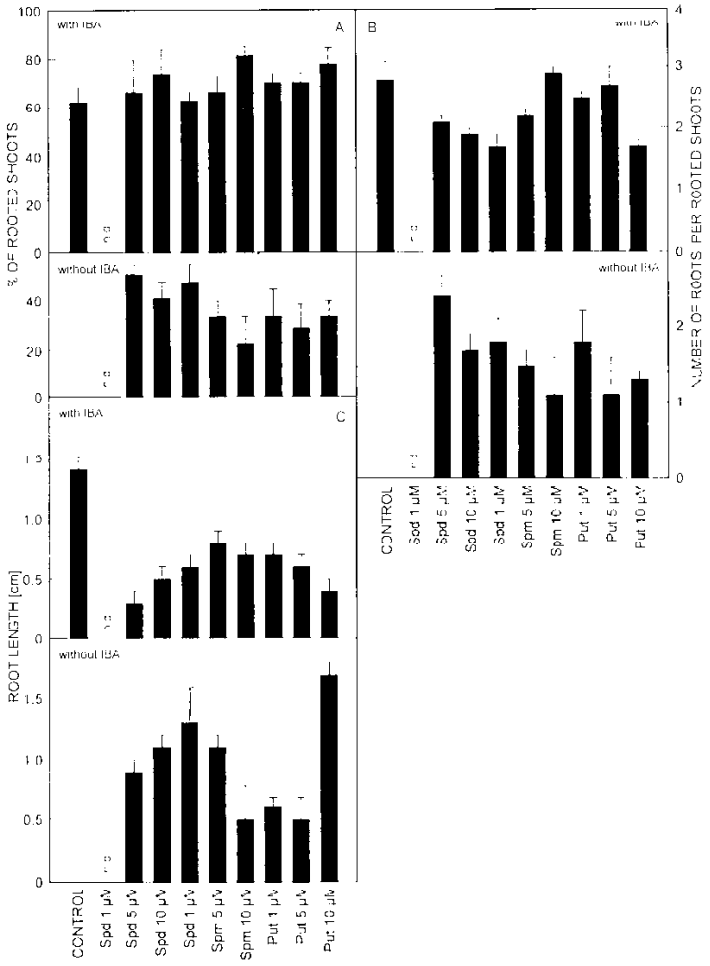

Fig. 2. Effect of polyamines on the rooting percentage (*A*), the root number (*B*), and the root length (*C*) at 3 weeks of root initiation. Each bar represents the mean ± SE of three replications (12 shoots for each replication).

rooting, indicating that the earlier protrusion of root apices exclusively depends on the early primordia formation as reported for the *Rosa* (Pierik 1997). To our knowledge there is no report concerning the role of polyamines on rooting ability of *in vitro*

formed shoots of *S. alternifolium*. According to our experiments, polyamines have a beneficial effect only on the rooting percentage. Further study is in progress with the aim to achieve increased root elongation with polyamines. In addition, *S. alternifolium* could be used as a model plant to study the metabolism of polyamines in root initiation.

The results presented above suggest that the use of half-strength MS medium supplemented with 1.0 mg dm^{-3} IBA, 2 % sucrose, 10 μM spermine and 0.8 % agar is optimal for the rooting of *in vitro* formed shoots of *S. alternifolium*. This knowledge on rooting ability and general health of plant could be helpful for the routine micropropagation of this rare endemic tree.

References

Altamura, M.M.: Root histogenesis in herbaceous and woody explants cultured *in vitro*. A critical review. - Agronomie **16**: 589-602, 1996.

Biondi, S., Diaz, T., Iglesias, I., Gamberini, G., Bagni, N.: Polyamines and ethylene in relation to adventitious root formation in *Prunus avium* shoot cultures. - Physiol. Plant. **78**: 474-483, 1990.

Davies, T.M., Haissig, B.E., Sankhala, N.: Adventitious Root Formation in Cuttings. Volume 2. - Dioscorides, Portland 1988.

Debergh, P.C., Harbaoui, Y., Lemeur, R.: Mass propagation of globe artichoke (*Cynara scolymus*). Evaluation of different hypotheses to overcome vitrification with special reference to water potential. - Physiol. Plant. **53**: 181-187, 1981.

De Klerk, G.J., Keppel, M., Ter Brugge, J., Meekes, H.: Timing of the phases in adventitious root formation in apple microcuttings. - J. exp. Bot. **46**: 965-972, 1995.

Gaspar, T., Coumans, M.: Root formation. - In: Bonga, J.M., Durzan, D.J. (ed.): Cell and Tissue Culture in Forestry. Volume 2. Specific Principles and Methods: Growth and Developments. Pp. 202-216. Martinus Nijhoff Publishers, Dordrecht 1987.

Hackett, W.P.: Donor plant maturation and adventitious root formation. - In: Davies, T.M., Haissig, B.E., Sankhala, N. (ed.): Adventitious Root Formation in Cuttings. Volume 2. Pp. 11-28. Dioscorides, Portland 1988.

Haissig, B.E.: Carbohydrate and amino acid concentrations during adventitious root primordium development in *Pinus banksiana* Lamb. cuttings. - Forest. Sci. **28**: 813-821, 1982.

Haissig, B.E.: Metabolic processes in adventitious rooting. - In: Jackson, M.B. (ed.): New Root Formation in Plants and Cuttings. Pp.141-189. Martinus Nijhoff Publishers, Dordrecht 1986.

Hausman, J.F., Kevers, C., Gaspar, T.: Involvement of putrescine in the inductive rooting phase of poplar shoots raised *in vitro*. - Physiol. Plant. **92**: 201-203, 1994.

Hausman, J.F., Kevers, C., Gaspar, T.: Auxin-polyamine interaction in the control of the rooting inductive phase of poplar shoots *in vitro*. - Plant. Sci. **110**: 63-71, 1995.

Hausman, J.F., Kevers, C., Evers, D., Gaspar, T.: Conversion of putrescine to γ-aminobutyric acid, an essential pathway for root formation by poplar shoots *in vitro*. - In: Altman, A., Waisel, Y. (ed.): Biology of Root Formation and Development. Pp. 133-139. Plenum Press, New York 1997.

Heloir, M., Kevers, C., Hausman, J.F., Gaspar, T.: Changes in the concentration of auxins and polyamines during rooting of *in-vitro* propagated walnut shoots. - Tree Physiol. **16**: 515-519, 1996.

Manzanera, J.A., Pardos, J.A.: Micropropagation of juvenile and adult *Quercus suber* L. - Plant Cell Tissue Organ Cult. **21**: 1-8, 1990.

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

Pierik, R.L.M.: Factors controlling adventitious root formation on stem explants of rose (*Rosa hybrida* "Motrea") *in vitro*. - In: Altman, A., Waisel, Y. (ed.): Biology of Root Formation and Development. Pp. 297-307. Plenum Press, New York 1997.

Plego-Alfaro, F.: Development of an *in-vitro* rooting bioassay using juvenile phase stem cuttings of *Persea americana* Mill. - J. hort. Sci. **63**: 295-301, 1988.

Pospíšilová, J., Solárová, J., Čatský, J.: Photosynthetic responses to stress during *in vitro* cultivation. - Photosynthetica **26**: 3-18, 1992.

Purohit, S.D., Kukda, G., Sharma, P., Tak, K.: *In vitro* propagation of an adult tree *Wrightia tomentosa* through enhanced axillary branching. - Plant Sci. **103**: 67-72;1994.

Rey, M., Diaz-Sala, C., Rodriguez: Exogenous polyamines improve rooting of hazel microshoots. - Plant Cell Tissue Organ Cult. **36**: 303-308, 1994.

Rugini, E., Di Francesco, G., Muganu, M., Astolfi, S., Caricato, G.: The effects of polyamines and hydrogen peroxide on root formation in olive and the role of polyamines as an early maker for rooting ability. - In: Altman, A., Waisel, Y. (ed.): Biology of Root Formation and Development. Pp. 65-73. Plenum Press, New York 1997.

Sha Valli Khan, P.S., Prakash, E., Rao, K.R.: *In vitro* micropropagation of an endemic fruit tree *Syzygium alternifolium* (Wight.) Walp. - Plant Cell Rep. **16**: 325-328, 1997.

Zimmerman, R.H.: Factors affecting *in vitro* propagation of apple cultivars. - Acta Hort. **131**: 171-178, 1983.