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RESEARCH ARTICLE

Conservation of Manjishtha—*Rubia cordifolia* L. through Nodal Culture

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ABSTRACT

Aim: Manjishtha—*Rubia cordifolia* L. (family: Rubiaceae) is an important medicinal plant and used in various Ayurvedic formulations. Plant parts like roots, stems, leaves and fruits are being used to treat various respiratory and skin diseases. Manjistha is excessively collected from natural habitat and becoming rare and vulnerable in different parts of country. Therefore, it is decided to develop a systematic *in vitro* protocol for rapid multiplication of the plant.

Materials and methods: Nodal segments collected from healthy, desease free plant were used as explants. Pretreated and surface sterilized nodal segments were implanted on to MS basal medium as well as MS fortified with different concentrations of plant growth regulators *viz.*, BAP, TDZ, Kn, NAA, IAA, IBA singly or in combinations. Then, the cultures were incubated at 22°C ± 2°C for 8 hours photoperiod with light intensity of 3000 lux.

Results: Maximum number of shoots (20–25) developed from the nodal segments inoculated on MS + TDZ (0.5 mg/l) + 0.1% PVP liquid medium. The best rooting (2–3 roots) were developed in MS + IBA (2 mg/l) in 8 to 14 days.

Conclusion: The *in vitro* protocol developed would be beneficial to multiply the plants of *R. cordifolia* on large scale within the short period with low cost and to conserve the plant.

Keywords: Conservation, Growth regulators, *In vitro* propagation, Micropropagation, Node.

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INTRODUCTION

The World Health Organization is emphasizing the people to use herbal medicine to avoid the side effects of allopathic medicine. European countries have also drastically changed their lifestyle and are now preferring pure, natural, herbal medicines. Among the Asian countries, China is the biggest producer and supplier of the herbal extracts, raw drugs, and medicines. India has rich flora and established its own medicine systems. Ayurveda is being practiced since thousands of years, using herbal medicines.

Due to overexploitation of medicinal plants from wild source, majority of the plants are becoming rare, endangered, and vulnerable. To meet the demand and supply of medicinal raw drugs, efforts are being made to conserve these plants through cultivation, *ex situ* conservation, and *in vitro* propagation.

Manjishtha—*R. cordifolia* L. (family: Rubiaceae) is an important medicinal plant used in Ayurveda. It is a perennial climber found throughout India. It is an important ingredient of Manjishthadi Kwath, Manjishthadi taila, Chandanasav, Brihanmanjishthadi Kwath, Arvindasava, and Ashwagandharishta.[1] It exhibits antioxidant, anticancer, antitumor, hypoglycemic, antiviral, antibacterial activity.[2] Plant parts, viz., roots, stems, leaves, and fruits, are being used as medicine by many tribal folk of India, China, Korea, South Africa, and Philippines to cure blood diseases and to treat rheumatism, diabetes, skin diseases, acne, bronchitis, pneumonia, cough, cold, jaundice, urinary disorders, abnormal uterine bleeding, chest inflammation, fever, stomachache, dysentery, and constipation.[3,4]

This plant contains major chemical constituents, like purpurin, xanthopurpurin, manjistin,[2] anthroquinones, naphthoquinones, pseudopurpurin.[4]

Root, stem, leaf, and fruit of Manjishtha are being used to prepare various traditional and modern medicines. It is also used to adulterate *Swertia chirayata* (Roxb.) ex Flem Karst.[5] Due to excessive collections from wild sources, Manjishtha is depleting and becoming vulnerable.[6-9] Keeping in view the importance of medicinal value and tremendous collection from available sources, it is felt necessary to conserve this plant. Therefore, efforts were made to develop its *in vitro* propagation technique and achievements are communicated in the present study.

MATERIALS AND METHODS

Plant Material and Source of Explants

Plantlets were collected from forest area of Mahabaleshwara, Satara district, Maharashtra state of India. Seedlings

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were established in nursery maintained at Regional Ayurveda Institute for Fundamental Research, Pune.

Identification and Authentication of Plant

Collected plant was identified by Botanist of the Institute and authenticated with the help of floras.[10,11] Herbarium specimens were prepared[12] and deposited in the herbarium section of the Institute with the voucher specimen number 3739.

Explants Preparation

Healthy, disease-free explants were collected from the plants maintained in the Institute nursery. Nodal segments were prepared by cutting the stem part at internodal portion. Explants were soaked in 5% Tween 20 solution (Hi Media) for half an hour and washed thoroughly under running tap water for 30 minutes. Further, these explants were sterilized with 0.1% mercury chloride solution (Hi Media) for 1 minute and rinsed thrice with sterile distilled water under aseptic conditions.

Media Preparation and Inoculation

MS salt medium supplemented with 3% sucrose (w/v; Hi Media, India) was used in all experiments. pH of the medium was adjusted to 5.7 to 5.8 with 0.1 N NaOH or HCl prior to adding agar 0.8% (w/v; Hi media, India). A total of 20 mL of digested medium was poured in test tubes and plugged with nonabsorbent cotton covered with double-layered gauze cloth. The media was autoclaved for 20 minutes at 121°C at 15 lbs/inch^2 pressure. The MS salt medium fortified with different concentrations of benzyl amino purine (BAP), TDZ, kinetin (Kn), α-naphthalene acetic acid (NAA), indol-3-acetic acid (IAA), IBA singly or in combinations with other hormones.[13]

Decontaminated nodal segments were cut into 2 cm long sections and implanted onto MS basal medium as well as MS fortified with different concentrations of plant growth regulators.

Culture Conditions

All cultures were incubated at 22 ± 2°C for 8 hours photoperiod with light intensity of 3,000 lux using cool-white fluorescent tubes. Observations were recorded after 3 to 4 weeks. All the experiments were repeated thrice with 21 replicates.

RESULTS

Shoot Regeneration from Nodal Segment

Explants were obtained from the plants grown in the nursery. After complete sterilization under aseptic conditions, explants were trimmed 1 to 2 cm and inoculated onto MS basal and MS augmented with different growth hormones, viz., IBA (1–4 mg/L), Kn (1–4 mg/L), TDZ (0.5–2 mg/L), IAA (1–4 mg/L), and NAA (1–4 mg/L) alone and MS supplemented with BAP (1–5 mg/L) + NAA (0.1 mg/L) + 0.1% polyvinylpyrrolidone (PVP), MS + Kn (1–3 mg/L) + NAA (0.1 mg/L) + 0.1% PVP. Bud swelling and leaf formation was observed in explants inoculated on MS + BAP (1–4 mg/L), MS + Kn (1–4 mg/L) with or without 0.1% PVP. Explants inoculated on MS + IAA (1–4 mg/L), MS + NAA (1–4 mg/L) fortified with or without 0.1% PVP, did not show bud swelling or shoot formation. The explants inoculated on MS + IBA (1–4 mg/L) showed induction of callus at cut ends.

Shoot formation and multiple shoots were developed from the nodal segments inoculated on MS (solid and liquid) medium enriched with TDZ (0.5–1 mg/L). Maximum 20 to 25 number of shoots developed on MS + TDZ (0.5 mg/L) + 0.1% PVP liquid medium and 2 to 3 shoots on solid medium. Whereas, 3 to 4 and 1 to 2 shoots developed from nodal explants inoculated on MS + TDZ (1 mg/L) + 0.1% PVP liquid and solid medium respectively. Details are shown in Tables 1 to 3, Figure 1, Graphs 1 and 2.

Root Formation

In vitro grown shoots were transferred on rooting medium ½ MS, MS basal, solid and liquid MS + IBA (1–4 mg/L),

Table 1: Effect of different phytohormones on nodal segment of *R. cordifolia* L.

Medium	*Explant*	*Callusing*	*Shooting*	*Rooting*	*Number of shoots*	*Average height of shoots (cm)*
MS Plain	Nodal segments	–	–	–	–	–
MS + BAP (1–4 mg/L)	Nodal segments	–	+	–	–	–
MS + Kn (1–4 mg/L)	Nodal segments	–	+	–	–	–
MS + TDZ (0.5–1 mg/L)	Nodal segments	–	+	–	1–2	7.52
MS + TDZ (0.5–1 mg/L) (Liquid medium)	Nodal segments	–	+	–	20–25	6.61
MS + IAA (1–4 mg/L)	Nodal segments	–	–	–	–	–
MS + IBA (1–4 mg/L)	Nodal segments	+	–	–	–	–
MS + NAA (1–4 mg/L)	Nodal segments	–	–	–	–	–

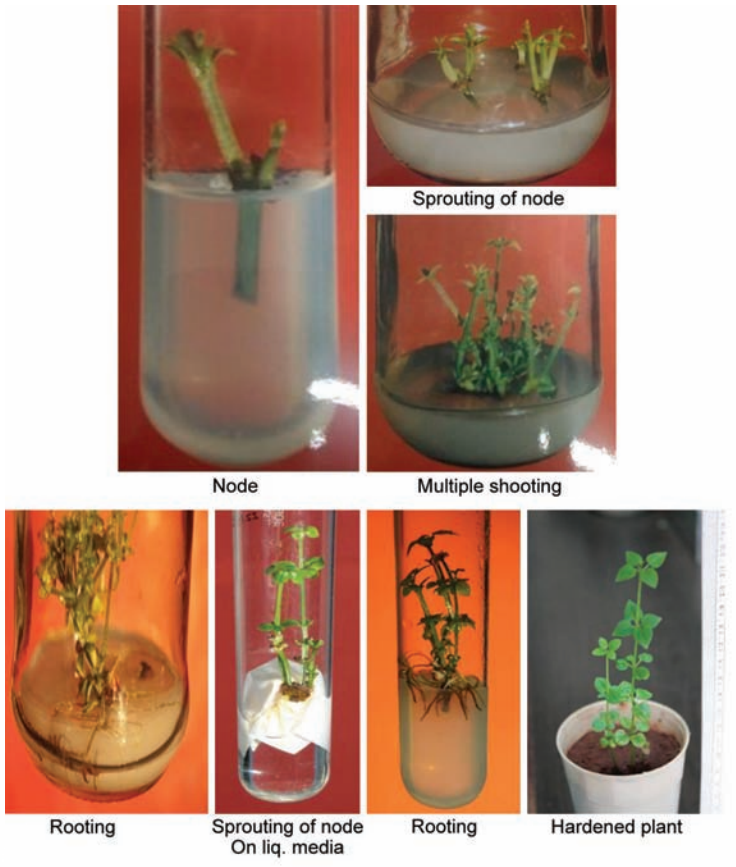
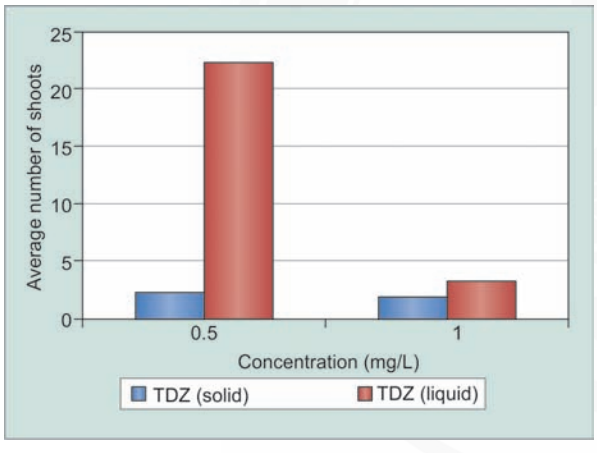
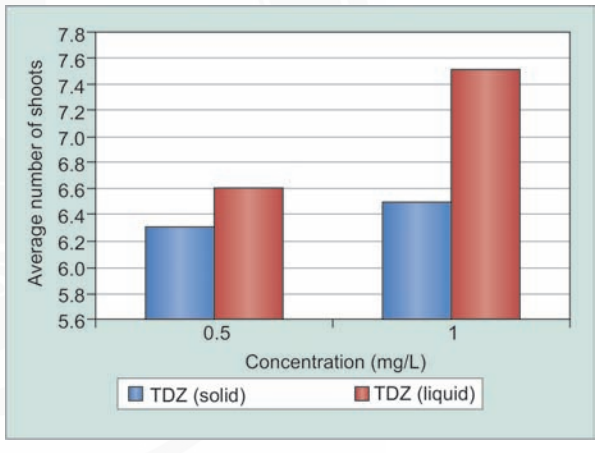


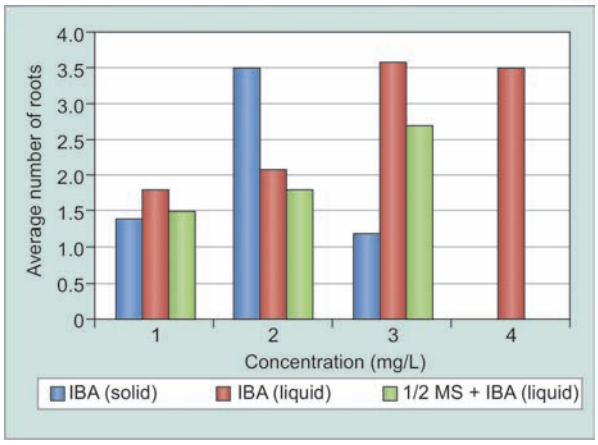
Fig. 1: *In vitro* grown shoots, roots, and hardening of the plant



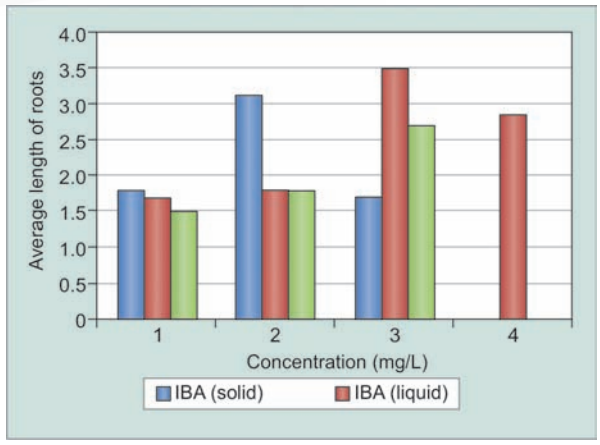
Graph 1: Effect of TDZ on number of shoots of *R. cordifolia* L.



Graph 2: Effect of TDZ on height of shoots of *R. cordifolia* L.



Graph 3: Effect of IBA on number of roots of *R. cordifolia* L.



Graph 4: Effect of IBA on length of roots of *R. cordifolia* L.

Table 2: Effect of BAP, Kn, and NAA on nodal segment of *R. cordifolia* L.

Medium	*Explant*	*Callusing*	*Shooting*	*Rooting*	*Average number of shoots*	*Average height of shoot (cm)*
MS + BAP (1 mg/L) + NAA (0.1 mg/L) + 0.1% PVP	Nodal segments	+	–	–	–	–
MS + BAP (2 mg/L) + NAA (0.1 mg/L) + 0.1% PVP	Nodal segments	+	–	–	–	–
MS + BAP (3 mg/L) + NAA (0.1 mg/L) + 0.1% PVP	Nodal segments	+	+ (Bud sprouting at node)	–	–	–
MS + BAP (4 mg/L) + NAA (0.1 mg/L) + 0.1% PVP	Nodal segments	+	–	–	–	–
MS + BAP (5 mg/L) + NAA (0.1 mg/L) + 0.1% PVP	Nodal segments	+	–	–	–	–
MS + Kn (1 mg/L) + NAA (0.1 mg/L) + 0.1% PVP	Nodal sectors	–	+ (Bud sprouting at node with slow growth)	–	–	–
MS + Kn (2 mg/L) + NAA (0.1 mg/L) + 0.1% PVP	Nodal sectors	–	+ (Bud sprouting at node with slow growth)	–	–	–
MS + Kn (3 mg/L) + NAA (0.1 mg/L) + 0.1% PVP	Nodal sectors	–	+ (Bud sprouting at node)	–	–	–

Table 3: Effect of TDZ on nodal segment of *R. cordifolia* L.

Medium	*Explant*	*Callusing*	*Shooting*	*Rooting*	*Average number of shoots*	*Average height of shoots (cm)*
MS + TDZ (0.5 mg/L) + 0.1% PVP	Nodal sectors	–	+	–	2.4	6.31
MS + TDZ (1 mg/L) + 0.1% PVP	Nodal sectors	–	+	–	2 .0	6.50
MS + TDZ (0.5 mg/L) + 0.1% PVP (liquid media)	Nodal sectors	–	+	–	22.2	6.61
MS + TDZ (1 mg/L) + 0.1% PVP (liquid media)	Nodal sectors	–	+	–	3.4	7.52

MS + IAA (1–4 mg/L), and liquid ½ MS + IBA (1–3 mg/L). Root induction was achieved in MS + IBA (1–3 mg/L) solid and MS + IBA (1–4 mg/L) liquid medium within 2 to 4 weeks. The best rooting (2–3 roots) was observed in MS + IBA (2 mg/L) in 8 to 14 days. 1 to 2 roots developed in IBA (1 and 3 mg/L) after 4 weeks; however, IBA (4 mg/L) did not induce rooting during the present study. Liquid culture medium fortified with IBA (3 mg/L) showed better response as compared with the IBA (1 and 2 mg/L). Details are given in Figure 1, Graphs 3, 4 and Table 4.

Hardening

After 6 weeks on rooting media, completely developed plantlets were carefully removed from culture tubes, washed with sterile distilled water to remove agar and sucrose. Plantlets were kept in sterile distilled water for 30 minutes before transferring to plastic pots containing sterile soil and sand in 1:1 ratio. Pots were covered with transparent plastic bags to maintain humidity and kept in culture room for 2 to 3 weeks. These plants were then exposed to the nursery conditions for 2 to 3 weeks before transferring in the field conditions. 70% plants survived in the field trials.

DISCUSSION

Efforts have been made to propagate the plant through seed germination to conserve vulnerable and endangered plants of *R. cordifolia* L. from North East and Western Ghats of Maharashtra region of India.[14,15] *In vitro* propagation trials conducted using nodal explants inoculated on MS basal medium supplemented with benzyl adenine (1 mg/L) and IAA (0.02 mg/L); produced maximum of 5.9 and 5.2 shoots per explants respectively, and maximum 8.9 number of roots with 6.4 cm length.[16]

Indirect *in vitro* propagation protocol was established by inducing callus from leaf, internode, and node explants. Induction of 85% callus was reported on 2,4- Dichlorophenoxyacetic acid (2.5 mg/L) + NAA (2.0 mg/L) and maximum 1.9 number of shoots per culture were achieved on BAP (4 mg/L) and Adenine sulphate (5 mg/L). The best rooting response was recorded on IBA (2 mg/L) with 8 number of roots with 4.6 cm length.[17]

Maximum 8.1 ± 1.2 number of shoots per node and 3.9 ± 0.1 cm shoot length was recorded when nodal segments were inoculated on MS fortified with TDZ (4 mg/L) and maximum 4.9 ± 0.7 numbers of roots with 4.7 cm length were noted on IBA (1 mg/L),[18] whereas in our experiment

Table 4: Effect of IBA on *in vitro* grown shoots of *R. cordifolia* L.

Medium	*Explant*	*Callusing*	*Shooting*	*Rooting*	*Average number of roots*	*Average length of roots (cm)*
½ MS	*In vitro* grown shoots	–	–	–	–	–
MS plain	*In vitro* grown shoots	–	–	–	–	–
MS + IBA (1 mg/L)	*In vitro* grown shoots	–	+	+	1.4	1.79
MS + IBA (2 mg/L)	*In vitro* grown shoots	–	+	+	3.5	3.13
MS + IBA (3 mg/L)	*In vitro* grown shoots	–	+	+	1.2	1.71
MS + IBA (4 mg/L)	*In vitro* grown shoots	–	–	–	–	–
MS (liquid) + 1 mg/L IBA	*In vitro* grown microshoots	–	+	+	1.8	1.69
MS (liquid) + 2 mg/L IBA	*In vitro* grown microshoots	–	+	+	2.1	1.81
MS (liquid) + 3 mg/L IBA	*In vitro* grown microshoots	–	+	+	3.6	3.48
MS (liquid) + 4 mg/L IBA	*In vitro* grown microshoots	–	+	+	3.5	2.86
½ MS (liquid) + 1 mg/L IBA	*In vitro* grown microshoots	–	+	+	1.5	2.61
½ MS (liquid) + 2 mg/L IBA	*In vitro* grown microshoots	–	+	+	1.8	2.58
½ MS (liquid) + 3 mg/L IBA	*In vitro* grown microshoots	–	+	+	2.7	2.43
MS + IAA (1–4 mg/L)	*In vitro* grown shoots	–	–	–	–	–

maximum average 22.2 number of shoots with 6.61 cm length was achieved on MS + TDZ (0.5 mg/L) + 0.1% PVP liquid medium. There was five-fold increase in the number of shoots than Khadke et al.[18]

This clearly showed that use of TDZ (0.5 mg/L) liquid medium supplemented with 0.1% PVP enhanced induction of shooting and growth of shoots. Maximum rooting and root number was also at par with the findings of Khadke et al.[18] The use of liquid TDZ (0.5 mg/L) + 0.1% PVP proved to be more effective than the solid TDZ and other growth hormones tried for induction of shoots.

CONCLUSION

To conserve and propagate vulnerable, endangered medicinal plant of Ayurvedic importance, an effective *in vitro* protocol has been developed. It would be more beneficial to multiply the plants of *R. cordifolia* L. on large scale within the short period with low cost as compared with the earlier protocols.

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हिन्दी सारांश

नोडल संवर्धन के द्वारा मंजिष्ठा–रुबिआ कोर्डिफोलिआ एल. का संरक्षण

उद्देश्यः मंजिष्ठा–रुबिया कोर्डीफोलिया एल. (परिवारः रुबिएसी) एक महत्वपूर्ण औषधीय पादप है जिसका विभिन्न आयुर्वेदिक योगों में प्रयोग किया जाता है। विभिन्न श्वसन और त्वक रोगों की चिकित्सा के लिए इस पादप के मूल, तना, पत्र व फलों का उपयोग किया जाता है। मंजिष्ठा देश के विभिन्न हिस्सों के प्राकृतिक जगह से अत्यधिक संग्रहित किये जाने के कारण दुर्लभ तथा लुप्तप्रायः हो गया है। इसलिए, पौधे के त्वरित गुणन के लिए व्यवस्थित इन विट्रो प्रोटोकॉल विकसित करने का निर्णय लिया गया है।

सामग्री और विधिः स्वस्थ्य, रोगमुक्त पादपों से संग्रहित नोडल खण्डों को एक्सप्लाण्ट के रूप में प्रयुक्त किया। पूर्वउपचारित एवं विसंक्रमित सतह नोडल खण्डों को एमएस आधारीय माध्यम के साथ–साथ पादप वृद्धि नियामक विभन्न सांद्रणों जैसे बीएपी, टीडीजेड, केएन, एनएए आईएए, आईबी, एकल या संयुक्त के साथ एमएस दृढ़ माध्यम पर प्रत्यारोपित किया गया। इसके बाद 3000 एलयूएक्स की प्रकाश तीव्रता के साथ 8 घण्टे के प्रकाश समय के लिए संवर्धनों का 22 डीग्री सेण्टीग्रेड ± 2 डीग्री सेण्टीग्रेड पर ऊष्मायन किया गया।

परिणामः एमएस+टीडीजेड (0.5 मिग्रा/ली) + 0.1 प्रतिशत पीवीपी तरल माध्यम पर संरोपित नोड़ल खण्डों से अधिकतम संख्या में टहनियाँ (20–25) विकसित हुई। सबसे अच्छे मूल (2–3 मूल) एमएस+आईबी, (2 मिग्रा/ली) पर 8 से 14 दिनों में विकसित हुए।

निष्कर्षः विकसित इनविट्रो प्रोटोकाल कम लागत के साथ अल्प समय में बड़े पैमाने पर रुबिया कार्डिफोलिया के पादपों की गुणन वृद्धि करने तथा पादपों के संरक्षण में लाभकारी होगा।

मुख्य शब्दः संरक्षण, वृद्धि नियामक, इन विट्रो संचरण, सूक्ष्मसंचरण, नोड़।