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South African Journal of Botany 73 (2007) 60 – 63
SOUTH AFRICAN JOURNAL OF BOTANY
www.elsevier.com/locate/sajb

Micropropagation of *Hoslundia opposita* Vahl—a valuable medicinal plant

S. Prakash, J. Van Staden *

Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

Received 9 June 2006; received in revised form 25 July 2006; accepted 25 July 2006

Abstract

A rapid micropropagation protocol was developed for *Hoslundia opposita* using nodal explants derived from mature trees. Multiple shoots were induced directly from the axis of nodal explants on MS medium containing 30 g l^{-1} sucrose, 0.2% gelrite and different concentrations (0, 2.2, 4.4, 6.6, 8.8 or 11 μM) of N^6-benzyladenine (BA). BA produced healthy and normal shoots when compared to those developed on kinetin supplemented medium. MS medium supplemented with 4.4 μM BA gave the best caulogenic response with an average of 7.3±0.4 shoots per explant developed on 91% of the cultures, after 30 days. *In vitro* regenerated shoots developed roots directly from the basal cut ends of shoots without an intervening callus phase, either on MS medium alone or MS medium supplemented with 3.6 μM indole-3-acetic acid (IAA), after 30 days of incubation. More than 90% of the rooted plantlets survived in the greenhouse, after 3 months of transfer. These plantlets are growing well without any phenotypic aberrations. This standardized protocol will ensure the mass propagation of *H. opposita* plants in limited time and space, thereby ruling out the dependence on natural stands for the plant material required for therapeutic purposes.

Keywords: Lamiaceae; Micropropagation; Multiple shoots; Orange berry; Plantlets

1. Introduction

Hoslundia opposita Vahl, commonly known as orange bird berry or bird gooseberry, is a herbaceous perennial shrub (1–2 m tall) belonging to the Lamiaceae. It is distributed in tropical and subtropical open lands of Africa. Various parts of *H. opposita* are used traditionally in many parts of Africa to treat gonorrhea, cystitis, coughs, fever, wounds, convulsions, sores, mental disturbances, abdominal pains, snake bites and for the relief of swellings (Watt and Breyer-Brandwijk, 1962). A recent study had reported that leaves of this plant could be potentially used in treatment of epilepsy and convulsions (Risa et al., 2004).

At present herbalists are depending on naturally occurring stands of the plants to fulfill their demands for plant material. This practice holds a threat for natural stands. Propagation of the elite tree through conventional methods *via* stem cuttings is limited due to a low survival rate. Therefore, there is an urgent need to look for alternate means of propagation, which could ensure large-scale production of plants to fulfill the growing demands. *In vitro* propagation is a feasible alternative for the rapid multiplication and maintenance of germplasm. *In vitro* propagation ensures the production of true-to-type plants in limited time and space. The propagation from elite mature plants is preferred for this purpose as they are selected on past performances. This communication reports an efficient protocol for regeneration of plants of *H. opposita* using nodes derived from mature trees.

2. Materials and methods

Young offshoots were collected during the months of March–April from 6- to 7-year-old trees of *H. opposita* Vahl growing in the Botanical garden of the University of KwaZulu-Natal, Pietermaritzburg, South Africa. The offshoots were defoliated and washed with ultra liquid bleach (Quality Hygiene, South Africa) for 20 min to remove all surface adherents and then left in running tap water for 30 min. Following this, the defoliated twigs were

* Corresponding author.
E-mail address: rcpgd@ukzn.ac.za (J. Van Staden).

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doi:10.1016/j.sajb.2006.07.001

surface decontaminated with 0.01% (w/v) mercuric chloride for 15 min. The treated twigs were washed several times with sterile distilled water. Prior to inoculation, the twigs were trimmed into 1-cm-long pieces, each having two nodes.

The nodal explants (1 cm) were implanted into MS medium (Murashige and Skoog, 1962) supplemented with 0.2% (w/v) gelrite (Sigma-Aldrich) and 3% (w/v) sucrose (Merck). The media were supplemented with different concentrations (0.0, 2.2, 4.4, 6.6, 8.8 or 11.0 μM) of either BA or (0, 2.4, 4.7, 7.0, 9.3 or 11.7 μM) Kin (Sigma-Aldrich). The pH of the media was adjusted to 5.8 with 1 N HCl or 1 N NaOH, before addition of gelrite. The tubes were closed with metallic caps and contained 10 ml of medium. Media were autoclaved at 1.05 kPa at 121 °C for 20 min. All cultures were maintained at 25±2 °C under a 16 h photoperiod at a photosynthetic flux of 12.6 μmol m^{-2} s^{-1}, provided by cool daylight fluorescent lamps.

For rooting of *in vitro* regenerated shoots, the shoots were either implanted on MS medium alone or MS supplemented with different concentrations (0, 5, 10 or 15 μM) of IBA or (0, 3.6, 7.3 or 10.9 μM) IAA (Sigma-Aldrich). All media were supplemented with 3% (w/v) sucrose and 0.2% (w/v) gelrite.

For hardening-off, 7- to 8-week-old rooted shoots were removed from the culture tubes. After washing away the gelrite with water they were transferred to small pots containing garden soil/vermiculite (1:1) and kept in a mist house. After acclimatization in the mist house for 2 months, they were transferred to a greenhouse.

The culture responses were expressed in terms of percentage responding explants, number of regenerants (shoots or roots) per explant, average length of shoots or roots. A completely randomized block design with three replications was used. Percentage of response data was arcsine transformed before analysis. All data were subjected to one-way analysis of variance (ANOVA) using the MINITAB version 14 (Minitab Ltd., USA). Fisher's least significant difference (LSD) at the 5% level was used to analyze the differences between the means. For each treatment of a replicate experiment, 24 explants were cultured.

3. Results and discussion

Initial establishment of cultures of the explants derived from the mature tree was thwarted by a high degree of contamination and polyphenol oxidation. To control the high incidences of microbial contamination a composite approach of washing the explants first with ultra liquid bleach for 20 min followed by mercuric chloride (0.01%) for 15 min was employed. By using this method, 57% aseptic cultures were obtained. The regenerative potential of explants was greatly affected by the time of collection of offshoots. The offshoots collected during the months of March to April elicited the best morphogenic response compared to those collected at other months of the year. A similar differential seasonal responsiveness of the explants derived from the twigs collected from mature trees was encountered in *Alnus cremastogyne* (Tang et al., 1996) and *Feronia limonia* (Purohit et al., 1998).

After successful decontamination of cultures, the other major constraint was the slow growth and low percentage of morphogenic response. On MS basal medium, the dormant

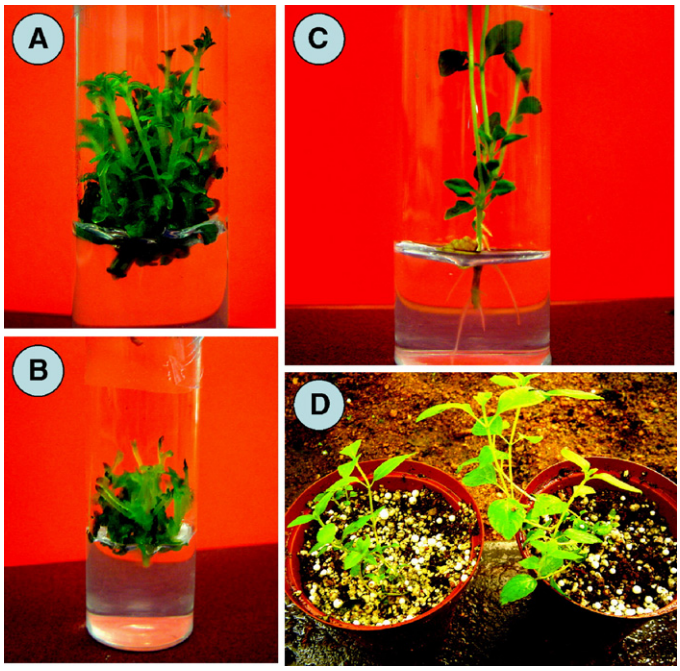

Fig. 1. *In vitro* regeneration of nodal explants derived from mature tree of *H. opposita*. (A) Nodal explants with multiple shoot development on MS medium supplemented with 4.4 μM BA after 30 days of culture. (B) Stunted and malformed shoots developed on MS medium supplemented with 4.7 μM Kin after 30 days of culture. (C) Development of roots from the base of an *in vitro* regenerated shoot on MS medium supplemented with 3.6 μM IAA after 21 days. (D) Three-month-old regenerated plants in the greenhouse.

Table 1
Caulogenic response of nodal explants of *H. opposita* on MS medium supplemented with different concentrations of BA, after 30 days of culture

BA (μM)	Percentage response	Number of shoots/explant	Average shoot length (cm)
0	89 a	2.3±0.3e	4.1±0.3a
2.2	91 a	4.0±0.3d	3.7±0.2b
4.4	91 a	7.3±0.4a	3.9±0.1b
6.6	90 a	6.9±0.4ab	3.2±0.2bc
8.8	88 a	6.1±0.41bc	2.0±0.2cd
11.0	89 a	5.2±0.38c	1.9±0.3cd

Values represent mean±standard error. Values followed by the same letter in each column are not significantly different ($p \leq 0.05$).

Table 2
Rhizogenic response of *in vitro* regenerated shoots of *H. opposita* on MS medium supplemented with different concentrations of IAA, after 30 days of culture

IAA (μM)	Percentage response	Number of roots/shoot	Average root length (cm)
0	43 bc	3.8±0.5c	3.2±0.2a
3.6	68 a	5.3±0.4a	3.6±0.2a
7.3	47 b	4.8±0.4b	3.3±0.3a
10.9	39 c	3.3±0.5c	3.0±0.2a

Values represent mean±standard error. Values followed by the same letter in each column are not significantly different ($p \leq 0.05$).