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Screening of Different Carbon Sources for *in vitro* Shoot Production of *Asparagus racemosus* Willd

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Abstract: Effect of different sugars on *in vitro* shoot multiplication of *Asparagus racemosus* was investigated. Six different carbon sources were incorporated separately in 6-benzyladenine containing Murashige and Skoog medium. Two of these carbon sources were non-synthetic ones- coconut milk and de-oiled rice bran. The de-oiled rice bran was utilized first time in plant tissue culture. Both the sources- coconut milk and de-oiled rice bran supported good number of shoot production, equivalent to lactose, dextrose and maltose. However, minimum shoot elongation was observed in the de-oiled rice bran containing medium. Shoot multiplication rate as well as shoot elongation were significantly high in the sucrose containing medium in comparison to all the other tested carbon sources.

Key words: Carbohydrates · Shoot multiplication · Rice bran · Sucrose · Micropropagation

INTRODUCTION

In vitro culture of plant cell, tissue and organ is greatly influenced by the composition of nutrient medium. One of the important component of nutrient medium is carbohydrate that strongly affect the growth and morphogenesis of *in vitro* cultured plant as it acts as a carbon source and osmoticum both [1]. *In vitro* cultures require sugars due to the observed phenomenon of heterotrophy of cultured cells, to replace the carbon, which is fixed from atmosphere during photosynthesis *in vivo* [2, 3]. Among the sugars, sucrose is most popular carbon source used in plant tissue culture. However, other carbon sources have also been reported to enhance the growth of different plant species [4, 5]. The aim of this study was to optimize the best carbon source for efficient shoot multiplication of *Asparagus racemous*, a valuable medicinal plant.

A. racemosus Willd, a member of Asparagaeceae family, is an important medicinal plant distributed in tropical and subtropical parts of India. The plant could be considered as female friendly herb due to its wide use in treatment of menstrual related disorders and increasing lactation [6, 7]. The plant, commonly known as Shatavari, contains steroidal saponin (Shatavrin I-X) [8] which imparts phtyoestrogenic properties to it. Apart from this, it has also been reported to be used as immunomodulant, galactogauge, adaptogen, antitussive, anticarcenogenic, antioxidant and antidiarrhial [9-15] and thus present as major constituent in numerous Ayurvedic formulations. Therefore, it is included in the list of 32 nationally prioritized plants of India, by National Medicinal Plant Board of India [16]. Due to escalating demand natural population of plant is shrinking and thus regarded as vulnerable in its native habitat [17]. There are some reports available on the *in vitro* studies of *A. racemosus* [18, 19] but none of the researchers have studied the effects of different types of carbon sources. Therefore, the present study was undertaken with a view to optimize best carbon sources for *in vitro* shoot proliferation of *A. racemosus*.

MATERIALS AND METHODS

Collection of Explants: The single nodal segments were used for establishment of cultures collected from the plants of *A. racemosus* maintained in the greenhouse of School of Studies in Biotechnology, Pt. Ravishankar Shukla University, Raipur, C.G., India.

Establishment of *In vitro* Cultures: Nodal segments were washed thoroughly in running tap water and then divided into approx. 1.5 cm pieces. The explants were surface disinfected by treating with 0.1% mercuric chloride for 10

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minutes and washed three times with sterile distilled water under laminar air flow cabinet. Surface disinfested nodal explants were cut to final size approx. 1 cm long and were inoculated aseptically onto Murashige and Skoog medium [20] with 3% sucrose and 0.75% agar supplemented with 0.5 mg/L 6-Benzylaminopurine, BA. The pH of medium was adjusted to 5.8 before autoclaving at 121°C and 15lbs pressure for 20 minutes.

***In Vitro* Shoot Multiplication.** The *in vitro*-formed shoots after 4 weeks on initiation medium were excised from the nodal segment and cultured on semi-solid MS medium containing 0.25mg/L BA and 0.75% agar. To observe the effect of carbon sources on shoot multiplication MS medium was supplemented with different carbon sources (3%) viz. sucrose, dextrose, lactose, maltose, coconut milk and deoiled rice bran.

Culture Conditions: All cultures were maintained at 25°C ±2, under a 16-h photoperiod with a light intensity of approximately 2000 lux provided by cool, white fluorescent tubes. Explant establishment experiment was carried out in 25 × 150 mm glass tubes containing 15 ml medium each and 250 ml conical flasks containing 60 ml medium each were used for shoot proliferation studies. The number and length of shoots per culture were recorded after the 4 weeks of culture.

Data Analysis: In all the experiments, 10 replicates were taken in each treatment and each experiment was repeated thrice. The analysis of variance (ANOVA) appropriate for the design was carried out to detect the significance of differences among the treatment means and the treatment means were compared using Duncan's Multiple Range Test (DMRT) at a 5% probability level using software SPSS 10.0.

RESULTS

Six types of carbon sources were used for the shoot multiplication in MS + 0.25 mg/L BAP (Fig. 1 and 2). Out of six, two non-synthetic carbon sources were tested- coconut milk and rice bran. Coconut milk has been used as organic supplement in the different plant tissue culture media [21, 22]. On the other hand, rice bran is tested first time in plant tissue culture, which is a byproduct of rice mill. The de-oiled rice bran contains 43.12% carbohydrate, 12.6% protein, 21.13% fat, 5.59% crude fiber, 8.97% ash and 8.5% moisture [23]. Both the sources- coconut milk and rice bran supported very good rate of shoot

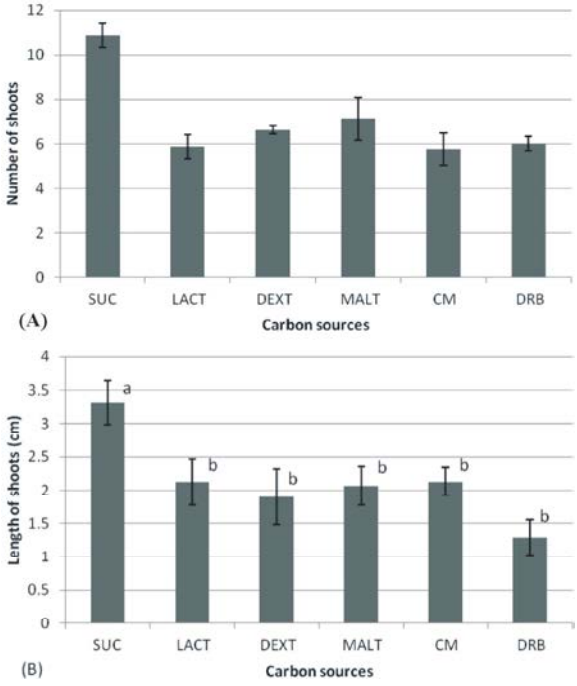

Fig. 1: Effect of different carbon sources on MS + 0.25 mg^{-L} (A) Shoot number (B) Shoot length of *in vitro* cultures of *A. racemosus*.
*Significance of difference among treatment means is analyzed by ANOVA.
(Shoot number) $df = 5$; $F=10.29$; $P<0.0001$
(Shoot length) $df = 5$; $F=4.46$; $P<0.005$
Values followed by the same letters with in the column are not significantly different at the 5% level (DMRT with SPSS 10.0).

production, approx 6 shoots per culture of *A. racemosus*. That is statistically at par of other tested synthetic sources except sucrose. Sucrose supported the production of significantly higher number of shoots compared to any other carbon source. Apart from number, proper shoot elongation is another important factor for shoot production. Minimum shoot length was recorded with the rice bran containing medium. In the presence of coconut milk, shoot length was statistically equivalent to other synthetic carbon sources except sucrose again; longest *in vitro* shoots were produced in the presence of sucrose.

DISCUSSION

Selection of carbon source that supports maximum growth of *in vitro* raised plant attains great importance. Numerous reports have been published in a number of

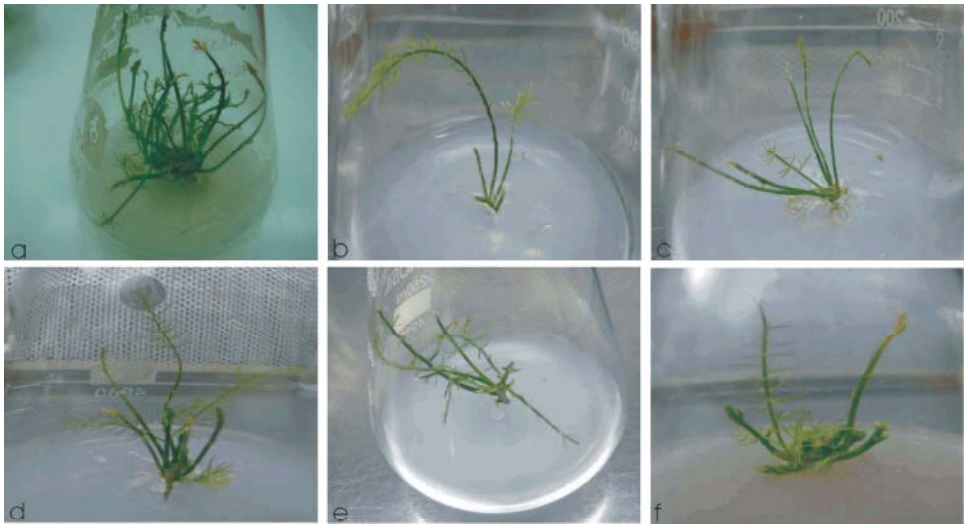

Fig. 2: Effect of different carbon sources on *in vitro* shoot proliferation of *A. racemosus* on MS + 0.25 mg^{-L} BA supplemented with 3% carbon source.
A. Sucrose, B. Lactose, C. Dextrose, D. Maltose, E. Coconut milk, F. Deoiled Rice bran.

plant species optimizing best carbon source. Many reports suggest that sucrose and glucose support maximum growth of the *in vitro* culture of Peach root stocks and Banana [24, 25] which is in accordance with our results. This may be probably because it is the most common carbohydrate in the phloem sap of many plants [26]. In coconut milk containing medium, the *in vitro* shoot production of *A. racemosus* was comparable to lactose, dextrose and maltose; although it was significantly lower than sucrose. Deoiled rice bran is found to be used as carbon source for many other processes eg. Bioethanol production [27]. For the first time it was used in plant tissue culture experiments. However, it was not found as efficient as other carbon sources particularly for shoot elongation but it may be supplemented along with other carbon sources in a cost effective manner.

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