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In vitro propagation of *Caralluma adscendens*: An ethnomedicinal plant

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Caralluma adscendens (Roxb.) R.Br. is a traditional medicinal plant which is conserved by in vitro technique of tissues culture using nodal explants. Among various concentrations, best response in terms of multiple shoot formation was observed on Murashige and Skoog medium supplemented with 1.0 mgl-1 Kn. It was produced an average shoot length of 2.98 ± 0.38 cm per explant . 1.0 mgl^{-1} IAA proved to be an optimal, producing an average root length of 4.98±0.4 cm per explant. The plantlets were successfully established in pots with 73% survival rate which did not show any morphological abnormalities in the field conditions during acclimatization period of 3 months.

Key words: *Caralluma adscendens, in vitro* propagation, IAA, Kn, tradicinal medicinal plant

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INTRODUCTION

Caralluma adscendens (Roxb.) R.Br. is a wild, succulent, perennial medicinal herb. The species is edible and its medicinal properties include anti-inflammatory, anti-nociceptive, anti-ulcer, anti-diabetic, carminative, febrifugal, antipyretic and antioxidant effects [1,2]. Ethnobotanicaly, it has been used for epilepsy ,diabetes, lung diseases, indigestion, kidney stone and skin diseases by Paliyar tribe [3]. Propagation through seed is an inadequate solution due to low viability, poor seed germination rate, scanty and delayed rooting of the seedlings. It is depleted due to urban activities like building constructions, road widening and grassing by sheep and goats. Because of vegetative propagation by stem is a very tedious procedure, Hence, the present study aimed to conserve through micro propagation.

MATERIALS AND METHODS

Plant materials

The plant materials were collected in Kamuthi, Ramanathapuram disrict, Tamilnadu from its natural habitat (Plate 1). The herbarium specimen was prepared using the methodology of Jain and Rao [4]. It was identified with the help of the Flora of the Presidency of Madras [5] and voucher specimen are deposited in research department of Botany, Pasumpon Thiru Muthuramalinga Thevar Memorial college, Kamuthi.

Plate 1: Habit of *Caralluma adscendens* (Roxb.) R.Br.

a.Habit b.Flower c.Fruit d. Seeds

Micropropagation Protocol

Preparation of hormonal stock:

100mg of Kn and NAA are dissolved into 0.1N HCl separately and made up of 100ml double strile distilled water for stock concentration.

Preparation of nutrient media:

The required volume of macronutrients, micronutrients and vitamins were taken from the stock solutions [6] and mixed well with the appropriate volume of double sterilized distilled water. MS medium containing 30 g/l sucrose and solidified with 5.5 g/l agar served as the basal medium. The media were adjusted to the p^H of 5.6 for shoot and 5.8 for root with 0.1N NaOH or HCl. The Plant growth regulators (PGRs) are used Kinetin for shoot multiplication and IAA for root initiation.

Inoculation of Explants:

Inoculation of explants was carried out in the laminar flow hood chamber aseptically. The surface sterilized explants were transferred to sterile petri plates with sterile filter papers. After drying, the appropriate size of the explants were excised (1.0-1.5 cm length) and inoculated.

Incubation (Culture conditions):

Explants were inoculated in 20 ml culture tube each containing 5 ml of medium. The cultures were incubated at 25±2°C with 16 hrs photoperiod under an illumination of 20 µmolm-2s-1 photosynthetic photon flux density which was provided by ordinary tube light. The whole experiment was repeated three times.

Shoot Regeneration

Nodal explants after an incubation period of 30 days were sub cultured on MS medium augmented with Indole acetic acid (IAA) 0.5mg/L and Kinetin 0.25mg/L either alone or in combinations for rapid shoot multiplication.

Multiple shoot proliferation

For direct adventitious shoot regeneration, nodal segments were used as explants, and inoculated on MS medium fortified with Kn (0.5-3.0 mg/L) separately and in combination with IAA (0.5-3.0mg/L).

Rooting

in vitro shoots were excised (3-5cm) from regenerated shoots of the nodal explant derived callus and transferred to MS medium with IAA (0.5mg/L) combined with Kn(0.5 to 2.5 mg/L).

Hardening

The well developed plantlets were taken from culture tubes and agar was removed carefully by running tap water. They were transferred to 1.5cm teapots with potting mixture of sterilized soil, sand, farmyard manure (1:1:1) and maintained in the green house with a relative humidity of 80-85%. Plants were irrigated well at 8 hrs interval for 3-4 week and the rate of establishment was recorded after six week.

RESULTS AND DISCUSSION

In the present study showed that explants were green on MS basal medium without plant growth regulators. Aseptic seedling explants such a cotyledonary node, node and shoot tip explants were cultured on different media to regenerate shoots. The type and concentration of Kinetin (Kn) influenced the average number of shoots produced per explants as well as mean length of the shoots. There was no result shown in internodal explants. There was no sign of growth when explants were cultured in either single IAA or combination with IAA in the media (Table 1). The concentrations (0.5, 1.0, 1.5, 2.0 mgl^{-1}) of Kn showed shoot production.

Among various concentrations best response in terms of multiple shoot formation was observed on MS medium supplemented with 1.0 mgl-1 Kn (Table 1). 1.0 mgl^{-1} Kn proved to be optimal, producing an average shoot length of 2.98 ± 0.38 cm per explant Table 1& Plate 2. The best result for shoot proliferation was obtained from MS medium supplemented with 1.0 mgl-1 Kn. Explants respond significantly when cultured on the medium containing Kn combination with IAA (Table 1). At low level of Kn concentration (0.5 mgl^{-1}), fewer shoots were obtained (1.3 ± 0.5). At high level of Kn concentration (2.0 mgl^{-1}), fewer shoots were recorded (1.2 ± 0.4).

The length of the shoots varied at different concentrations (0.5, 1.0, 1.5, 2.0 mgl^{-1}) of Kn. Maximum shoot length 2.98 ± 0.3 cm was obtained from explants on a MS medium with 1.0 mgl^{-1} KN. In 0.5 mgl^{-1} concentration, the average length of the shoots was 1.64±0.2, and 1.5 mgl^{-1} concentration, the length of the shoots was 1.86±0.2 cm. The higher concentration (2.0 mgl^{-1}) of KN, the average length of shoots was 1.56±0.2 cm (Table 1).IAA alone did not show any positive response on shoot formation and development. The combination of Kn with IAA treatment promote new shoot formation. It was evident of synergistic influence. There was no response in medium containing without Kn (Table 1). When MS medium supplemented with Kn was more effective for efficient shoot induction in *Caralluma*. Continued exposure of explants in concentrations higher than 1.0 mgl^{-1} Kn during shoot induction caused high accumulation of cytokinin which inhibit further shoots development.

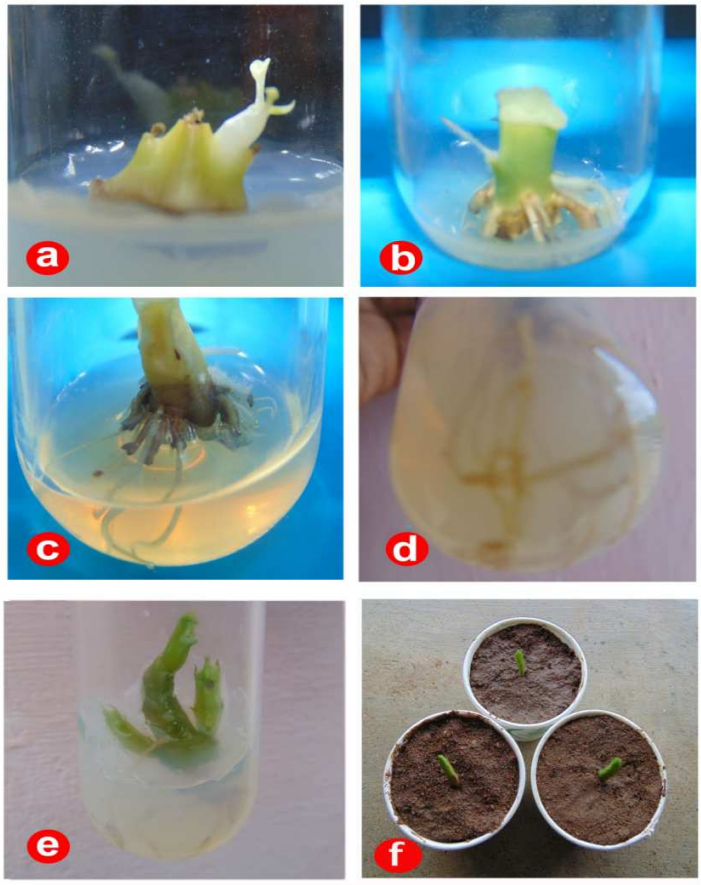
Table 1. Effect of growth regulators on *in vitro* shoot multiplication of *Caralluma adscendens*

Growth regulators(mg/l^{-1})		Average no of shoots per explant (mean±SE)	Average length of shoots(cm) mean± SE)
KN	IAA		
0.0	0.0	-	-
0.0	0.25	-	-
0.25	0.25	-	-
0.5	-	1.3±0.5	1.64±0.2
0.5	0.5	-	-
-	0.5	-	-
1.0	-	2.0±0.7	2.98±0.3
1.0	0.5	1.3±0.5	1.64±0.2
-	0.5	-	-
1.5	-	1.4±0.5	1.86±0.2
1.5	0.5	1.2±0.4	1.56±0.2
-	0.5	-	-
2.0	-	1.2±0.4	1.56±0.2
2.0	0.5	1.2±0.4	1.56±0.2
-	0.5	-	-

There was no sign of growth of roots when explants were cultured in either single KN or combination with IAA in the media (Table 2). The concentrations (0.5, 1.0, 1.5, 2.0 mgl^{-1}) of IAA showed root production. Among various concentrations best response in terms of multiple root formation was observed on MS medium supplemented

with 1.0 mgl-1 IAA (Table 2). 1.0 mgl^{-1} IAA proved to be optimal, producing an average root length 4.98±0.4 cm per explant Table 2& Plate2. The best result for root proliferation was obtained from MS medium supplemented with 1.0 mgl-1 IAA. Explants respond significantly when cultured on the medium containing IAA combination with Kn (Table 2). At low level of IAA concentration (0.5 mgl^{-1}), fewer roots were obtained (1.4±0.5). At high level of IAA concentration (2.0 mgl^{-1}), fewer roots were recorded (1.3±0.3).

Plate 2. Micropropagation of Caralluma adscendens (Roxb.) R.Br.



a. Shoot induction b. Root and Callus induction c.&d Well developed roots
e. Shoot multiplication f. Hardening

The length of the roots varied at different concentrations (0.5, 1.0, 1.5, 2.0 mgl^{-1}) of IAA. Maximum root length (4.98±0.4 cm) was obtained from explants on MS medium with 1.0 mgl^{-1} IAA. In 0.5 mgl^{-1} concentration, the average length of the roots was 1.86±0.2, and 1.5 mgl^{-1} concentration, the length of the roots was 1.56±0.2cm. In higher concentration (2.0 mgl^{-1}) of IAA, the average length of roots was 1.56±0.2 cm (Table 2). Kn alone did not show any positive response on root formation and development. The combination of IAA with Kn treatment also promote new root formation. There was no response in medium containing without IAA (Table 2). Continued exposure of explants in concentrations higher than 1.0 mgl^{-1} IAA during root induction caused high accumulation of auxin which inhibited further roots development.

Table 2. Effect of growth regulators on *in vitro* root multiplication of *Caralluma adscendens*

Growth regulators (mg/l^{-1})		Average no of roots per explant (mean±SE)	Average length of roots (cm) mean± SE)
IAA	KN		
0.0	0.0	-	-
0.0	0.25	-	-
0.25	0.25	-	-
0.5	-	1.4±0.5	1.86±0.2
0.5	0.5	-	-
-	0.5	-	-
1.0	-	4.0±0.7	4.98±0.4
1.0	0.5	1.4±0.5	1.56±0.2
-	0.5	-	-
1.5	-	2.6±0.4	1.56±0.2
1.5	0.5	1.4±0.5	1.56±0.2
-	0.5	-	-
2.0	-	1.3±0.3	1.56±0.2
2.0	0.5	1.3±0.3	1.56±0.2
-	0.5	-	-

The higher concentration of Kn decreased shoot multiplication in mulberry plant [7]. In our result , the maximum shoot length 2.98 ± 0.3 cm was obtained from explants on MS medium with 1.0 mgl-1 Kn. The maximum rate of shoot induction was recorded (80%) at 0.1 mgl-^{1} Kn in *Gladiolus hybridus* [8]. MS medium with BAP (0.5 mg/L) and Kn (1.0 mg/L) was suitable for multiple shoot induction recorded in *Jatropha curcas* L. [9]. Maximum number of multiple shoot bud (3.6±0.51) per explants was induced on MS medium supplemented with 2.0 mgl-^{1} KN combination with 0.5 mgl-^{1} IAA in *Vitex negundo* [10] But in our result even a single hormone 1.0 mgl-^{1} KN showed maximum number of multiple shoots (2.0±0.7) and shoot length (2.98 ± 0.3). *In vitro* regenerated plantlets with well developed shoots and roots were transferred to pots containing peat moss, farmyard manure and garden soil in 1:1:1 ratio. The plantlets were successfully established in pots with 73% survival rate. Plantlets did not show any morphological abnormalities in the field conditions during acclimatization period of 3 months.

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