

Effect of Different Carbon Sources and Elicitors on Shoot Multiplication in Accessions of *Centella asiatica*

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Abstract

Centella asiatica is a medicinal herb which has been extensively used in the treatment of nervous disorders and skin diseases. It is distributed throughout the tropical and sub-tropical region of the world. This plant contains various bioactive constituents such as asiaticoside, madecassic acid, flavonoids etc. *Centella* is in huge demand in International market for the preparation of pharmaceuticals and cosmetics which has resulted in its overexploitation. This situation demands the use of biotechnological methods to conserve as well as increase the production of biomass and bioactive compounds. The present study focused the use of different carbon sources (sucrose and fructose) and elicitors (Malt extract, Salicylic acid and Jasmonic acid) for the enhancement of biomass in five accessions of *Centella asiatica*. It was concluded that Murashige and Skoog (MS) medium supplemented with 1 mg/L concentration of Malt extract and 1.5 mg/L concentration of 6-Benzyl amino purine (BAP) showed highest biomass production among all elicitors. Among two carbon sources, MS media supplemented with Sucrose (3 mg/L) along with BAP (1.5 mg/L) showed better result than fructose.

Keywords: *Centella asiatica*; Carbon sources; Elicitors; Sucrose; Fructose; Malt extract; Salicylic acid; Jasmonic acid

Introduction

Centella asiatica is a perennial herb belonging to the family Apiaceae and found throughout tropical and sub-tropical regions of India. This plant is known by different names in different regions of India, Manimunni in Assam, Thankuni in Bengali, Vallari in Decan, Mandookaparni in Hindi and Gotukola in Sinhali [1]. It is a softly perfumed, creeping plant that attains height up to 15 cm. Stem is smooth and rooting occurs at the nodes. It grows extensively in marshy, damp and wet places and flowering occurs during April to June with white to purple or pink flowers. Fruits are approximately 2 inches long, globular in shape and strongly thickened pericarp [2]. This plant has been used since ancient days in Ayurvedic medicines. Use of this plant has been described in the ancient literature Charaka Chikitsa [3]. Whole plant is used for the preparation of drug and these drugs can be used internally or externally. Paste from fresh herbs can be used externally for the treatment of rheumatism and elephantitis. Leaf juice can be applied on forehead in case of headache. An ointment prepared from this plant can be used for the treatment of leprosy or any skin disorder [3]. One of the bioactive compound i.e., asiaticoside helps in collagen I synthesis in human [4]. Asiaticoside shows antitumor activity by apoptosis of cancer cells. This plant can also be used for the treatment of eczema. Internally, it can also be used for the treatment of various diseases such as bronchitis, asthma, gastric, leucorrhea, kidney disease, urethritis and dropsy [5]. *Centella asiatica* contain various types of bioactive compounds like Asiaticoside, Madecassoside, Brahmoside, Alkaloids (Hydrocotylin), triterpene glycoside, Triterpene acid, Anthrone of Asiaticoside, Asiatic acid, Madegascariac or madecassic acid Isothankuniside, Brahmie acid, Centelloside, Centic acid, Indocentoic acid, Indocentelloside, Oligosaccharide, etc. [6,7]. The plant also contains volatile and fatty oils. Glycosides of palmitic, stearic, oleic and linolenic acids are present in fatty oils.

Due to great medicinal importance of *Centella asiatica* in the preparation of different pharmaceutical compounds, this plant has been overexploited and marked as threatened plant species by the International Union for Conservation of Nature and Natural Resources (IUCN), and also as an endangered plant species [8,9].

Thus conservation of this plant is need of the hour. Biotechnological methods not only help in the conservation of endangered plants but also help in the enhancement of biomass and commercially important plant products.

Mass propagation of plants through *in vitro* micro propagation is the most successful commercial application of plant tissue culture technique. *In vitro* propagation may provide a potential solution for cloning of plant and secondary metabolite production. Application of elicitors is one of the most important method to increase the production of secondary metabolites in plants. Elicitors are the molecule that enhances the secondary metabolite production under stress [10]. In plant tissue culture, plants are grown under stress condition but the plant genome has the defense mechanism to protect the plant via some secondary metabolite production. Elicitors are the signal compound of plant defense mechanism [11]. It was reported that elicitors enhance bioaccumulation of asiaticoside in *C. asiatica* [12]. Sugars also have important role in metabolic activity [13] as they enhance the formation of auxiliary buds and branching of adventitious roots [14]. But the above mentioned effects are not universal and depend on the geographical conditions of plant to a great extent. Plant of same species is collected from different locations and is assigned an accession number. Accessions differ in their growth pattern and secondary metabolite content. Therefore the present study was undertaken to determine the effect of different carbon sources like sucrose and fructose and different elicitors, like Malt extract (ME), Salicylic acid (SA), Jasmonic acid (JA) on growth of five different accession of *Centella asiatica* and to assess the response of different accession to variable conditions of carbon sources and elicitors.

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Received April 19, 2016; Accepted May 20, 2016; Published May 23, 2016

Citation: Kundu K, Roy A, Saxena G, Kumar L, Bharadvaja N (2016) Effect of Different Carbon Sources and Elicitors on Shoot Multiplication in Accessions of *Centella asiatica*. Med Aromat Plants 5: 251. doi:[10.4172/2167-0412.1000251](https://doi.org/10.4172/2167-0412.1000251)

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Materials and Methods

Cultures of five different accessions i.e., 281374, 383913, 342109, 347492, 331514 of *C. asiatica* were collected from NBPGR New Delhi, India. To study the effect of carbon source and elicitors on shoot multiplication MS media was used. Murashige and Skoog (MS) [15] media was prepared and pH was adjusted to 5.8 using 1 N HCl or 1 N NaOH solution. 0.8% plant agar was used to solidify the media. Sterilization of the media was done by autoclaving for 20 min at 121°C and 15 lb pressure. For experiment on elicitors and carbon sources, MS media was supplemented with 1.5 mg/L 6- Benzyl amino purine along with different elicitors {Malt extract (1 mg/L), Salicylic acid (1 mg/L), Jasmonic acid (1 mg/L)} and different carbon sources {Sucrose (3%) and fructose (3%)} separately. After the solidification of media, sterile shoot nodes from each accession were inoculated in culture tubes (25 × 150 mm) separately. The cultures were incubated at 26 ± 2°C under 16 h photoperiod and light intensity of 3000 lux for four weeks. Each and every experiment was done in triplicates. Visual data was recorded after 4th week of inoculation in terms of number of shoots and length of shoots for *in vitro* growth measurement.

Data analysis

Observations were recorded and are presented as means ± standard deviation of 3 biological replicates to estimate the variability between the accessions.

Results and Discussion

Effect of carbon source and elicitors on shoot regeneration was observed in five accessions of *Centella asiatica*. Observation was recorded as mean value of triplicate samples after four weeks of inoculation (Tables 1 and 2). Among the elicitors used, MS media supplemented with malt extract (1 mg/ltr) showed maximum number and maximum length of shoots (Figure 1). Maximum effect of malt extract was found on accession no. 281374 as maximum number of shoots i.e., 25 were reported in this accession followed by 342109 (16), 331514 (15), 347492 (14) and 383913 (10). Maximum average length was reported in accession number 347492 (3.07) followed by 281374 (2.35), 342109 (1.69), 331514 (1.75), 383913 (1.5).

Two different carbohydrate sources i.e., sucrose and fructose were tested for their potential on shoot regeneration in five different accession of *Centella asiatica* and it was found that sucrose showed better shoot growth than fructose in different accession of *Centella asiatica* (Figure 2). Maximum number of shoots generation was recorded in MS media supplemented with Sucrose (3%). In case of sucrose as carbon source, maximum number of shoots was reported in accession number 347492 (16) followed by 331514 (10), 281374 (8), 342109 (5) and 383913 (3). Maximum average length was reported in accession number 347492 (1.66).

Order of other accession's average length of shoots in sucrose experiment is 281374 (1.5) and 331514 (1.5), 342109 (0.75),

S No	Accession Number	Treatment	No. of shoots (M ± SE)	Length of shoot (cm.) (M ± SE)
1	Accession No. 281374	MS+BAP (1.5 mg/ltr)+ME (1 mg/ltr)	25 ± 1.52	2.35 ± 0.12
		MS+BAP (1.5 mg/ltr)+SA (1 mg/ltr)	4 ± 1.15	1 ± 0.08
		MS+BAP (1.5 mg/ltr)+JA (1 mg/ltr)	2 ± 1	0.75 ± 0.12
2	Accession No. 383913	MS+BAP (1.5 mg/ltr)+ME (1 mg/ltr)	10 ± 0.57	1.3 ± 0.05
		MS+BAP (1.5 mg/ltr)+SA (1 mg/ltr)	1 ± 0.33	1.5 ± 0.12
		MS+BAP (1.5 mg/ltr)+JA (1 mg/ltr)	1 ± 0	0.5 ± 0.05
3	Accession No. 342109	MS+BAP (1.5 mg/ltr)+ME (1 mg/ltr)	16 ± 3.46	1.69 ± 0.09
		MS+BAP (1.5 mg/ltr)+SA (1 mg/ltr)	1 ± 0	2.5 ± 0.28
		MS+BAP (1.5 mg/ltr)+JA (1 mg/ltr)	2 ± 0.57	0.75 ± 0.07
4	Accession No. 347492	MS+BAP (1.5 mg/ltr)+ME (1 mg/ltr)	14 ± 1.15	3.07 ± 0.12
		MS+BAP (1.5 mg/ltr)+SA (1 mg/ltr)	5 ± 1.15	0.9 ± 0.10
		MS+BAP (1.5 mg/ltr)+JA (1 mg/ltr)	5 ± 1.52	0.52 ± 0.03
5	Accession No. 331514	MS+BAP (1.5 mg/ltr)+ME (1 mg/ltr)	15 ± 1.15	1.43 ± 0.07
		MS+BAP (1.5 mg/ltr)+SA (1 mg/ltr)	8 ± 2	1.75 ± 0.07
		MS+BAP (1.5 mg/ltr)+JA (1 mg/ltr)	12 ± 2.88	1.66 ± 0.07

Table 1: Effect of different elicitors on number and length of regenerated shoots in five different accession of *Centella asiatica* after four weeks of inoculation. Values are expressed as mean ± Standard Error (M ± SE). MS: Murashige and Skoog medium; BAP: 6-Benzyl amino purine; ME: Malt Extract; SA: Salicylic acid; JA: Jasmonic acid.

S No	Accession Number	Treatment	No. of shoots (M ± SE)	Length of shoot (cm) (M ± SE)
1	Accession No. 281374	MS+Sucrose+BAP (1.5 mg/ltr)	8 ± 1.15	1 ± 0.06
		MS+Fructose+BAP (1.5 mg/ltr)	2 ± 0.57	1.5 ± 0.16
2	Accession No. 383913	MS+Sucrose+BAP (1.5 mg/ltr)	3 ± 0.57	0.27 ± 0.04
		MS+Fructose+BAP (1.5 mg/ltr)	2 ± 0.57	0.75 ± 0.08
3	Accession No. 342109	MS+Sucrose+BAP (1.5 mg/ltr)	5 ± 1.2	0.52 ± 0.02
		MS+Fructose+BAP (1.5 mg/ltr)	2 ± 0.57	0.75 ± 0.10
4	Accession No. 347492	MS+Sucrose+BAP (1.5 mg/ltr)	16 ± 3.05	1.66 ± 0.15
		MS+Fructose+BAP (1.5 mg/ltr)	5 ± 0.57	0.7 ± 0.05
5	Accession No. 331514	MS+Sucrose+BAP (1.5 mg/ltr)	10 ± 1.15	1.5 ± 0.08
		MS+Fructose+BAP (1.5 mg/ltr)	3 ± 0.57	1 ± 0.09

Table 2: Effect of different carbon sources on number and length of regenerated shoots in five different accession of *Centella asiatica* after four weeks of inoculation. Values are expressed as mean ± Standard Error (M ± SE). MS: Murashige and Skoog medium; BAP: 6-Benzyl amino purine.

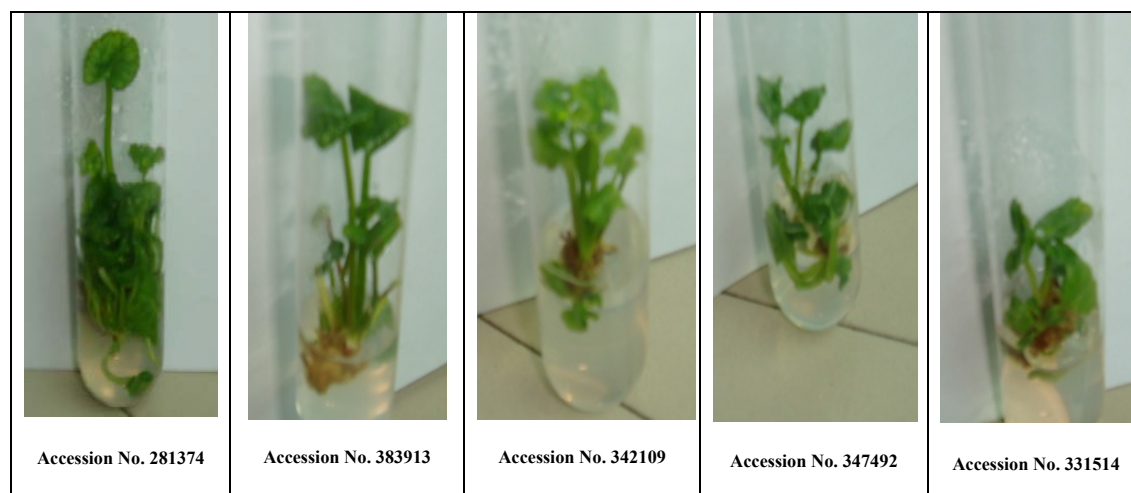


Figure 1: *In vitro* culture of five different accession of *Centella asiatica* in malt extract (After four weeks of inoculation).

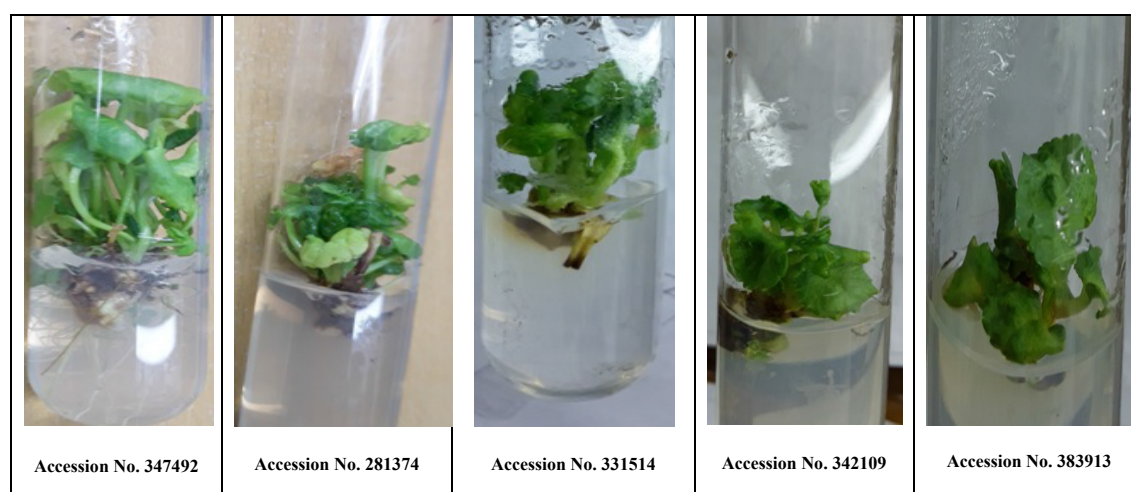


Figure 2: *In vitro* culture of five different accession of *Centella asiatica* in sucrose (After four weeks of inoculation).

383913(0.75). These parameters can be used for maximum production of biomass and bioactive compounds. Focus can be drawn on high yielding accessions for secondary metabolite production. Hossain et al. [16] also reported similar results in *C. asiatica*. They concluded that sucrose showed best results as it helped in the production of maximum shoot length in presence of BA and Kinetin.

Conclusion

From the above study, it can be concluded that different carbon sources and elicitors have significant effect on the shoot formation of *Centella asiatica* as they have the capacity to enhance shoot multiplication as well as shoot length which ultimately increases the growth of the plant. Among the carbon source, sucrose showed better capability to enhance the growth of *Centella asiatica* than fructose. Accession no. 347492 showed maximum number of shoots in MS media containing sucrose. Malt extract showed the highest number of shoots generation among the other elicitors used. Accession no. 281374 showed maximum number of shoots regeneration in MS media containing Malt extract. Shoot proliferation can be used for production

of important bioactive compound of this plant i.e., asiaticoside. This study not only finalized the appropriate elicitors and carbon source but also the high yielding accession of *Centella asiatica*. Further study will include the quantitative and qualitative analysis of various secondary metabolites like saponins, terpenoids, and alkaloids extracted from *Centella asiatica*.

Acknowledgements

The authors are grateful to the Department of Biotechnology, Delhi Technological University, Delhi for extending all the laboratory facilities towards successful completion of this investigation and National Bureau of Plant Genetic Resources for providing the plant materials.

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