https://africanjournalofbiomedicalresearch.com/index.php/AJBR
Afr. J. Biomed. Res. Vol. 28(3s) (April 2025); 42-47
Research Article

Callus Formation from Encapsulated Explants of Plant *Diplocyclos Palmatus* (Shivlingi)

Nikita Mishra[1], Ujjwala Supe[2*], Hemshankar Sahu[3]

[1]Plant tissue culture Laboratory, Department of Biotechnology, St. Thomas College, Bhilai, Chhattisgarh.
[2*]Plant tissue culture Laboratory, Department of Biotechnology, St. Thomas College, Bhilai, Chhattisgarh.
[3]Plant tissue culture Laboratory, Department of Biotechnology, St. Thomas College, Bhilai, Chhattisgarh.

***Author for Corresponding:** **Email: ujsupe@gmail.com*

Abstract- Conservation of plants through different methods is being done from many years because many important plants are getting reduced due to several reasons. *Diplocyclos palmatus* (Shivlingi) is a medicinal herb. To conserve Shivlingi, encapsulation and callus induction technique was performed. Encapsulation technique was done by preparing beads of explants like shoot tip and nodal segments by using different concentration of Sodium alginate and calcium chloride. Combination of 4% sodium alginate, 100 mM calcium chloride showed best initiation. Callus induction from encapsulated explants was done by preparing MS medium containing different concentration of BAP + NAA and control medium.

Keywords- Callus, Encapsulation, Shivlingi, MS medium, Conservation.

Author of correspondence: Email: ujsupe@gmail.com

Received- 4/03/2025 *Acceptance- 7/03/2025*

DOI: https://doi.org/10.53555/AJBR.v28i3S.7352

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).

Cultivation of medicinal plants especially high value medicinal plants is creating new dimension in the field of agriculture. Indian herbal industry is at blooming stage; however, cultivation of medicinal plant is not so easy. It is challenging task because less is known about their seed biology. Due to its therapeutic activity and diversified uses, demand of this plant is increasing in Indian and international market. Its seeds have poor germination percentage (5-11%), low viability and long dormancy period. Due to large scale and indiscriminate collection of its parts for gainful trade and insufficient attempts either to allow its replenishment or its cultivation. There is a greater need for commercial cultivation of this species is due to overwhelming use of this plant, nowadays this plant is in endangered condition (Vijaya Shalini *et al*., 2016). 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. Encapsulation technique is a most effective and convenient method to conserve plants. Sodium alginate and calcium chloride are mostly used as gelling agent and complex agent respectively. After successfully development of encapsulated beads regeneration can be done after short term cold storage (4°C). 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 encapsulated explants and this is performed for both encapsulated shoot tip and nodal segments.

MATERIALS AND METHODS

Explants collection and preparation

Young fresh shoot tip and nodal segment were collected from 4 month old plant. The mother plants were maintained in the St. Thomas College, Bhilai, Chattisgarh. Shoottip and nodal segments were excised 2.5 to 5 mm from mother plant. Young Shoot tips and nodal segments were washed under the running tap water thoroughly then again washed with distilled water for three times. For surface sterilization 0.1% mercury chloride and ethanol (70%) were used for 10 minute and 1min respectively. The sterilized explants were washed with sterilized distilled water for three times to remove excess traces of mercury chloride and ethanol. The explants were blotted on sterilized filter paper and inoculated in the Plant tissue culture medium for encapsulation and callus induction.

Encapsulation and storage

Sodium alginate with different concentration 1%, 2%, 3%, 4% , 5% and Calcium chloride with 50, 75, 100, 125, 150 mM were prepared for encapsulation in MS medium (Murashige and Skoog 1962) and autoclaved at 120°C for 15-20 min. Shoot tip and nodal segment were dipped in cooled sterilized sodium alginate and then dropped in chilled calcium chloride for the preparation of encapsulated beads. The beads were kept for incubation for 30 minutes for hardening.

Culture media and Regeneration

Culture medium was prepared by using Murashige and Skoog (1962) medium. pH of the medium was adjusted to 5.8. and for solidification 0.8% agar(Hi-media) 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°C ± 2. After 15-20 days callus formation and shoot initiation was started. 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 BAP + NAA) was prepared. The encapsulated explants were washed with sterilized distilled water and were inoculated in medium. The cultures were maintained at 25°C ± 2 in continuous darkperiod. After 20 days every culture medium showed callus induction.

RESULT AND DISCUSSION

In the present investigation, explants obtained from *in vivo* maintained plants were used for the encapsulation because somatic embryogenesis has not yet been documented in *Diplocyclos palmatus*. Shoot tips and nodal segments contain meristematic tissues with high capacity for cell division, thus synthetic seed developed from shoot tips generally yield good response than other non-embryogenic vegetative propagules. The reason for such a difference in response is not clear, but it may be related to mitotic activity being greater in the meristem of the shoot tips than in nodal segments, which are subject to apical dominance (Ballester et al., 1997). Encapsulation of plant explants in calcium alginate has also been reported in a wide range of plant species.

Various concentration of sodium alginate were used for encapsulation of *Diplocyclos palmatus* plant material. Explants that were encapsulated in 4% sodium alginate showed the highest regeneration percentage (**Table-l**) compared to other concentration of alginate. The concentration of alginic acid also influenced the frequency of conversion due to the hardness factor of the beads. At a higher percentage of sodium alginate (5%), beads were harder which may have suppressed the ability of shoots and roots to emerge. Beads of uniform size and shape were obtained when using 4 % sodium alginate. Similar reports of maximum plantlet regeneration were by many researchers.

Table 1-Effect of different concentrations of alginate on regeneration

Concentration of alginate matrix	% Regeneration	Average Frequency of plantlet regeneration
1%	17	32.22 ± 0.02
2%	25	36.25 ± 0.03
3%	49	43.85 ± 0.07
4%	70	57.92 ± 0.04
5%	54	21.01 ± 0.02

Relative growth rate of explants were increased significantly on increasing calcium chloride concentration to the growth medium from 50 mM to 100 mM at which growth rate reached to its maximum (55.67) followed by 42.83 at 125 mM, but at high concentration of 150 mM relative growth rate decreased to 17.60 that was almost equal to 20%, the relative growth rate at concentration of 50mM $CaCl_2$. Least relative growth rate was recorded at 150 mM concentration as shown is in Table 2.

Table 2-Effect of different concentrations of calcium chloride on regeneration

Concenrtation of CaCl2	% Regeneration	Average Frequency of plantlet regeneration
50 mM	20	20.05 ± 0.03
75 mM	35	34.12 ± 0.07
100 mM	50	55.67 ± 0.06
125 mM	89	42.83 ± 0.02
150 mM	46	17.60 ± 0.01

Callus fresh matter accumulation was proportional to the concentration of calcium nutrient in the growth medium. The results of this study are similar to those reported by Frett & Dirr (1986) working with *Petunia hybrida*. Those authors attributed that calcium effect on callogenesis to the decrease in lignifications of the cell wall caused by low level of calcium which facilitates callus initiation and growth *in vitro*. As the data reveals that increasing calcium chloride to the growth medium from 50 mM to 150 mM significantly increased the relative growth rate of calli. This might be due to Ca/pectate interaction as a regulator of growth that dominates the requirement for calcium ions, and as a factor involved in the control of cell growth. Current results are also in agreement with the findings of Brewbaker & Kwack (1963), and Mascarenhas & Machlis (1964), who found a compelling interaction between calcium ion, the cell wall and cell growth and reported that calcium must be present. Callus fresh matter accumulation was proportional to the concentration of calcium nutrient in the growth medium.

Increased growth rate of calli may be the result of calcium effect on plant hormone activity that affect growth *in vitro* as well, as action of each plant growth hormone can be altered by calcium salt. Further increasing of calcium chloride to the growth medium from 50 mM to 150 mM caused maximum increase in growth rate of calli. The present study results are in agreement with the research work of Arvin et al., (2005), who reported that the conventional calcium concentration in MS basal medium is not enough for regeneration.

The current findings further stated that 100 mM calcium chloride in the growth medium caused a significant reduction in the relative growth rate of calli that showed toxic effect of high Ca content on calli growth. High calcium negatively affects the growth of calli. Such typical calcifugic behavior might be related to insufficient compartmentation or physiological inactivation of calcium (precipitation of calcium oxalate). Ahmad et al., (2009) also determined the relationship between callus growth and mineral nutrients uptake in salt-stressed. The current findings further reveal that 9mM calcium chloride in the growth medium caused a significant reduction in the relative growth rate of calli that showed toxic effect of high Ca content on calli growth. High calcium negatively affects the growth of calli. Such typical calcifugic behavior might be related to insufficient compartmentation or physiological inactivation of calcium (precipitation of calcium oxalate)

Sodium alginate and calcium chloride play an important role in gel matrix formation and gel omplexation and capsule hardiness depends upon ion exchange of Sodium and Calcium ions.

The encapsulated explants (Fig. I A) showed signs of germination 8-10 days after culture. The alginate matrix ruptured and callus was emerged from the capsule (Fig. IB). The callus grew vigorously. Different basal media were tried to increase the germination rate of the encapsulated explants (Table 3). Maximum germination was obtained on MS basal medium alone.

Significant differences were observed in regeneration from encapsulated explants MS media with different concentration of auxin and cytokinin. This may be due to the nature of alginate or physical handling of encapsulated explants. The use of full-strength MS basal medium for conversion, also gave the highest conversion frequency of artificial seeds in *Solanum melongena* (Lakshmana Rao and Singh 1991) and

sandalwood (Bapat and Rao 1983).

Conversion of encapsulated shoot tips and nodes was significantly ($P < 0.05$) affected by MS medium formulation. Callus emerged after 1–2 weeks whereas shoots (Fig. 1C and D) were visible after 3–4 weeks. The reason for such a difference in response is not clear, but it may be due to more requirements of major and minor salts, iron and organics for the growth and conversion. Similar to our observation, Singh et al. (2006) also found maximum percentage response for conversion on full strength MS medium without plant growth regulator in *P. amarus*. Recently, a few workers have attempted liquid medium for the conversion of synthetic seeds to plantlets (Rai et al., 2008; Singh et al., 2009). In contrast to those reports, there was no significant ($P < 0.05$) difference observed on conversion between full-strength agar-solidified and liquid MS medium in the present investigation. Encapsulated shoot tip and nodes were inoculated in MS medium supplemented with different concentration of BAP + NAA. Proliferation of explants and their performance were shown inthe table 3 & 4. About 90% regeneration was observed on medium supplemented with BAP, however, BAP inhibited rooting in emerged shoots from encapsulated shoot tips resulting that none of the explants converted into plantlets. Similar result was also observed in *Spilanthes acmella* (Singh et al., 2009).

Table 3: Effect of sowing medium formulation on conversion and on shoot length of the plantlets for Encapsulated shoot tips)

MS basal Medium			
BAP (mg/l)	NAA (mg/l)	% of conversion	Shoot length
0.5	0.5	15	1.3 ± 0.04
1.0	0.5	17	2.3 ± 0.17
1.5	0.5	22	2.5 ± 0.03
2.0	1.0	43	2.7 ± 0.12
2.5	1.0	47	3.0 ± 0.06
3.0	**1.0**	**65**	**3.3** ± 0.08
3.5	1.5	53	2.5 ± 0.03
4.0	1.5	42	2.3 ± 0.01
4.5	1.5	38	1.2 ± 0.02
5.0	1.5	29	1.1 ± 0.09

Table 4: Effect of sowing medium formulation on conversion and on shoot length of the plantlets for Encapsulated Nodes)

MS basal Medium			
BAP (mg/l)	NAA (mg/l)	% of conversion	Shoot length
0.5	0.5	21	1.1 ± 0.14
1.0	0.5	37	1.2 ± 0.15
1.5	0.5	42	1.3 ± 0.02
2.0	1.0	46	1.6 ± 0.04
2.5	1.0	57	2.0 ± 0.08
3.0	**1.0**	**75**	**2.3** ± 0.04
3.5	1.5	67	1.9 ± 0.11
4.0	1.5	41	1.4 ± 0.06
4.5	1.5	39	1.2 ± 0.03
5.0	1.5	15	1.1 ± 0.02

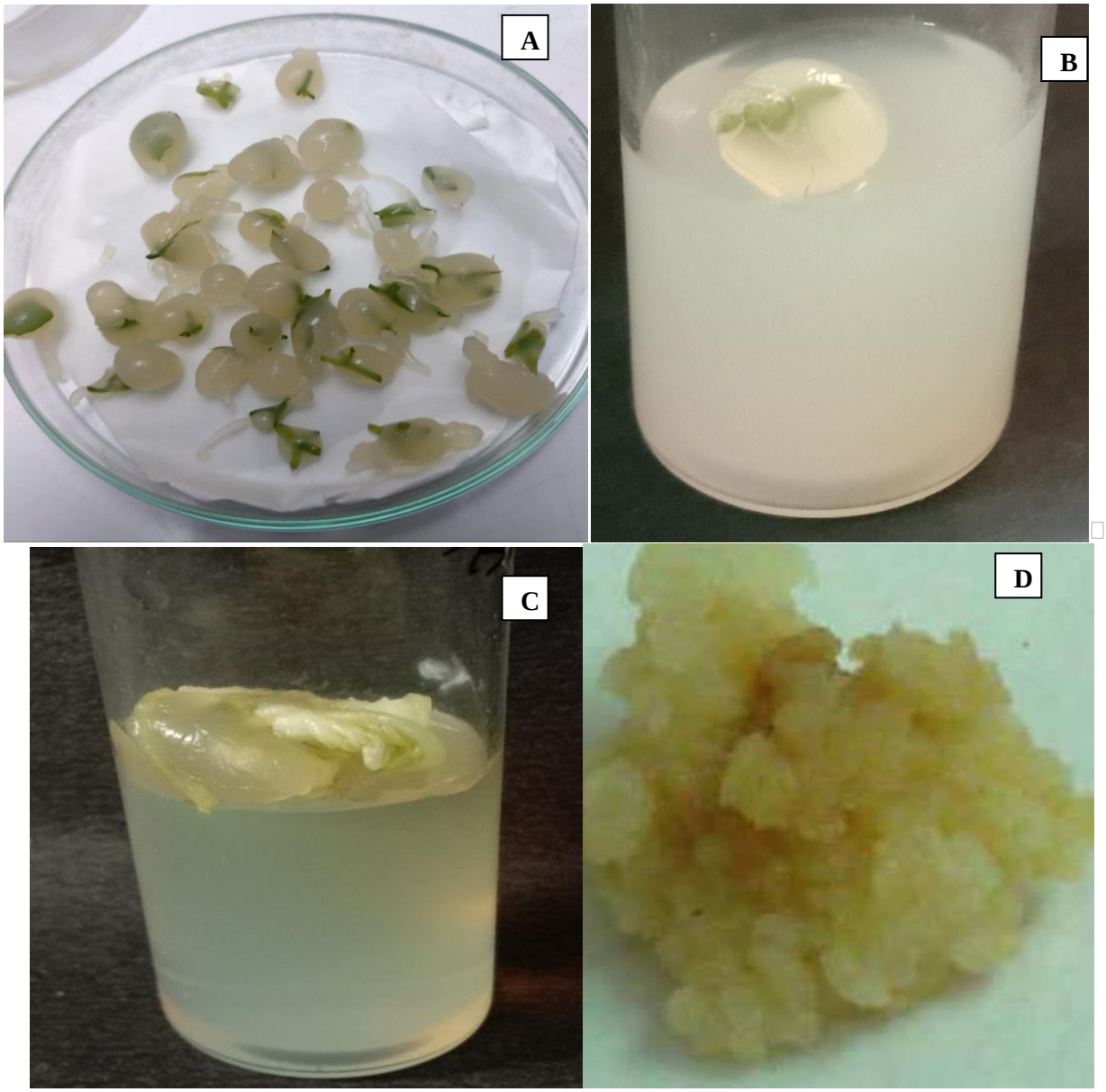

Figure 1. Plant Regeneration from encapsulated explants of *Diplocyclos palmatus* A- Encapsulated explants, B- Inoculated explants In MS Basal Medium with growth regulator, C- Regeneration of Encapsulated explants, D- Callus formation from encapsulated explants.

REFERENCES

1. Ahmad, M.S.A., F. Javed, S. Javed and A.K. Alvi. 2009. Relationship between callus growth and mineral nutrients uptake in salt-stressed Indica rice callus. J. Plant Nutr., 32: 382-394.
2. Arvin, M., A. Habib and J. Donnelly. 2005. Effect of calcium concentrations in medium on microtuberization of potato (Solanum tuberosum L.). Iranian J. Biotech., 3: 3.
3. Ballester A. , Janeiro, L.V. Vieitez A.M. 1997 Cold storage of shoot cultures and alginate encapsulation of shoot tips of *Camellia japonica* L. and *Camellia reticulata* Lindley Scientia Horticulturae. 71, (1–2), 67-70.
4. Brewbaker, J.L. and B.H. Kwack. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. Am. J. Bot., 50: 859-865.
5. Chauhan, M., Sharma, V., Himanchal., Kumar, D. (2018). A scientific review on shivlingi beej (*Bryonopsis laciniosa)*: *Bryonia laciniosa* Amystical ethno-medicine for infertility. *IOSR Journal of Applied Chemistry*, **11**(5), 40-44.
6. Chauhan, N. S., Dixit, V.K. (2010). Effect of *Bryonia laciniosa* seeds on sexual behavior of male rats. *International Journal of Impotence Research*, **22**, 190-195.
7. Chavhan, S. A., Wathurkar, R. N., Shinde, S. A. (2019). Phytopharmacognostic Review of *Bryonia laciniosa* (Shivlingi Beej). *International Journal of Pharmacognosy and Chinese Medicine*, **3**(3), 1-7.
8. Faisal, M., Anis, M. (2007). Regeneration of plants from alginate - encapsulated shoot of *Tylophora indica* (Burm.f.) Merrill, an endangered medicinal plant. *Journal of Horticultural Science & Biotechnology*, **82**, 351 - 354.
9. Frett, J.J. and M.A. Dirr. 1986. Effect of nitrogen

and calcium stock plant nutrition on Petunia hybrida leaf and anther explant growth In vitro. Sci. Hort., 28(3): 289-298.

10. Kumar, R.D., Jaya, K., Vishal, K. (2016). A review of *Bryonia laciniosa. International Journal of Advanced Research in Medical Pharmaceutical Science*, **1**(2), 23-26.
11. Lakshmana Rao, P. V. and Singh, B. 1991. **Plantlet regeneration from encapsulated somatic**. embryos of hybrid Solanum melongena L. Plant Cell Rep., 10: 7:11.
12. Mascarenhas, J.P. and L. Machlis. 1964. Chemotropic response of the pollen of Antirrhinum majus to calcium. Plant Physiol., 39(1): 70-77.
13. Mishra, N., Verma, P. (2018). Review: Tissue culture techniques in different plants of Chhattisgar. *International Journal of Advances in Science Engineering and Technology*, **6**(4), 112-118.
14. Misra, A., Shukla, P. K., Kumar, B., Niranjan, A., Rawat, AKS., Shriwastava, S.(2017).Simultaneous- HPLC quantification of phenolic acids in traditionally used ayurvedic herb *Diplocyclos palmatus* (L.) jeffery. *A Multifacetated Journal in The Field of Natural Products and Pharmacognosy*, **9**(4) 483-487.
15. Murashige, T and Skoog, F. (1962). A revised medium for rapid growth and bio assay with tobacco tissue culture. *Physiological Plantarum, Phytochemistry*, **16**, 1519-1522.
16. Rai, M. K., Jaiswal, V.S., Jaiswal, U. (2008). Encapsulation of shoot tips of guava (*Psidium guajava* L.*)* for short - term storage and germplasm exchange. *Scientia Horticulturae*, **118**(1), 33-38.
17. Rao P S & Bapat V A, 1993,Micropropagation of sandalwood and mulberry, in Synthetic seed technology, edited by M R Ahuja (Kluwer Academic Publishers, Dordrecht) 317.
18. Sarowar, S., Oh, H. Y., Hyung, N. I., Min, B. W., Harn, C. H., Yang, S. K., Ok, S. H. (2003). *In vitro* micropropagation of a cucurbita interspecific hybrid cultivar- a root stockplant. *Plant cell tissue and organ culture*, ***75***, 179-182.
19. Singh AK, Sharma M, Varshney R, Agarwal SS, Bansal KC (2006a) Plant regeneration from alginate-encapsulated shoot tips of Phyllanthus amarus Schum and Thonn, a medicinally important plant species. In Vitro Cell Dev Biol Plant 42:109–113.
20. Singh AK, Varshney R, Sharma M, Agarwal SS, Bansal KC (2006b) Regeneration of plants from alginate-encapsulated shoot tips of Withania somnifera (L.) Dunal, a medicinally important plant species. J Plant Physiol 163:220–223.
21. Singh S k., Rai, M.K., Asthana, P. (2009) Plant regeneration from alginate-encapsulated shoot tips of *Spilanthes acmella* (L.) Murr., a medicinally important and herbal pesticidal plant species. Acta Physiol Plant 31:649–653
22. Sud, K., & Sud, S. (2017). A Scientific review on shivlingi beej (*Bryonia laciniosa)* : A mystical ethno- medicine for infertility. *European Journal of Biomedical and Pharmaceutical Science*, **4**(8), 1098-1102.
23. Supe, U. (2015). Callogenesis, rhizogenesis and cell suspension culture of *Bryonia laciniosa*. *Research Journal of Biotechnology*, **10**(12), 64-69.
24. Supe, U. (2016). *In vitro* characteristics of callus induction of *Bryonia laciniosa* - A medicinal plant. *International Journal of Innovation in Engineering and Science*, **1**, 01-03.
25. Supe, U. (2018). Direct rhizogenesis, Phytochemical analysis and Antimicrobial activity of *Bryonia laciniosa*. *International Journal of Advances in Science Engineering and Technology*, **6(**1), 18-20.
26. Thakur, S., Tiwari, K. L., Jadhav, S.K. (2015). *In vitro* approaches for conservation of *Asparagus racemosus* willd. *In Vitro Cellular Developmental Biology*, ***51***(6), 619-625.
27. Vijaya Shalini, P., Anjanadevi, N., Abirami, P. (2016). Preliminary phytochemical screening and GC-MS profiling of an endangered medicinal plant *Bryonia laciniosa* L. *International Journal of Applied and Advanced Scientific Research*, **1**(1), 181-185.
28. Vijaya Shalini, P., Anjanadevi, N., Abirami, P., Sharmila, M. (2017). *In vitro* Propogation of endangered medicinal plant *Bryonia laciniosa* L. through leaf explant. *International Journal of Current Research and Modern Education*, **2**(1), 33-36.