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In vitro Seed Germination and Plantlets Development of Canthium Coromandelicum (Burm. f.) Alston.

Priyadarshini S. Ekambe[1], Babasaheb S. Surwase[2]
[1, 2]School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra, India-431606
Email address: [1]priyaekambe@gmail.com

***Abstract*—**A protocol for in vitro seed germination and plantlets development of Canthium coromandelicum (Burm. f.) Alston a medicinal plant belonging to family Rubiaceae was developed which is used in the treatment of scabies, ringworm, cough, indigestion, snake bite. It shows various pharmacological properties like antioxidant, anti-inflammatory, antirhuematic, wound healing and diuretic activity. High frequency in vitro clonal propagation protocol was standardized from the nodal explants derived from in vitro raised 30 days old seedlings. Knudson's C (KnC) medium was the best suitable medium for seed germination and seedling development. Maximum shoot multiplication frequency was obtained on Murashige & Skoog medium supplemented with 4 mg/l 6-benzyl amino purine (BAP) alone. The elongated shoots were subcultured for successful rooting on ¼ MS with 3 mg/l NAA. The in vitro raised plantlets were acclimatized in green house and progressively transplanted to natural conditions with 70% survival.

***Keywords*—**BAP; Canthium coromandelicum (Burm. f.) alston; knudson's C medium; micropropagation; murashige and skoog medium; NAA.

I. INTRODUCTION

Canthium coromandelicum (Burm. f.) Alston is a wild plant which belongs to family Rubiaceae. Locally, it is known as Kara, Mullukara with synonyms as Canthium parviflorum, Plectronia parviflorum, one of the important medicinal plant which plays a versatile role in traditional medicines. Canthium coromandelicum (Burm. f.) Alston is erect, armed, rigid shrubs, 2-3 m tall, branches many with opposite, supra-axillary, horizontal, sharp, straight thorns. Leaves ovate to the orbicular, obtuse, glabrous, and green above paler beneath. Petioles are long, slender; stipules triangular. Flower 4-merons, small, in many flowered cymes; drupes globose [1], [2].

C. coromandelicum is traditionally used for snake bite in some villages in Shimoga district of Karnataka [3]. The leaves, bark and stem are antimicrobial [4]. Leaves are used in the treatment of scabies and ringworm [5]. Leaves are also used as functional food [6], [7]. Phenolics and flavonoids of this plant have considerable antioxidant activity [8]. It is used as laxative and to cure gout. The tribal in Orissa use its fruits to treat headache. Leaf paste has wound healing property [9]. As per our knowledge, there is no standardized protocol on in vitro propagation of C. coromandelicum (Burm. f.) available. Hence this study was proposed to achieve the appropriate medium for seed germination, seedling development and micropropagation of C. coromandelicum.

II. MATERIALS AND METHODS

Plant Material

Mature fruits of *Canthium coromandelicum* were collected from place 'Udgir' in Latur district, Maharashtra state in India. It was authenticated by using regional flora. The herbarium sheets of medicinal plant under study are deposited at Dept. of Botany, S.R.T.M.University, Nanded (MS).

Preparation of Explant

The shade dried seeds were used for seed germination. The seeds were thoroughly washed under running tap water in the tissue culture bottle for about 30 minutes. Seeds were disinfected in 1% Sodium hypochlorite solution for 1-2 min. Seeds were washed with sterile distilled water. After that they were treated with 70 % v/v ethanol for 1 min. Again washed with distilled water. Surface sterilized in 1 % $HgCl_2$ for 1 min. Thereafter, the seeds were washed thrice with sterile distilled water to remove the traces of mercuric chloride prior to placing onto different medium. Since seeds show seed coat dormancy, seeds were mechanically scarified to remove seed coat to facilitate the intake of water and nutrients from the surrounding medium.

Seed Germination and Seedling Development

Seeds were inoculated in different media like half strength of Murashige and Skoog (MS) (1962) medium, full strength MS medium, Knudson's C medium for seed germination. After four weeks, there was development of seedlings observed. These were used as a source of explants. Nodal explants were prepared by cutting the parts in aseptic conditions.

Culture Media & Conditions

Murashige & Skoog (MS, 1962) medium supplemented with 0.9% (w/v) Agar-agar, 3% sucrose with cytokinin like 6-Benzylaminopurine (BAP) (1-10 mg/l) was used for regeneration of shoots. The pH of the medium was adjusted to 5.8 before adding agar-agar. Molten medium (15 ml) was poured into each test tube and was autoclaved at 15 lbs and 121^{o}C for 15-20 mins. Nodal explants were inoculated and incubated at 25±2^{o}C at a relative humidity of 70-80% under 16 h photoperiod of 2000 lux light intensity provided by cool white fluorescent tubes. For each treatment 20 replicates were used. The growing explants were sub cultured after every 2 weeks. All the experiments were repeated in triplicate.

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Rooting and Acclimatization

The healthy regenerated shoots of about 3 cm in height were transferred to ¼ MS medium with different concentrations of Naphthalene 3- acetic acid (NAA) for induction of roots. The rooted plantlets were transferred to plastic pots containing a mixture of sterile soil and sand (1:1) in the lab conditions. Humidity was maintained at 70- 80 % by covering the plants with polythene bags. The polythene bags were progressively removed to reduce the humidity. After acclimatization for 15 days, plantlets were transferred to green house for one month with an average temperature of 25 ±2 ^{o}C. Following hardening, the plantlets were transplanted to pots containing garden soil alone.

III. Results and Discussion

In vitro regeneration of this medicinal plant species has successfully been reported using nodal explants. In the present investigation, high frequency in vitro clonal propagation protocol of the selected plant species has been reported using nodal explants.

Nodal explants were inoculated on MS medium containing different concentrations of BAP for optimization of cytokinin concentration and to elicit the best regeneration response. Induction of multiple shoots took place in all concentrations of BAP used. Maximum shoot multiplication i.e. 9 shoots per explants was achieved on MS medium containing 4 mg/l BAP with 90% response. Either decrease or increase in concentration of BAP reduced the shoot number, percentage of response and an average shoot length. The promotive role of BAP for multiple shoot induction has been reported in Cyperus rotundus [10], [11].

Similarly, multiple shoots were induced on MS medium supplemented with BAP in many other medicinal plants species like Chlorophytum species [12]; Cercis canadensis var. Mexicana [13]; Schinopsis balansae [14]; Holarrhena antidysentrica [15]; Sersia dentata [16] and Acacia nilotica [17]. Two week old healthy shoots with 3-4 cm in height were cut and were inoculated on ¼ MS medium containing different concentrations of NAA for induction of roots. Roots developed from the base of shoots after one week of culture. Amongst different concentrations of NAA tried 3 mg/l concentration was the best and showed maximum root number i.e. 7.16±0.16 per shoot with an average root length of 3.11±0.20 cm. NAA is widely used for induction of roots on regenerated shoots of medicinal plants like Chlorophytum borivilianum [12], Curculago orchoides [18], Taverniera Cuneifolia [19]. Similarly, IBA promoting rooting in Momordica cymbalaria [20]. In contrat to this IAA is best for induction of roots [17].

The well-developed healthy rooted plantlets were used for the acclimatization. The in vitro generated plantlets were taken out from the rooting medium after 4 weeks of incubation and washed thoroughly with water. They were later transferred to pot containing autoclaved sandy soil and covered with polythene bags under culture room conditions for two weeks. The plantlets were irrigated with sterile distilled water. After the development of new leaves, the polythene bags were progressively removed. Then plantlets were transferred to soil and were kept under culture room conditions for two weeks. Finally after two weeks, they were transferred to pot containing soil only. Such plantlets were transferred to shade house and were irrigated with tap water. The survived plantlets showed 70% survival without any morphological aberrations.

The plants have continuously been ever exploited from their natural strands for medicinal purpose. Hence, standardization of regeneration protocols of such medicinal plants has become important. The developed regeneration protocol may facilitate the conservation of the selected species, which is extensively used in traditional medicines.

TABLE I. Effect of different media on seed germination of C. coromandelicum

S. No.	Medium	% of Seed germination
1	MS Medium	80
2	Half MS Medium	70
3	Knudson's Medium	100

TABLE II. Influence of Full MS, Half MS medium in combination with BAP on in vitro callus formation and shoot regeneration by using nodal explant of C. coromandelicum after four weeks of culture.

Medium	MS with Growth Hormones BAP (mg/l)	Percentage Response (%)/ explant	Shoot Number/ Explant Mean ± SE
Half MS	-	60	4.16±0.40
Full MS	-	90	5.33±0.55
Full MS	1	50	4.16±0.40
Full MS	2	60	5.16±0.60
Full MS	3	70	5.33±0.55
Full MS	4	90	9.00±0.36
Full MS	5	60	5.00±0.57
Full MS	6	60	4.83±0.47
Full MS	7	60	4.50±0.42
Full MS	8	50	4.33±0.42
Full MS	9	40	4.00±0.25
Full MS	10	40	3.33±0.71

TABLE III. Rhizogenic response of in vitro regenerated shoots of C. coromandelicum on full, half and ¼ strength MS medium supplemented with different concentrations of NAA after 30 days of culture.

Medium	Growth Hormone (mg/l) NAA	Percentage Response (%)	Average No. of Roots/ Explant (Mean± SEM)	Average Root length (cm) (Mean± SEM)
MS	-	30	1.08±0.08	1.16±0.16
½ MS	-	30	2.00±0.25	1.16±0.16
¼ MS	-	50	2.00±0.25	1.08±0.08
¼ MS	1	60	4.83±0.47	1.00±0.12
¼ MS	2	70	5.00±0.25	2.08±0.15
¼ MS	3	90	7.16±0.16	3.11±0.20
¼ MS	4	80	5.33±0.21	2.83±0.10
¼ MS	5	80	5.16±0.30	1.83±0.30

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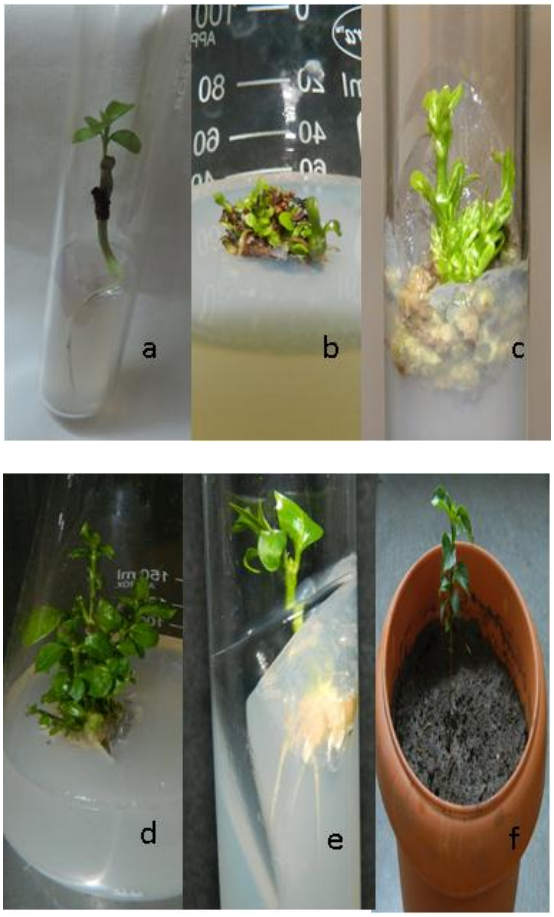

Fig. 1. (a) Seed germination and seedling development of C. coromandelicum on Knudson's medium. (b) Initiation of callus through regeneration by using nodal explant. (c) Induction of multiple shoots. (d) Development of shoots after four weeks. (e) Rhizogenic response of in vitro regenerated shoots. (f) Acclimatized plantlets of C. coromandelicum.

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