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Review Article

MICROPROPAGATION OF *CARALLUMA STALAGMIFERA* FISCHER.- REVIEW

***Anitha S.**
Department of Biotechnology, Sri Krishnadevaraya University,
Anantapur-515 001, Andhra Pradesh, India
**Author for Correspondence*

ABSTRACT
Caralluma stalagmifera Fischer. is a medicinal plant belonging to Family Apocynaceae. It is a succulent edible plant used as a vegetable by the local tribal people. There are a number of chemical compounds like carumbellosides, lasianthosides, steroidal glycosides and stalagmosides in their phytochemical extractions. This is an endemic plant of South India, growing in the dry areas. In the present review article a detailed micropropagation work done till now was given which will be helpful for the young researchers to take further work.

Keywords: *Micropropagation, Caralluma stalagmifera*

INTRODUCTION
Caralluma Genus belongs to family Apocynaceae. All plants of this genus secrete milky latex so it is known as milk weed family (Bader *et al.*, 2003). Plants in this genus are perennial, succulent, usually leafless and edible. These are mostly present in India and grow in dry areas. Phytochemicals present in these plants are flavonoids, alkaloids, glycosides, saponins and polyphenols. These are used in traditional medicinal preparation, for the treatment of skin diseases, Rheumatism, paralysis, hepatoprotection, fever, migraine, snake and scorpion bites, inflammation, diabetes and cancer (Venkatesh *et al.*, 2003; Latha *et al.*, 2004; Abdel–Sattar *et al.*, 2007; Habibuddin *et al.*, 2008; Kumar *et al.*, 2011; Maheshu *et al.*, 2012; Shanmugam *et al.*, 2013). *Carallullma stalagmifera* Fischer. is a endemic succulent herb of South India. It is an edible plant and used as a vegetable. Phytochemicals isolated from this plant are carumbelloside III, stalagmosides I-V, Steroidal glycosides, lasianthoside A & B (Kunert *et al.*, 2006). This plant is found to have significant antiarthritic and anti-inflammatory activities (Reddy *et al.*, 1996).

Micropropagation protocols
Naik *et al.*, (2014) reported for the first time micropropagation of *C. stalagmifera* using nodal explants of mature shoots. They have sterilised the plant explants by washing under running tap water for 15 minutes (min), to remove dust and soil particles. Excised the internodes and rinsed with Tween 20 (1%v/v) for 5 min. Rinsed with water 3 times and washed with sterile double distilled water in laminar chamber. Later these explants were dipped in 30% ethanol for 3 min and washed with sterile water. Finally treated with mercuric chloride ($HgCl_2$) (0.1%) for 2 min and thoroughly washed with several sterile water. 0.6-10 mm explants were inoculated on MS (Murashige and Skoog, 1962) medium containing BAP (6-benzylaminopurine), KN (Kinetin), and NAA (Naphthalene Acetic Acid) and IAA (Indole-3-acetic acid). Subculturing was done after every 30 days. BAP (2mg/l) was found to be best without callus formation. They have recorded 2.25 shoots/explants with a 3.11 cm, mean length. When combination of BAP (2.0 mg/l) + KN (0.5mg/l) + IAA (0.3mg/l) was used 2.06±0.16 shoots were developed with 3.96 ±0.20 cm shoot length.

Callus induction was done by placing internodes on MS medium containing 2,4-D (2,4 dichlorophenoxyacetic acid and NAA (0.5-2.0mg/l) alone. In these 2,4-D (1.5mg/l) was best with 90% response. When this callus was subcultured on to a combination of hormones like, 2,4-D (1.5mg/l) + BAP (1.0mg/l) + IAA (0.2mg/l) produced maximum embryos. For rooting IAA (0.3mg/l) was found to be best with 4.05±0.34 root/shoot, 3.25±0.33cm root length and 80% response. After acclimatization for 4 weeks the plantlets were transferred to green house and 75% survivability was recorded.

Sreelatha *et al.,* (2014) reported the micropropagation of *C. stalagmifera* using nodal explants of mature shoots. They have sterilised these explants first by washing under the running tap water and washed with Tween-20 (1%) for 10 min, washed once again thoroughly under tap water. Later, washed with sterilised double distilled water in laminar chamber. Then treated with disinfectant $HgCl_2$ (0.1%) for 5 min. Rinsed several times. Then treated with the ethanol (70%) for 1 min and washed with sterilized double distilled water 3-4 times. After removing the damaged ends these explants were inoculated on MS medium. They have tested with different growth regulators such as BAP, 2Ip (isopentenyl adenine), GA_3 (Gibberellic acid), IAA, IBA and NAA either individually or in combinations for shoot multiplication.

Best hormone for shoot formation was found to be BAP (2.0 mg/l) with 2.88 shoots/explants and a shoot length of 2.80 cm. When combinations of hormones was used, BAP (2.0 mg/l+2iP (2.0mg/l) + NAA (0.5mg/l was found to be best with 88% of response, 8.47 shoots/explants and average shoot length of 2.50cm. For rooting half strength MS (1/2MS) medium supplemented with IAA, NAA, IBA were used. Of the tested concentrations NAA (0.5mg/l) was found to be best with an average of 8.42 roots and 73% response.

For acclimatization the regenerated shoots were first washed with water and potted in plastic pots containing peat mass, sand and farmyard manure in equal quantities. These 10 cm pots were covered with polythene cover and watered with ½ MS macro salts free of Sucrose for 2-3 weeks. After 10 days removed covers and pots were shifted to green house with 70% survival rate.

Sreelatha *et al.,* (2015a) sterilised selected nodal explants first by washing under the tap using Tween-20 (1%) for 5-10 minutes (min). Later washed with sterilised distilled water, followed by dipping in the ethanol (70%) for 1 min. Rinsed 3-4 times and treated with disinfectant $HgCl_2$ (0.1%) for 5 min. Finally, rinsed several times with sterilized double distilled water. After removing the damaged ends the explants were blotted on sterile paper and inoculated on MS medium supplemented with different growth regulators such as BAP, KN, IAA, IBA and NAA either individually or in combinations for shoot multiplication.

Among the tested combinations BAP (2mg/l) found to be best with 80% sprouting and 2.54 shoots/explants and with 1.80 cm shoot length. For enhancing the shoot number combinations were tried and BAP (2mg/l) + NAA (0.5mg/l) was found to be better with 6.72 shoots and 4.16cm shoot length. After subculturing on to the same medium, and observation for 25 days, the shoot number increased to 12. These shoots were excised and transferred to full and half strength MS medium for root induction supplemented with auxins like IAA, IBA and NAA (0.1mg/l– 3.0mg/l). Among the tested combinations ½ MS+NAA (0.5mg/l) was found to be best Rooted plantlets were hardened after transferring to the pots containing sterile sand, soil and farmyard manure in 1:1:1 ratio. These acclimatized plants were transferred to soil with 73% survival rate.

Sreelatha *et al.,* (2015b) sterilized the internodal shoot segments of *C. stalagmifera* as mentioned in Sreelatha *et al.,* (2015a). Later inoculated on the MS medium containing different auxins like 2,4-D, 2,4,5-T (2,4,5-Trichlorophenoxy acetic acid, 2,4,5-T P (2,4,5-Trichloro Phenoxy Picolinic acid, Dicamba (3,6-Dichloroanisic acid), Picloram (4 Amino, 3,5,6-Trichloro Picolinic Acid), NAA, IAA, IBA and Cytokinins BAP, KN, 2iP and Zeatin (4-hydroxy-3-methyl-trans-2-butenylaminopurine). Eventhough callus was produced on all combinations of tested hormones. Highest response was observed on 2,4-D (2mg/l), which was green in colour. Among combination of hormones 2,4-D (2.0mg/l) + BAP (0.5mg/l) showed highest response of 80%. For shoot regeneration BAP (2.0 mg/l) individually and BAP (2.0mg/l) + NAA (0.5mg/l) produced maximum shoots in 20 days. Shoots were later transferred for rooting to ½ MS medium containing NAA (0.5mg/l). Rooted plantlets were acclimatized after transferring to the plastic cups containing sterile sand (1): soil (1): farmyard manure (1), covered with perforated polythene covers. After 15 days removed these covers and transferred to green house. After a month transferred them to field conditions, with 70% of survival rate.

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