



MICROPROPAGATION OF *MERREMIA QUINQUEFOLIA* (L.) HALLIER F. FROM NODAL EXPLANTS

Short communication

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ABSTRACT

Merremia quinquefolia, is an important medicinal plant of the family Convolvulaceae known for its vasoconstrictor, uterotonic, neurohormonic, sympatholytic and sedative effects. In the present investigation effect of cytokinins 6-benzylaminopurine (BAP), kinetin (Kn) and thidiazuron (TDZ), at concentrations 1.0, 2.0, 3.0, 4.0 and 5.0 mg·dm⁻³ on *in vitro* shoot multiplication from nodal explants of *M. quinquefolia* was evaluated. Bud breaking and emergence of shoots started within 10-15 days of inoculation in all media containing cytokinin. Murashige and Skoog (MS) medium supplemented with 4.0 mg·dm⁻³ BAP resulted in maximum number of shoots from single node within 45 days. *In vitro* raised shoots were successfully rooted on ½ mineral salts of MS medium with 3% sucrose supplemented with 2.0 mg·dm⁻³ indole-3-butyric acid (IBA). This is the first report on *in vitro* propagation of *Merremia quinquefolia*. This study can be useful for development of micropropagation protocols for related taxa.

Key words: axillary shoot multiplication, Convolvulaceae, *Merremia quinquefolia*

INTRODUCTION

Merremia is a genus of the family Convolvulaceae. According to The Plant List (2015), 54 species of *Merremia* are reported around the world, and are distributed in tropical region of Africa, Asia, Australia, North and South America, and China (Simpson 2015). In India *M. quinquefolia* is distributed in Maharashtra, Gujarat, Orisa and Rajasthan (Khare 2007). Seeds of *M. quinquefolia* contain ergoline alkaloids that are reported to produce vasoconstrictor, uterotonic, neurohormonic, sympatholytic and sedative effects (Khare 2007). *M. quinquefolia* is an herbaceous climber characterized by palmately compound leaves, white flowers and with straw colored globose capsules having brown black seeds (Rao & Safui 1963). *M. quinquefolia* is used as diuretic, deobstruent, antirheumatic and alterative; the root is used as mouthwash; leaves are used for burns, scalds and sores (Khare 2007).

Antibacterial activity of *M. emarginata* was reported by Elumalai et al. (2011), and antidiabetic activity by Arunachalam and Parimelazhagan (2012). Merresectine alkaloid was isolated from *M. dissecta* root (Weigl et al. 1992). *M. quinquefolia* is cultivated at commercial scale for medicinal purposes in West Africa (Quiroz et al. 2014).

Plant tissue culture techniques are used for *in situ* conservation of many medicinally important plants (Bharvad & Mohan 2012; Inamdar et al. 1990; Kher et al. 2014; Mehta & Subramanian 2005; Sharma & Mohan 2006a, b; Sharma et al. 2007; Varghese et al. 1993a, b, 1992). Axillary shoot proliferation technique is popularly used for clonal propagation in order to produce true to type and a high quality plants. Micropropagation and genetic transformation using tissue culture technique was reported in other Convolvulaceae genera including sweet potato (*Ipomoea batatas*) (Afreen-Zobayed et al. 2000; El Abidine Triqui et al. 2008;

Yang et al. 2011) and *M. tridentata* (Kaladhar 2010). In the present paper results from study of plant growth regulators influence on *in vitro* shoot multiplication from nodal explants of *M. quinquefolia* and on *in vitro* rooting of raised shoots are presented.

MATERIALS AND METHODS

Nodal explants were taken from healthy *M. quinquefolia* plant from nearby BRD School of Biosciences campus, Sardar Patel University. The explants were washed thoroughly with running tap water for 30 min, and then treated with 3% Neutral Rankleen detergent (v/v) (RFCL – India) for 10 min followed by 30 minutes washing in tap water. Thereafter the explants were sterilized with 0.1% mercuric chloride (w/v) solution for 3 minutes and then rinsed 3-4 times with sterile distilled water (DW).

Murashige and Skoog – MS medium (Murashige & Skoog 1962) supplemented with 3% (w/v) sucrose and 0.8% (w/v) plant tissue culture grade agar (Merck, India) was used throughout the experiments. The pH of the medium was adjusted to 5.8. All the cultures were maintained at 25 ± 2 °C under 16 hours photoperiod with a photosynthetic photon flux density (PPFD) of $35 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ provided by cool white fluorescent tubes (36 W, Philips, India).

To study the effect of cytokinin 6-benzylaminopurine (BAP), kinetin (Kn) and thidiazuron (TDZ) of concentration 1.0, 2.0, 3.0, 4.0 and 5.0 $\text{mg} \cdot \text{dm}^{-3}$ on shoot bud formation, nodal explants were cultured on 20 ml medium in borosilicate glass test tube (25×150 mm). *In vitro* raised shoots of 3-4 cm length were divided for single node explants and routinely cultured on MS medium supplemented with $4.0 \text{ mg} \cdot \text{dm}^{-3}$ BA for one month in borosilicate glass bottles of 400 ml capacity with 50 ml of medium.

The shoots of 4-5 cm in length raised from this medium were transferred to $\frac{1}{2}$ mineral MS salts with 3% sucrose, and supplemented with indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA) at concentrations 0.5, 1.0, 1.5 and $2.0 \text{ mg} \cdot \text{dm}^{-3}$ to optimize rooting conditions.

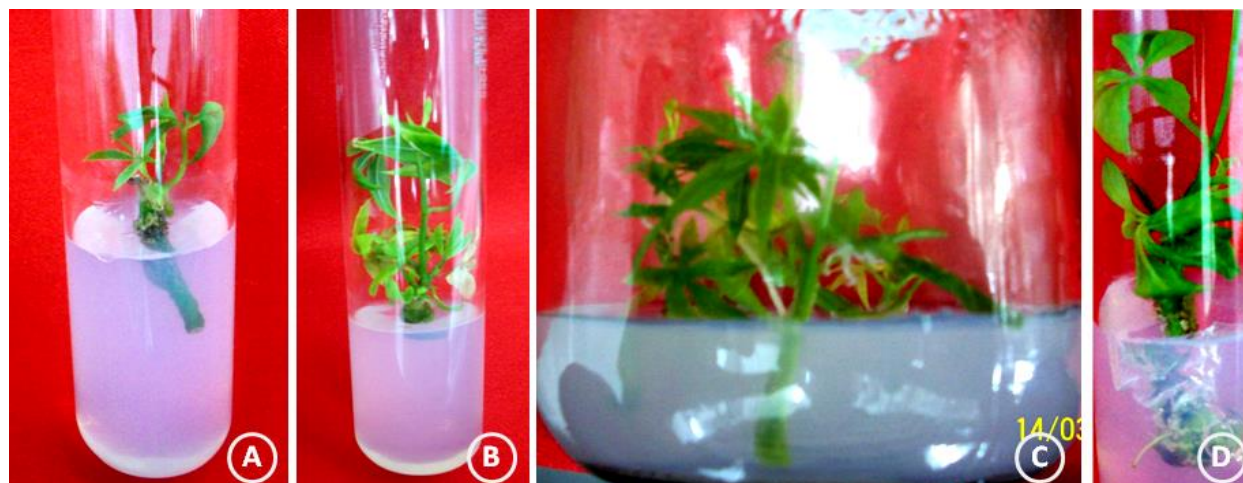
Experiments having 12 replicates (explants) per treatment were repeated at least three times. Observations from all experiment were recorded after 45 days. Mean values were subjected to one way

analysis of variance (ANOVA) using SPSS Inc., Chicago, USA. The significance of difference among mean values was carried out using Duncan's multiple range test (DMRT) at $p < 0.05$.

RESULTS AND DISCUSSION

There is no report available on tissue culture studies on *M. quinquefolia*, and only two reports on *in vitro* responses of entire *Merremia* genus concerning excised embryo reaction of *M. dissecta* to inositol (Sankhla et al. 1966) and callus mediated regeneration from shoot tips and floral explants of *M. tridentata* (Kaladhar 2010). It is well accepted that more chances of getting somaclonal variations is in callus mediated organogenesis (Bairu et al. 2011). Therefore axillary shoot multiplication is popular choice, however callus mediated organogenesis can be used for genetic transformation studies.

The response of nodal explants cultured on MS medium supplemented with various concentrations of 6-benzylaminopurine (BAP), kinetin (Kn) and thidiazuron (TDZ) is presented in Table 1. Nodal explants showed minimal response in the cytokinin free medium, where only 12.5% of nodal explants produced average 1.8 shoots per node. MS medium supplemented with TDZ, Kn and BAP caused bud breaking within 10-15 days of inoculation (Fig. 1A). In the medium supplemented with $5.0 \text{ mg} \cdot \text{dm}^{-3}$ TDZ, 58.3% of cultures responded with average 4.5 shoots per node, on the medium supplemented with $5.0 \text{ mg} \cdot \text{dm}^{-3}$ Kn, average 3.6 shoots per node was obtained in 62.5% of cultures, and 70.8% cultures were produced average 6.11 shoots per node on the medium supplemented with $4.0 \text{ mg} \cdot \text{dm}^{-3}$ BAP (Fig. 1B) within 45 days. Propagation from nodal explants of a sweet potato *Ipomoea tuberculata* 'Carmen Rubin' and 'White Triumph', a plant of the same family, was recommended on MS medium supplemented with 1 and $0.1 \text{ mg} \cdot \text{dm}^{-3}$ Kn and rooting on $0.5 \text{ mg} \cdot \text{dm}^{-3}$ of IAA (Doliński & Olek 2013). The variation in requirement of cytokinin type and concentration differs between plant species, genotypes, cultivars and explant types. Therefore it is essential to optimize plant growth regulator type and concentration for successful establishment of micropropagation protocol for any plant species.



A – Culture initiation after 15 days; B – Elongated shoots after 45 days;
C – Culture multiplication after 45 days; D – Rooting of shoots

Fig. 1. Stages of micropropagation from culture initiation to rooting of *M. quinquefolia*

Table 1. Effects of type and concentrations of cytokinin on shoot multiplication on nodal explants of *M. quinquefolia* evaluated after 45 days

Cytokinin	mg·dm ⁻³	% of node response	Shoot number per node
Control	0.0	12.5	1.8 ± 0.50 ^h
TDZ	1.0	25.0	3.0 ± 0.89 ^{gh}
TDZ	2.0	33.3	2.6 ± 0.91 ^{gh}
TDZ	3.0	45.8	3.2 ± 1.00 ^{efg}
TDZ	4.0	50.0	4.0 ± 1.50 ^{cdefg}
TDZ	5.0	58.3	4.5 ± 1.60 ^{bcdef}
Kn	1.0	37.5	2.9 ± 1.16 ^{gh}
Kn	2.0	45.8	3.0 ± 1.13 ^{fgh}
Kn	3.0	54.1	3.3 ± 1.31 ^{efg}
Kn	4.0	58.3	3.4 ± 1.50 ^{efg}
Kn	5.0	62.5	3.6 ± 1.40 ^{defg}
BAP	1.0	54.1	4.6 ± 1.70 ^{bcde}
BAP	2.0	58.3	5.8 ± 1.71 ^{ab}
BAP	3.0	66.6	4.9 ± 1.43 ^{bcd}
BAP	4.0	70.8	6.1 ± 1.50 ^a
BAP	5.0	79.1	5.3 ± 1.60 ^{abc}

Means ± SD in each column followed by same letters at superscripts are not significantly different at $p < 0.05$ according to DMRT ($n =$ minimum 12 replicates). Experiment was repeated thrice.

In vitro raised shoots (Fig. 1C) were successfully rooted on ½ strength MS medium but number of roots depended on type and concentration of auxin. 1-2 tap roots were produced on auxin free medium (Fig. 1D). There were no differences in rooting on the media containing 0.5 mg·dm⁻³ IAA or IBA. The medium supplemented with 2 mg·dm⁻³

IBA yielded maximum number of roots per shoots although no statistical differences were between media supplemented with 1.5, 2.0 and 2.5 mg·dm⁻³ IBA or 1.5 and 2 mg·dm⁻³ IAA (Fig. 2). Kaladhar (2010) investigated auxin IAA at 0.1, 0.3, 0.5, 1.0, 2.0 and 3.0 mg·dm⁻³ and found that 1 mg·dm⁻³ resulted in formation of roots in 85% cultures of *M. tridentata*. This study can be useful for development of micropropagation protocols for related taxa.

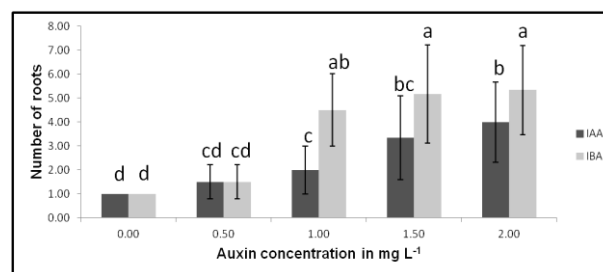


Fig. 2. Effect of concentration of auxins on *in vitro* rooting of *M. quinquefolia* as evaluated after 45 days. Bars represent means ± SE ($n=12$) repeated thrice. The same letters show means that are not significantly different at < 0.05 according to DMRT

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