Saudi Journal of Biological Sciences xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Auxin-cytokinin synergism *in vitro* for producing genetically stable plants of *Ruta graveolens* using shoot tip meristems

Mohammad Faisal [a,*], Naseem Ahmad [b], Mohammad Anis [b], Abdulrahman A. Alatar [a], Ahmad A. Qahtan [a]

[a] Department of Botany & Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
[b] Department of Botany, Aligarh Muslim University, Aligarh 202002, India

ARTICLE INFO

Article history:
Received 30 June 2017
Revised 11 September 2017
Accepted 25 September 2017
Available online xxxx

Keywords:
Auxin
Cytokinin
Genetic stability
In vitro
Plant regeneration
Shoot tip

ABSTRACT

An efficient micropropagation protocol was developed for *Ruta graveolens* Linn. using shoot tip meristems derived from a 4-month-old field grown plant. *In vitro* shoot regeneration and proliferation was accomplished on Murashige and Skoogs (MS) semi-solid medium in addition to different doses of cytokinins viz.6- benzyl adenine (BA), Kinetin (Kn) or 2-isopetynyl adenine (2iP), singly or in combination with auxins viz. indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) or α-naphthalene acetic acid (NAA). Highest regeneration frequency (27.6%) was obtained on (MS) medium composed of BA (10 μM) with maximum number (9.4) of shoots and 4.3 cm shoot length after 4 weeks of incubation. Among various combinations tried best regeneration frequency (71%) of multiple shoot formation with highest number (12.6) of shoots per shoot tip explants were achieved in MS medium augmented with a combination BA (10.0 μM) and NAA (2.5 μM) after 4 weeks of incubation. The optimum frequency (97%) of rhizogenesis was achieved on half-strength MS medium having 0.5 μM IBA after 4 weeks of incubation. Tissue culture raised plantlets with 5–7 fully opened leaves with healthy root system were successfully acclimatized off in Soilrite™ with 80% survival rate followed by transportation to normal soil under natural light. Genetic stability among *in vitro* raised progeny was evaluated by ISSR and RAPD markers. The entire banding pattern revealed from *in vitro* regenerated plants was monomorphic to the donor. The present protocol provides an alternative option for commercial propagation and fruitful setting up of genetically uniform progeny for sustainable utilization and germplasm preservation.
© 2017 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Ruta graveolens Linn (Rutaceae) is a multipurpose perennial medicinal herb, commonly known as 'rue' or 'herb of grace', native to Mediterranean region. It is also grown worldwide as an ornamental plant, due to its bluish green foliage with yellow flowers and for its tolerance in dry soil and hot climatic condition (Bohidor et al., 2008). The herb has resolving, diuretic, antispasmodic, emmenagogue, stimulant, antispasmodic properties and useful in hysteria, amenorrhea, earache and toothache. The rue plant has drawn special attention due to an essential oil called as 'rue oil' extracted by steam distillation of fresh materials of this plant. The rue oil contains several biologically active constituents like bergaptene, butanone, nonanone, nonyl acetate, psoralen, undecanone, and xanthotoxin (Anonymous, 2003).

Conventional cultivation via seeds or shoot cuttings is not adequate to accommodate the growing need of this species. The pharmaceutical companies are excavating the herb from the wild posing a threat to its existence and disturbing the ecological balance. Also, the variability in the constituents and chances of weed contamination reduce the profitability of the companies. Therefore, it is essential to develop an effective and reproducible method for clonal propagation for *Ruta graveolens,* which can provide contamination free supply of the herb that could suffice the demand.

Nowadays, tissue culture technique is proving to be very useful tool for mass propagation and conservation of various important medicinal species and the modern approaches of this technique emerge as an important production link between multiplication, conservation and sustainable utilization. Plant tissue culture technique is a key technology for production of large quantities of planting material of selected genotypes and chemotypes (Anis

* Corresponding author.
E-mail addresses: faisalm15@yahoo.com, mofaisal@ksu.edu.sa (M. Faisal).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2017.09.009
1319-562X/© 2017 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Please cite this article in press as: Faisal, M., et al. Auxin-cytokinin synergism *in vitro* for producing genetically stable plants of *Ruta graveolens* using shoot tip meristemsin vitro for producing genetically stable plants –>. Saudi Journal of Biological Sciences (2017), https://doi.org/10.1016/j.sjbs.2017.09.009

et al., 2008). Shoot tip culture technique is a very interesting tissue culture approach, as it eliminates the probability of endogenous contamination and provides a high frequency shoot regeneration system.

Clonal nature of regenerants is the most essential requirement in micropropagation of selected medicinal plant. Genetic variations in terms of somaclonal variation is a hindrance in attempts to maintain the advantages of elite germplasm. Consequently, it is crucial to ascertain genetic stability of the *in vitro* regenerated plantlets in order to substantiate the superiority of the regenerants.

Recently, PCR (Polymerase chain reaction) based molecular markers viz. RAPD (Random amplified polymorphic DNA) and ISSR (inter-simple sequence repeat) are extremely functional in establishing genetic constancy among micropropagated plantlets in many plant species (Ahmad and Anis, 2011; Faisal et al., 2012). RAPD and ISSR techniques are very simple, rapid, consistent, extremely discriminative and commercial. These necessitate only a very minute quantity of DNA sample and also, they do not need any prior sequence information to design the primer. Since, it is immensely essential to maintain genetic uniformity of *in vitro* raised progenies, therefore, we have adopted DNA based RAPD/ISSR molecular techniques for the appraisal of inherited stability among *in vitro* regenerated progeny.

Clonal propagation through shoot apices is advantageous over other explants as it eliminates any chance of contamination. Owing to the importance of the species *in vitro* regeneration has been attempted using nodal segments (Faisal et al., 2005) and leaf explants (Ahmad et al., 2010). However, to our knowledge there is no data published on direct regeneration from shoot tip meristems of this plant.

Hence, considering an enormous potential exhibited by the relevance of *in vitro* propagation procedures and existing situation of *R. graveolens*, the current research work was attempted to examine the effects of different concentration of cytokinins and auxin: cytokinin interactions on shoot regeneration using shoot tip meristems and determination of genetic fidelity of the regenerant progenies using molecular markers.

2. Materials and methods

2.1. Plant material establishment of in vitro cultures

Juvenile and fresh shoots of *R. graveolens* procured from a healthy plant were rinsed properly with normal laboratory tap water for 20 min, followed by 10 min treatment with a liquid detergent 5% (v/v) Labolene™ (a liquid detergent; Qualigens Fine Chemicals, Mumbai, India and cleansing thrice in tap water. After proper washing the explants were sterilized by 0.1% (w/v) mercuric chloride (Central Drug House, New Delhi, India) under Laminar Air Flow (Macro Scientific Works, Pvt. Ltd. New Delhi, India) for 5 min. After washing (5 times) with distilled water (sterile), the shoots were trimmed-off and shoot tip meristems (0.3–0.6 cm) were exterminate aseptically and cultured on pre-sterile culture medium (20.0 ml) in culture tubes (125 mm × 25 mm; Borosil, Agra, India). The culture medium contained MS nutrients (Murashige and Skoog, 1962) with 3% sucrose (a carbon source) and 1.0% (w/v) agar-agar (a gelling agent; Thermo Fisher Scientific India Pvt. Ltd, Mumbai, India) supplemented with 6-benzyladenine (BA), 6-furfurylaminopurine (Kn), 2 isopentenyl adenine (2iP) either singly or in different amalgamation at various doses of auxins viz. indole-3-acetic acid (IAA), indole-3- butyric acid (IBA) or α- naphthalene acetic acid (NAA). The pH of the medium was fixed to 5.8 with 1N NaOH before sterilization (autoclaving) at 121 °C and 15 psi for 15 min. The cultures were incubated in a culture room at 25 ± 2 °C with 16/8 h light/dark cycle. The light intensity of 50 μmol m^{-2} s^{-1} provided by 40 W fluorescent Lamps (Philips Electronics India Ltd. Kolkata, India) and the relative humidity was about 50–60%.

2.2. Sub-culturing

4 weeks after incubation, responded shoot tip meristems were sub-cultured to the culture media composed of same concentrations and combinations of plant growth regulators (PGRs) as was used in the respective inoculation media, after collecting the initiated shoots from cultures. The frequency of responded explants, the shoot number with length were documented after 8 weeks of culture.

2.3. In vitro rooting and hardening

In vitro propagated microshoots (approx. 4–5 cm) were removed from shoot cultures and submitted to full and half strength MS basal medium augmented with 0.5 μM. After proper root formation, plantlets were washed cautiously with normal water, transferred to pots composed of sterile potting mixture, Soilrite™ (Keltech Energies Ltd., Bangalore, India), moisturized with 0.5x MS, lacking organic supplements, and covered with clear polyethylene covers to ensure a high humidity. The covers were evacuated gradually after 2 weeks, in order to hardened the plantlets. The acclimatized plants were then shifted to a green-house, net house and finally to the field condition.

2.4. Genetic stability analysis

The genetic constancy of the *in vitro* raised plants was examined by RAPD and ISSR techniques. Randomly selected 10 *in vitro* raised plants alongwith mother plant was investigated for genetic integrity. Genomic DNA from young leaves of selected plants was isolated following the cetylmethylammonium bromide (CTAB method) defined by Doyle and Doyle (1987). The isolated genomic DNA was certified for purity (A260/280 ratio) on a Nanodrop Spectrophotometer (UV-1700 Implen, Germany).

A set of 10 RAPD primers (Kit C, Operon Technologies, California, USA; Table 1) and 10 ISSR primers (UBC, Vancouver, BC, Canada; Table 2) were used for initial screening. PCR reactions for ISSR/RAPD marker based amplification were executed on a PCR Machine (Biometra, T Gradient Thermoblock, Germany). The mixture (20 μl) for PCR contains 10X buffer (2 μl), 25 mM $MgCl_2$ (1.2 μl), 10 mM dNTPs (0.4 μl), 2 μM primers, 3 Unit Taq polymerase (0.2 μl) and 25 ng Template DNA. The PCR amplification schedule contains 45 cycles inclusive of 94 °C denaturation segments of 5 min, an annealing (35 °C) for 1 min and a 72 °C extension of 1 min. A final extension was followed at 72 °C for 10 min. The amplification results of DNA were separated by electrophoresis on agarose gels (0.8%) with 4 μl ethidium bromide in TAE buffer

Table 1
RAPD primers used for the assessment of genetic stability of *R. graveolens* plantlets.

S. no.	Name of the primer	Sequence
1.	OPC-1	TTCGAGCCAG
2.	OPC-2	GTGAGGCGTC
3.	OPC-3	GGGGGTCTTT
4.	OPC-4	CCGCATCTAC
5.	OPC-5	GATGACCGCC
6.	OPC-6	GAACGGACTC
7.	OPC-7	GTCCCGACGA
8.	OPC-8	TGGACCGGTG
9.	OPC-9	CTCACCGTCC
10	OPC-10	TGTCTGGGTG

Please cite this article in press as: Faisal, M., et al. Auxin-cytokinin synergism *in vitro* for producing genetically stable plants of *Ruta graveolens* using shoot tip meristemsin vitro for producing genetically stable plants –>. Saudi Journal of Biological Sciences (2017), https://doi.org/10.1016/j.sjbs.2017.09.009

Download English Version:

https://daneshyari.com/en/article/8849901

Download Persian Version:

https://daneshyari.com/article/8849901

Daneshyari.com