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In vitro regeneration of Rangoon creeper (*Quisqualis indica* Linn.)

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The present study describes an efficient and reproducible protocol for the regeneration of shrubby climber *Quisqualis indica* Linn. from nodal segments of mature plant. *In vitro* shoot regeneration was achieved within 21 d of culture initiation. The best shoot multiplication response (100%) was recorded on MS medium supplemented with 1.0 mg L^{-1} 6-benzyladenine (BA) and 0.5 mg L^{-1} gibberellic acid (GA_3) with the highest production of 20 shoots per nodal explant. Further, *in vitro* regenerated shoots showed the highest root induction (79.9 %) on MS medium supplemented with 0.5 mg L^{-1} indole-3-acetic acid (IAA) and 0.5 mg L^{-1}indole-3-butyric acid (IBA). Regenerated plantlets were acclimated in the culture room before transplanting to field conditions.

Keywords: Benzyl adenine (BA), Combretaceae, gibberelic acid (GA_3), indole acetic acid (IAA), indole bytyric acid (IBA), *in vitro* propagation, MS medium, *Quisqualis indica*

Introduction

Quisqualis indica Linn., commonly known as Chinese honey suckle or Rangoon creeper, is a large scandent shrub of the family Combretaceae. In India also, it is popularly known in many vernacular languages including *Madhumalti* (Hindi) and *Radha Manoharam* (Telugu). The plant is grown as an ornamental climber throughout the greater part of India, though it is a native to South East Asia and grows best in tropical areas[1]. Among many countries, it is grown in Africa, Philippines, Vietnam, Malaysia, India, Bangladesh and Thailand. Besides unique demand for beautiful flowers among the plant lovers and gardeners, this species is therapeutically significant and used as a potential remedy for human ailments. The leaf decoction alleviates flatulent distension of abdomen and has free radical scavenging activity[2]. Fruits and seeds are used to combat nephritis, whereas roots cure rheumatism[1,3]. Flowers and seeds are eaten as famine food in Southern Asia[3]. Fruits, leaves and seeds contain chemicals like 'quisqualic acid' and anthelmintic tannins, which are used for expelling round worms and thread worms[4-7]. Moreover, methanol extract of flowers contain high polyphenol contents that have strong antioxidant activity[8,9]. The leaves and flowers also contain rutin, pelargonidin-3-glucoside[10-12] and 25-O-acetyl-23,24-dihydro-cucurbitacin F; the latter one acts as an anticancer agent and has shown significant cytotoxic activity[13].

The commercial propagation of *Q. indica* is typically done through conventional techniques, such as, from seeds or by shoot cuttings. This species also produces suckers from roots, which can be detached from mother plants for multiplication. Propagation through seeds, though possible, is not practical as the seeds are genetically variable and add undesirable variation to the production system. The propagated plants also have a long period of juvenility and delayed flowering cycle[14]. In contrast, *in vitro* techniques permit rapid, large scale multiplication to meet the demand of plant material. In addition, tissue culture protocols are efficient tools and prerequisite for genetic transformation in breeding programms and producing cultivars with desirable characteristics. Although there is no established protocol for *in vitro* propagation of *Q. indica*, root induction in shoots was reported on medium supplemented with 0.5 mg L^{-1} IBA[15].

Apart from increasing interest as an ornamental, *Q. indica* also holds promise for medicinal potentials. Therefore, the objective of the present study was to develop an efficient protocol for rapid and large scale multiplication of *Q. indica* using nodal segment explants.

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Materials and Methods

Source of Explants and Establishment of *In Vitro* Culture

A 10-yr-old mature shrub growing in the Experimental Medicinal Garden of Regional Institute of Education, National Council of Educational Research and Training (NCERT), Bhopal was the source of explant material. Node explants, 1-2 cm long (1st to 3rd from shoot tip), were excised. The leaves present on the nodes were removed and washed with tap water for 10 min with continuous stirring. Then these explants were surface disinfected using 0.1% $HgCl_2$ for 3 min with continuous stirring under aseptic condition in Laminar air flow chamber and rinsed 5 times, each of 2 min, with sterilized double distilled water. The sterilized explants were placed on the MS basal medium[16] containing different cytokinins like 6-benzyladenine (BA), thidiazuron (TDZ) and 6-(γ,γ-dimethylallylamino) purine (2iP) at various concentrations (0.25, 0.5 or 1.0 mg L^{-1}) either alone or in combination with gibberellic acid (GA_3) (0.5 mg L^{-1}).

All media were adjusted to pH 5.8 using 1 N HCl or 1 N NaOH before adding agar. The media were gelled with 8.0 g L^{-1} agar (Qualigens, India) and dispensed into 100 mL conical flasks (Borosil, India) and test tubes (20 mm × 150 mm). Quantity of medium was 30 and 20 mL in conical flasks and test tubes, respectively. These culture vessels containing media were autoclaved at 104 kPa and 121°C for 20 min and stored until use. All cultures were maintained at 29±2°C under cool white fluorescent light (16 h photoperiod) providing 40 µmol m^{-2} s^{-1} light intensity and 8 h dark period.

Shoot Proliferation and Elongation

Nodal explants from i*n vitro* regenerated shoots of 30-d-old were placed into conical flasks containing MS medium supplemented with 1.0 mg L^{-1} BA or TDZ in combination with 0.5 mg L^{-1} GA_3 for further multiplication. Elongation of shoots was attempted on MS medium containing 0.5 mg L^{-1} BA. *In vitro* shoot proliferation and elongation were evaluated after 30 d of culture under the same culture conditions as used for establishment of *in vitro* culture. Subculture was done on every 2 wk on the same medium for mass scale shoot production. Bud break percentage, shoot number and shoot length were recorded from the nodal explants after 30 d of the culture period. In each treatment 20 replicates were tested and the experiments were repeated 3 times.

Rooting and Acclimatization

The elongated shoots (~3 to 8 cm) with 2 to 4 nodes were excised and cultured, one shoot per test tube, on either growth regulator free MS plant medium or MS medium supplemented with IAA, IBA or NAA (0.5 or 1.0 mg L^{-1}) or IAA+IBA (each 0.5 or 1.0 mg L^{-1}) for root induction. The culture tubes were then placed in a growth room under the same environmental conditions used for *in vitro* shoot regeneration. The percentage of shoots producing roots, number of roots per shoot and root length were recorded after 30 d of the culture period. Rooted plantlets were transferred into plastic cups, containing sterilized garden soil, wrapped with polyethylene bags for maintaining high humidity and kept under growth room conditions at 29±2°C for 4 wk. The plants were watered on every alternate day with full strength MS solution for the first 2 wk and with ½ strength MS solution for another 2 wk. Then the plants were transplanted into 6 cm diameter plastic pots containing garden soil and kept in shade for 3 wk before placing them to field conditions.

Statistical Analysis

For culture studies, 20 explants were cultured in 4 conical flasks, each containing five explants per treatment. All the experiments were carried out in a completely randomized design and repeated thrice. Means and standard error of means for all the dependent variables, such as, shoot regeneration, shoot number, shoot length, root regeneration, root number and root length, under different plant growth concentrations were computed for the significance of difference of means. Significant differences between means were determined using Tukey test (SPSS-17.0).

Results and Discussion

Initiation of *In Vitro* Cultures

Pre-examination of nodal explants collected round the year on *in vitro* response revealed that explant materials from newly sprung off shoots collected during the period of March to May were the most suitable for *in vitro* initiation as they were contamination free and responsive for the induction of best results. Nodal explants exhibited the best bud-break response (100%) on MS medium supplemented with either 1.0 mg L^{-1} BA or 2.0 mg L^{-1} TDZ within 15 to 20 d of *in vitro* culture (Figs 1a & 2). Within the same period of culture, 1.0 mg L^{-1} BA and 0.5 mg $L^{-1}GA_3$

also induced 100% bud-break response but with a larger number of shoots (20 shoots per explant) as compared to other cytokinins (Figs 1b & 2). On the other hand, the combinations of TDZ and GA_3 or 2 iP and GA_3 produced 75% bud-break response with a maximum number of 2-3 shoots per explants. Moreover, the frequency of shoot multiplication was also found to be the highest (100%) on MS medium supplemented with 1.0 mg L^{-1} BA and 0.5 mg $L^{-1}GA_3$, producing the highest number of shoos (20 shoots per explants) and shoot length (2.0 cm) as compared to other cytokinins added either individually or in combination with GA_3 (Figs. 3 & 4). Thus, the combination of BA and GA_3 was proved superior to the combinations of TDZ and GA_3, 2iP and GA_3, or either BA, TDZ or 2iP alone for multiple shoot

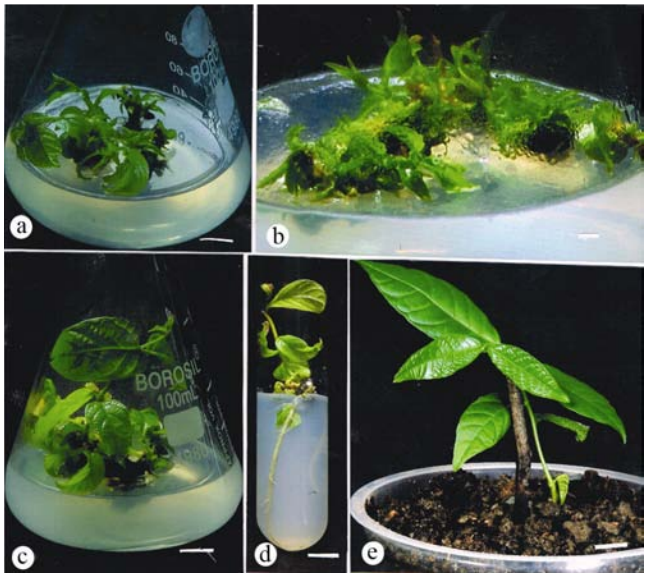

Fig. 1 (a-e)—*In vitro* propagation of *Q. indica*: a. Shoot initiation from nodal explants on MS medium supplemented with 0.5 mg L^{-1} BA after 10 d of culture; b. Induction of multiple shoots from nodal explants on medium containing 1.0 mg L^{-1} BA and 0.5 mg L^{-1} GA_3 after 15 d of culture; c. Shoot elongation on the medium supplemented with 0.5 mg L^{-1} BA after 21 d of culture; d. Rooting of *in vitro* regenerated shoot on MS medium supplemented with 0.5 mg L^{-1} each of IAA and IBA within 30 d of culture; e. A healthy *in vitro* regenerated plantlet acclimatized 30 d after transfer to soil. [Bars: 5 mm (a), 50 mm (b), 53 mm (c), 80 mm (d) and 100 mm(e)].

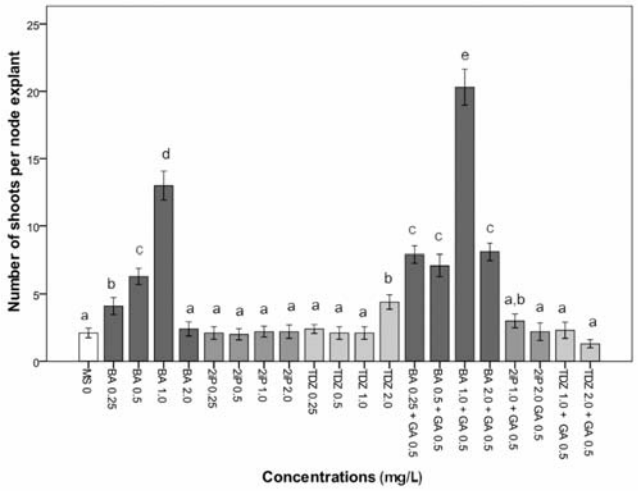

Fig. 3—Morphogenic response of nodal explants (no. of shoots per nodal explants) of *Q. indica* on MS medium supplemented with BA, 2iP or TDZ either alone or in combination with GA_3 after 30 d of culture.

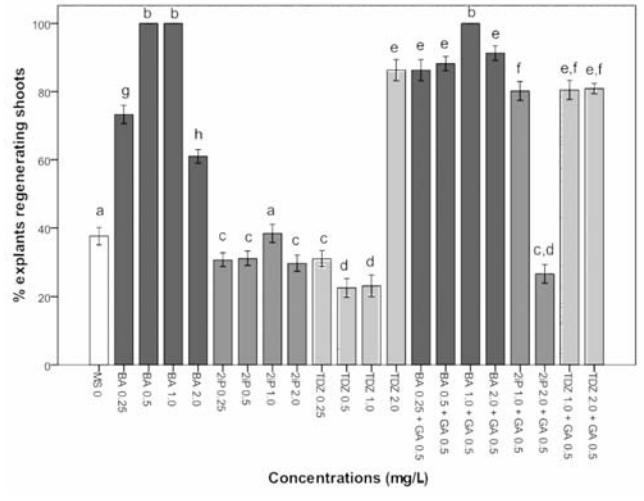

Fig. 2—Morphogenic response of nodal explants (% explants regenerating shoots) of *Q. indica* on MS medium supplemented with BA, 2iP or TDZ either alone or in combination with GA_3 after 30 d of culture.

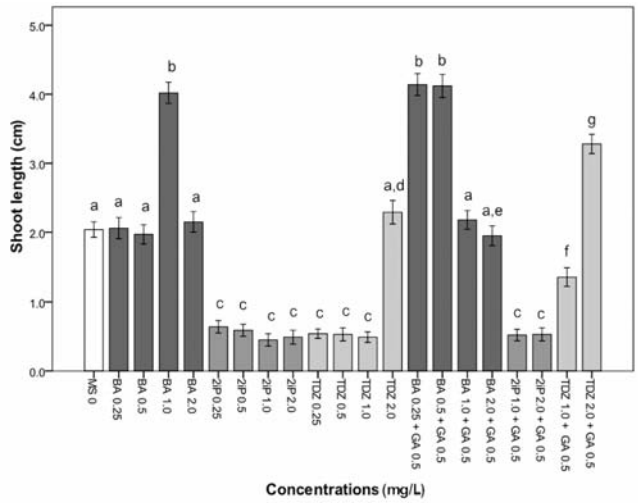

Fig. 4—Morphogenic response of nodal explants (shoot length in cm) of *Q. indica* on MS medium supplemented with BA, 2iP or TDZ either alone or in combination with GA_3 after 30 d of culture.

production. In earlier studies, the highest shoot proliferations in *Pisum sativum* from nodal explants was observed on MS3 medium supplemented with GA_3 plus TDZ[17]; while in *Terminalia arjuna*, the best shoot multiplication response from node explants was observed on MS medium supplemented with 4.44 µM BA and 0.53 µM NAA[18].

Shoot Proliferation and Elongation

In vitro regenerated 8-wk-old *Q. indica* microshoots were transferred to shoot multiplication media and a suitable medium was selected based on the rate of shoot production after 30 d of culture. The MS medium supplemented with 1.0 mg L^{-1} BA and 0.5 mg $L^{-1}GA_3$ was routinely being used for subculture of microshoots and shoot proliferation. However, the up-scaling of shoot proliferation was further boosted following transfer of *in vitro* regenerated microshoots to elongation medium based on MS medium supplemented with 0.5 mg L^{-1} BA (Fig. 1c). In every 3 wk, nodal explants from elongated axenic shoots were further cultured on proliferation medium for induction of shoot regeneration and proliferation. The addition of GA_3 to the MS medium supplemented with BA significantly enhanced shoot proliferation, which was similar to the *in vivo* developmental processes[19]. Both shoot proliferation and elongation were ascribed to the stimulatory effects of gibberellic acid (GA_3) in many species including *Pisum sativum*[17], *Ocimum americanum* and *O. sanctum*[20].

Rooting and Acclimatization

Rhizogenesis of *in vitro* regenerated shoots of *Q. indica* was observed both on growth regulator free MS medium and MS medium supplemented with IAA or IBA, while rooting was not induced on medium containing NAA (Table 1). The efficiency of IAA or IBA as root inducing substances has been documented for many medicinal and ornamental plants[21,22]. The highest root induction (79.9%) was achieved when both IAA and IBA in combination (each at 0.5 mg L^{-1}) was added to MS medium, producing an average 2.3 roots per shoot with an averaging root length of 8.1 cm after 4 wk (Fig. 1d; Table 1). An increase in the IAA level from a 0.5 to 1.0 mg L^{-1} led to decrease in root production, while IBA at 1.0 mg L^{-1} inhibited root induction. This inhibitory effect was ascribed to the slower metabolism of IBA compared to that of IAA[23]. The combined action of IAA and IBA increased the number of root formation but optimum results were always at the lower concentration of 0.5 mg L^{-1}. A similar observation was reported in the rooting of *Ailanthus triphysa*[24] where a combination of IAA and IBA increased rooting. The adverse effect of IAA and IBA at higher concentrations was attributed to either less rapid metabolic[23] or increased production of ethylene[25], showing inhibitory or lethal effect on root induction.

Table 1—Effect of auxins on rooting of *in vitro* regenerated shoots of *Q. indica* on MS medium after 30 d of culture

Auxins (mg L^{-1})	% rooted shoots	Av. no. of roots per shoot	Av. root length (cm)
IAA	19.5±1.7	1.4±0.52	3.0±0.19
0.5	40.2±1.5	1.7±0.67	5.1±0.19
1.0	39.3±1.9	1.1±0.43	4.9±0.17
IBA			
0.5	60.5±1.0	1.8±0.63	7.1±0.29
1.0	-	-	-
IAA+IBA			
0.5+0.5	79.9±2.7	2.3±0.48	8.1± 0.20
1.0+1.0	-	-	-

Data represent mean±SE based on three independent experiments, each of 10 replicates per treatment.

The plantlets with healthy root system were transplanted into a plastic cup containing sterilized garden soil, wrapped with polythene bags and kept in the growth room for 4 wk. The rooted plantlets were readily acclimatized under *ex vitro* condition with 80% survival (data not shown) after 8 wk of transfer to the soil (Fig. 1e). The plants looked healthy with no observable phenotypic variation with the mother plant.

Thus, in the present study, a reproducible and efficient *in vitro* regeneration system of *Q. indica* has been developed. The system produces about 20 shoots per nodal explants, which could further be up scaled by using axenic nodal explants from the elongated *in vitro* produced plants.

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