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Encapsulation and Callus production of most important plant *diplocyclos palmatus* (L.) C. Jeffrey (Shivlingi)

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ABSTRACT

Conservation of plants through different methods is being done for years because many important plants are getting reduced due to many reasons. Diplocyclos palmatus (Shivlingi) is a medicinal herb. Slowly this plant is also getting extinct. To conserve Shivlingi, encapsulation and callus induction technique was performed. Callus induction (leaves as explants) was done by preparing MS medium containing different concentration of 2, 4-D, kinetin and control medium. Best callus was induced in combination of Kin (2.5 mg/l) and 2,4-D(1.0 mg/l). Encapsulation method was successfully done for shoot tips by preparing beads by using different concentration of Sodium alginate and calcium chloride. At incubation time 30 minute combination of 3% sodium alginate, 100 mM calcium showed best initiation

Keywords- Conservation; encapsulation; Shivlingi; Callus; MS medium.

INTRODUCTION

All the plants in the world have both good and bad qualities, most of the plants have medicinal properties, and some plants are poisonous or harmful. Humans and some animals are dependent on trees and plants. Herbivorous are dependent on plants to fill their stomach. Plants are used as medicine in many places. These plants are considered as medicinal plant because of its high medicinal value. There are many such plants about which the whole world doesn't know, but the plant grown by the local people know the value of that plant one such plant is *Diplocyclos palmatus* commonly known as Shivlingi, Gurgumaru Its synonyms are *Bryonia laciniosa, Bryonopsis laciniosa,* (Sud *et al.,* 2017). The seeds of this plants look like the shivling of lord Shiva, hence it is named as Shivlingi (Chauhan *et al.,* 2010). *Diplocyclos palmatus* is a medicinal herb and annual climber and belongs to the family cucurbitaceae and generally distributed throughout India (Chauhan *et al.,* 2018). Different parts are being used to treat different kinds of disease and this plant is traditionally healer (Misra *et al.,* 2017). The seeds of the plant are used to treat sterility, female infertility, oligospermia, constipation, obesity and weight loss. The fruits are used to cure abdominal disease, anti - inflammation and leucoderma, other parts are used for antifungal, antimicrobial, antipyretic, anticancer or cytotoxic activity (Kumar *et al.,* 2016). *Diplocyclos palmatus* whole plant is useful and its every part contains high medicinal value. This plant contains many chemical constituents, main chemical compound is Bryonin and other photochemical are Punicic acid, goniothalamin, glucomannan, saponin, flavonoids, phenolic acids (Chavhan *et al.,* 2019). Due to overwhelming use of this plant, nowadays this plant is in endangered condition (Vijaya Shalini *et al.,* 2016). Root exudates shape nutrient availability and microbial composition in the rhizosphere, with multi-microbial inoculants showing promise for crop improvement. Tailored microbial consortia offer potential for long-term soil health and crop-specific benefits (Srivastava *et al.,* 2014). A field study (2020–22) on litchi cv. Shahi found that ethrel @ 400 ppm accelerated flowering and fruiting, while K_2HPO_4 @ 1% increased flower count per panicle. GA_3 @ 100 ppm improved sex ratio and fruit quality, including higher sugar content and lower acidity (Kumar *et al.,* 2023).

Because this is an important medicinal plant it is necessary to conserve this plant. For conservation of plants many methods such as plant tissue culture, encapsulation, cryo-preservation are being used.

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Shoot tip encapsulation is a biotechnological technique used for the preservation, propagation, and transportation of plant material. It involves encapsulating the shoot tips, or meristems, of plants in a protective, gel-like matrix, often composed of sodium alginate. This matrix acts as a synthetic seed coat, providing physical protection and maintaining the viability of the encapsulated plant tissues.The encapsulation process typically combines the advantages of in vitro culture and seed-based propagation methods, making it a valuable tool in plant tissue culture, germplasm conservation, and commercial micropropagation. Encapsulated shoot tips can be stored for extended periods, shipped easily without requiring complex infrastructure, and germinated under suitable conditions to produce whole plants.This method is particularly beneficial for species with limited seed production, recalcitrant seeds, or for plants that do not produce seeds at all. Additionally, shoot tip encapsulation supports genetic uniformity and facilitates the exchange of plant material across regions or laboratories while minimizing contamination risks. Encapsulation technique is a most effective and convenient method to conserve plants. After successfully development of encapsulated beads regeneration can be done after short term cold storage (4℃). For regeneration stored beads are inoculated in MS medium containing growth hormone, sucrose and agar. The aim of this study is to observe regeneration capacity of stored seeds and this is performed for both encapsulated and non encapsulated shoot tip.

MATERIALS AND METHODS

Explants collection and preparation

Fresh shoot cluster and leaves were collected from 4 week old plant maintained in the Garden of St. Thomas College, Ruabandha, Chattisgarh. Shoot cluster were washed under the running tap water then washed in sterilized water for two to three times. Explants were dipped in 0.1% mercury chloride for 10 minute. To remove excess traces of mercury chloride explants were washed thoroughly in sterilized water for three times after then for surface disinfectant 70% ethanol were used for 30-40 second and washed with sterilized water for two-three times. Shoot tip and leaves were excised in appropriate size for encapsulation and callus induction.

Encapsulation and storage

Gelling complex, sodium alginate was prepared in different concentration of 1, 2, 3, 4 5% and 50, 75, 100, 125, 150 mM of calcium chloride were prepared for encapsulation of shoot tips and autoclaved at 120°C in 15PSI for 15-20 minute. Shoot cluster was excised from mother plant and shoot tips were cut in appropriate size. Shoot tips were coated with sodium alginate for gelling complexation and then dropped in sterile calcium chloride and left for 20 - 30 minutes. Oval or round shaped beads developed and then beads were kept in a culture jars containing wet cotton to maintain humidity in jars and kept for storage for 2, 4 and 6 months at 4℃. Best beads were prepared in combination of 3% sodium alginate and 100mM calcium chloride.

Regeneration and culture media

Culture medium was prepared by using Murashige and Skoog (1962) medium pH was adjusted to 5.8 and for solidification 0.8% agar powder was used. For regeneration, encapsulated beads were transferred in the test tubes (in every 15 days intervals) containing MS medium supplemented with different concentrations of BAP + NAA and maintained at 25℃ ± 2. After 15-20 days shoot initiation has started as shown in figure 3. Proliferated shoots were sub cultured in fresh MS medium for proper shooting and rooting.

Callus Induction

For callus induction, MS medium (Control and with different concentration of Kin + 2,4-D) was prepared and leaves were sterilized in $HgCl_2$ (0.1 %) and in 70% ethanol. When explants are proper washed they were inoculated in culture medium and maintained at 25℃ ± 2 in dark. After 20 days every explants converted into callus in MS culture medium as shown in figure 5.

RESULT AND DISCUSSION

Encapsulated shoot tips were inoculated in MS medium supplemented with different concentration of BAP + NAA and kin+ 2,4-D in every 15 days response was observed. After 6 weeks BAP + NAA showed 85 % of response as shown in Table 1 and Kin + 2,4-D showed high response of 80% as showed in Table 2 and Fig.1.

Table 1: Shoot initiation from encapsulated shoot tip with growth hormone BAP + NAA (2.5+1)

BAP + NAA (mg/l)	% of initiated explants (Encapsulated Shoot tip)	No. of response Mean ± SD
0.5 + 0	40	2.00 ± 0.82
1 + 0.5	35	1.33 ± 0.49
1.5 + 0.5	55	0.67 ± 0.49
2 + 1	45	1.83±1.09
2.5 + 1	85	2.17 ± 0.90
3 + 1	70	1.67 ± 0.75

Greenish compact nodular calli obtained from leaf explants were selected for regeneration studies. These calli were transferred to regeneration medium, before that these calli were cut into 2 or 3 pieces and subcultured. After subculture on the parental medium shoot buds were initiated between 10-15 days.

Table 2: Shoot initiation from encapsulated shoot tip with growth hormone Kin + 2,4-D (3+1)

kin + 2,4-D (mg/l)	% of initiated explants (Encapsulated Shoot tip)	No. of response Mean ± SD
0.5 + 0	25	1.17 ± 1.09
1 + 0.5	40	1.67 ± 0.94
1.5 + 0.5	35	1.33 ± 0.75
2 + 1	60	1.83±0.90
2.5 + 1	55	2.33 ± 0.48
3 + 1	80	2.5 ± 0.5

The shoots were maintained on the same medium for 20 to 25 days to get sufficient growth. The regeneration frequency ranged between 20.0 to 68.8%. Encapsulation technique can be one of the best method for long term preservation of plants. These calli were maintained on parental medium for long time (or) subcultured on parental medium where they induced shoot buds between 15 to 25 days. After that the shoots were elongated and established into plantlets. There are differences in shoot regeneration frequency among different concentrations and combinations of auxin and cytokinin. Moreover the combination of higher auxin and was found to be more effective for callus induction.

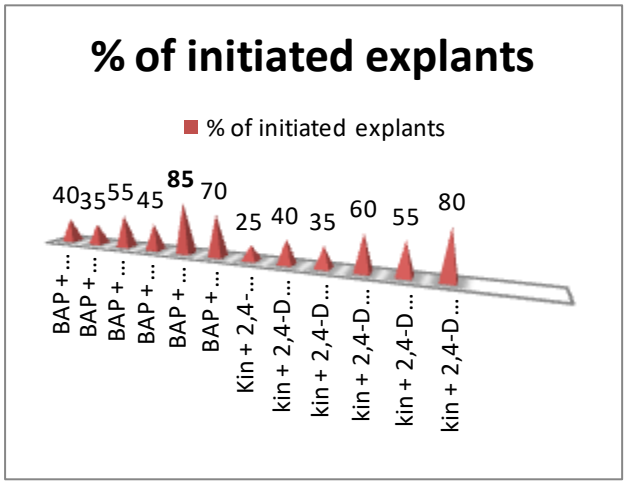

Fig 1: Percent of initiated shoot tip in BAP + NAA and Kin + 2, 4-D in different concentration

In this study organogenic callus induction was achieved from explants by using the above said concentration. But in other cases, the concentration of phytochormones varies for the induction of organogenic callus depending on the explants. Shoot tip of Kober 5BB grapevine rootstock was encapsulated by Benelli, 2016. Thakur *et al, 2015* worked on *Asparagus racemosus* willd, Rai *et al,* 2008 and many other researchers worked on encapsulation of different plant species because it gives high viability rate of plant after storing at cool temperature for long or short time period. MS medium was supplemented with different concentration of Kin + 2, 4-D for callus induction of *Diplocyclos palmatus*.

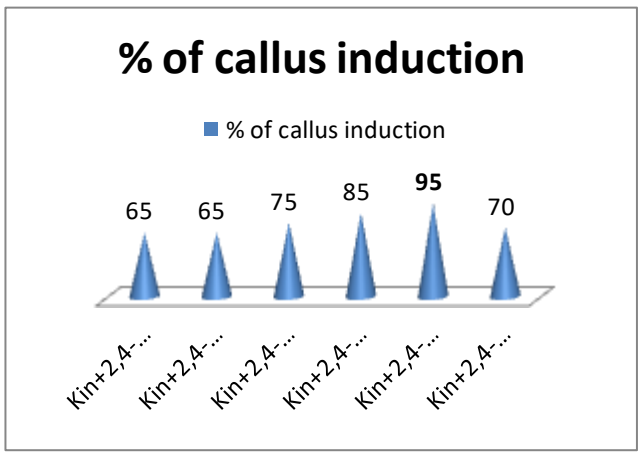

Fig. 2: Percent of callus induction from leaf explants with growth hormone Kin + 2,4-D (2.5+1)

Table 3: Callus induction from leaf explants with growth hormone Kin + 2,4-D (2.5+1)

Kin + 2,4-D (mg/l)	% of callus induction	No. of response Mean ± SD
0.5 + 0.5	65	1.83 ±1.54
1 + 0.5	65	2 ±0.89
1.5 + 0.5	75	2.17 ± 1.54
2 + 1	85	2 ± 1.55
2.5 + 1	95	3.17 ±1.25
3 + 1	70	2.17 ± 1.08

Same plant growth hormone combination was taken by Supe, 2018 for leaf explants of *Bryonia laciniosa*. This variation in the callogenesis may be due to the explant dependent response as shown in Fig.3 Regeneration frequency was very low at the lower concentration treatments. Higher concentrations of auxin reduced the shoot regeneration frequency. The increase and decrease in regeneration frequency is attributed to the cytokinin concentrations. Proliferation of leaves and their results are showed in Table 3. After 4 weeks combination of Kin and 2, 4-D showed higher response in concentration 2.5+1 and 2+1as shown in Fig. 2.

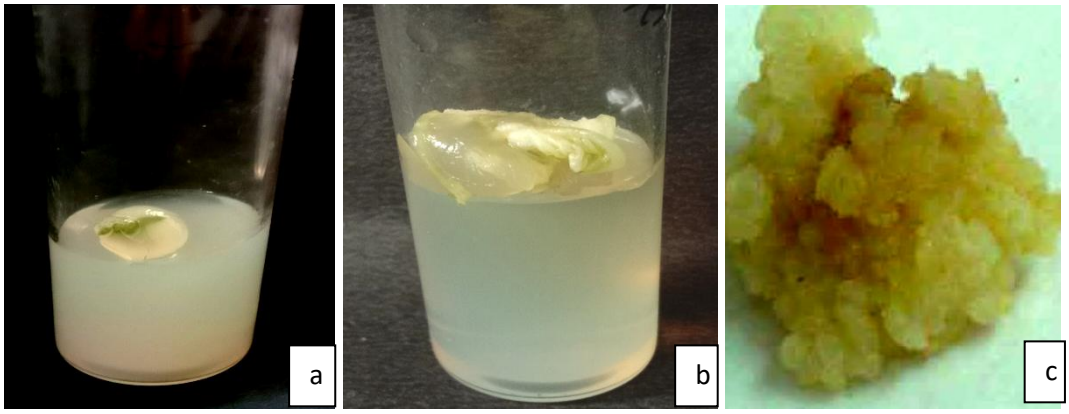

Fig. 3: Encapsulated shoot tip and callus induction in MS medium

a). Inoculated Bead in MS medium, b). Initiation of encapsulated shoot tip (Bead), c). Callus induction via leaf explant

CONCLUSION

In plant tissue culture, encapsulation making isodiametric beads by covering shoot tips through protecting elements such as sodium alginate and calcium chloride and storing them at cool temperature like 4℃ and when needed can be culture in culture medium. This technique may increase survival rate of explants. Callus induction using leaf explant or any other explants are intermediate phase for growth of full plant. Callus can be cultured in MS medium for mass production. These methods are being used to conserve plant germplasm. For regeneration different plant growth hormones in different concentration can be used.

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