



REGULAR ARTICLE

EFFECT OF DIFFERENT CARBON SOURCES ON *IN VITRO* MORPHOGENETIC RESPONSE OF PATCHOULI (*POGOSTEMON CABLIN BENTH.*)

M. Kumara Swamy^{1,2*}, K. M. Sudipta¹, S. Balasubramanya² and M. Anuradha^{1,2}

¹Padmashree Institute of Management and Sciences, Kommagatta, Kengeri, Bangalore- 560060

²Rishi Foundation, #234, 10th C main, 1st Block, Jayanagar, Bangalore- 560011

SUMMARY

The effect of various carbon sources, sucrose, glucose, fructose, table sugar and sugarcane juice was investigated on *in vitro* growth and physiology of *Pogostemon cablin* Benth. The entire morphogenetic pattern was influenced by nature and concentration of carbon source used. The maximum shoot length (4.87 ± 0.41 cm) and higher number of multiple shoots (61.43 ± 0.19) was observed on MS media fortified with 20% sugarcane juice. The maximum fresh weight of shoots was recorded on MS medium containing 2% sucrose (4.89 ± 0.19 g). Sugarcane juice at 20% resulted in maximum chlorophyll content (0.81 ± 2.0 mg/g tissue). The protein content was maximum on media supplemented with 20% sugarcane juice (18.8 ± 0.24 mg/ml) followed by 2% sucrose (18.5 ± 0.25 mg/ml). The least content was observed on media supplemented with 3% fructose (12.2 ± 0.32 mg/ml). Maximum accumulation of carbohydrate content was observed on MS supplemented with 3% sucrose (18.2 ± 0.30 mg/ml) and the least carbohydrate content (11.6 ± 0.53 mg/ml) was observed on MS media with 1% glucose. This is the first report on the use of sugarcane juice in tissue culture studies of patchouli.

Key words: Patchouli, Carbon sources, Sugarcane juice, Multiple shoots, Physiology

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*Corresponding Author, Email: swamy.bio@gmail.com

1. Introduction

Patchouli (*Pogostemon cablin* Benth.), belonging to the family Lamiaceae is an aromatic plant. The oil, obtained by steam distillation of shade dried leaves is commercially used in perfumes and cosmetics because of its strong fixative property. It possesses anti insecticidal activities, anti-fungal and bacteriostatic properties [1, 2]. In aromatherapy, it is used to calm nerves, relieve depression and stress. In Chinese medicine decoction from the leaves are used with other drugs to treat nausea, vomiting, diarrhea, cold and headaches [3]. Feasibility of mass propagation of high yielding and disease/pathogen resistant patchouli through tissue culture has been envisaged by several authors [1, 4, 5, 6 and 7].

The growth and multiplication of shoots *in vitro* are affected by many factors, one of which is the type of exogenous carbon source added to the medium [8]. The carbon sources serve as energy and osmotic agents to support the growth

of plant tissues [9]. There have been various opinions on the beneficial effects of various carbon sources (sucrose, fructose, glucose, table sugar etc.) on the growth of plants *in vitro*.

Sucrose (2-5%) is the most popular carbohydrate used in tissue culture [10]. In general most of the tissue culture studies are performed using sucrose as the sole carbon source due to its efficient uptake across the plasma membrane. Glucose also has been reported to have various effects on the *in vitro* growth of plants. Cunha and Fernandes-Ferreira (1999) on *Linum usitatissimum* showed that medium supplemented with monosaccharides (glucose or fructose) at concentrations of 4% gave consistently highly embryonic culture with higher somatic embryo frequencies and higher growth rate compared with medium supplemented with either sucrose or maltose [11]. The use of fructose is considered as an excellent source of carbohydrate for embryo culture [12].

Kaufman et al. (1962) and Dickinson (1966) used fructose as a good source for the culture of stem segments and pollen [13, 14]. However, use of fructose in the medium results in hyperhydricity which leads to low chlorophyll contents and abnormal nitrogen and sugar metabolism [15]. Table sugar is used as an alternative low cost medium component for *in vitro* micropropagation of potato [16]. It has been reported that in many plant species, addition of plant extracts/ juices of coconut, tomato, banana, orange, apple and yeast to the culture medium enhanced the growth of tissues [17, 18, 19 and 20].

It is evident that various carbon sources affect the growth of *in vitro* cultured plants differently. Therefore the present work was undertaken to study the effects of sucrose, fructose, glucose, table sugar and sugarcane juice on the growth of patchouli *in vitro*. Since the use of analytical grade sucrose contributes to the higher costs of media (34% of the production cost) [16], an attempt is made to reduce the cost of the medium by using low cost supplements like locally available table sugar and sugarcane juice.

2. Materials and Methods

Healthy patchouli plants grown in herbal garden of Rishi Herbal Technologies Pvt. Ltd., Bangalore were selected. Nodal segments and shoot tips of these selected plants were surface sterilized with 0.1% (w/v) HgCl_2 for 10 min and washed thoroughly with sterile distilled water. Later, the explants were implanted on Murashige and Skoog (MS) [21] medium supplemented with 0.5 mg/L 6-benzylaminopurine (BA). The pH of the medium was adjusted to 5.7 prior to autoclaving at 121°C for 20 min. Cultures were maintained at a temperature of 25±2°C under 16 h light/ 8 h dark photoperiod and sub cultured every 4 weeks. Multiple shoots regenerated on this medium were used for further studies. Established cultures were subjected to sub culturing in same media. Uniform proliferated shoots (4-5 cm in length) resulted from direct organogenesis were transferred to MS basal

medium supplemented with 0.5 mg/L BA and kinetin (KN). The media was further supplemented with different carbon sources viz., sucrose, glucose, fructose, commercially available table sugar (1, 2 and 3%) and sugarcane juice (10, 20 and 30%).

Data was taken on the following parameters; fresh shoot weight (g), number of shoots, shoot length (cm), number of roots and root length (cm). The total chlorophyll content of the regenerated plantlets was measured by following the method explained by Yadava [22] and was expressed in mg/g tissue. The total protein content of the regenerated plantlets was measured using standard Lowry's method [23] and was expressed in mg/ml. The total sugar content of the regenerated plantlets was measured using Anthrone method explained by Sadasivam and Manickam [24] and was expressed in mg/ml. The experiments were set up in completely randomized design with different treatments replicated thrice. Data recorded after 30 days of culture were subjected to Fisher's method of analysis of variance.

3. Results

The growth, multiplication rate and other physiological parameters were affected by type and concentration of carbon source used.

Number of shoots regenerated per explant

Sugar cane juice at 20% level is not only effective in elongating the shoots, but also regenerated higher number of multiple shoots (61.43±0.19). The next best rate of multiplication was recorded on MS medium supplemented with 2% sucrose (60.4±0.26) followed by 10% sugarcane juice (56.1±0.22). The use of table sugar at 2% showed 55.7±0.36 shoots while at 1% level resulted in 50.57±0.49 shoots per explant. However, at higher concentrations of carbon sources in the medium resulted in more of callus proliferation than shoot differentiation (Table 1). There was no significant difference in multiplication of shoots when glucose and fructose were used in the medium.

Table 1: Effect of carbon sources on *in vitro* shoot proliferation and growth of *Pogostemon cablin* Benth. after 30 days in MS medium supplemented with 0.5mg/L BA and 0.5mg/L KN.

Carbon sources		Number of multiple shoots \pm SD ^a	Shoot Length (cm) \pm SD ^a	Fresh weight of the shoots (g) \pm SD ^a	Callus formation
Sucrose	1%	45.00 \pm 0.78	3.57 \pm 0.15	3.10 \pm 0.17	-
	2%	60.40 \pm 0.26	4.57 \pm 0.22	4.89 \pm 0.19	-
	3%	30.13 \pm 0.67	2.43 \pm 0.34	3.30 \pm 0.07	+
Glucose	1%	32.50 \pm 0.95	3.27 \pm 0.44	2.73 \pm 0.10	-
	2%	36.50 \pm 0.43	3.63 \pm 0.04	2.80 \pm 0.38	-
	3%	36.00 \pm 0.38	3.20 \pm 0.08	3.73 \pm 0.10	+
Fructose	1%	32.10 \pm 0.94	2.87 \pm 0.39	2.67 \pm 0.14	-
	2%	32.63 \pm 0.13	3.30 \pm 0.10	3.40 \pm 0.13	+
	3%	35.53 \pm 0.53	2.77 \pm 0.29	3.43 \pm 0.11	+
Table sugar	1%	50.57 \pm 0.49	3.47 \pm 0.45	4.53 \pm 0.31	-
	2%	55.77 \pm 0.36	4.20 \pm 0.10	4.81 \pm 0.12	+
	3%	33.80 \pm 0.60	2.20 \pm 0.46	3.47 \pm 0.16	+
Sugarcane juice	10%	56.10 \pm 0.22	4.03 \pm 0.37	4.60 \pm 0.35	-
	20%	61.43 \pm 0.19	4.87 \pm 0.41	4.87 \pm 0.56	-
	30%	49.13 \pm 0.62	3.37 \pm 0.07	4.80 \pm 0.19	+
Control		-	-	-	
F- value		384*	5.66 *	9.08*	

CI at 95%

*Significant at 5% level

+: Callus induction, -: No callus

^a Data indicate mean \pm standard deviation. Ten replicates were used per treatments and experiment was repeated trice.

Mean length of the shoots

Shoots induced on MS media supplemented with 20% sugarcane juice resulted in maximum shoot length (4.87 \pm 0.41cm) when compared to other carbon sources (Table -1). The second best carbon source which exhibited positive influence is sucrose at 2 % level (4.57 \pm 0.22cm). The plants grown on fructose and glucose showed reduced shoot length.

Fresh weight of the shoots

The maximum average fresh weight of shoots was recorded on MS medium containing 2% sucrose (4.89 \pm 0.19g) followed by 20% sugarcane juice (4.87 \pm 0.56g). Glucose and fructose at 3% level exhibited 3.73 \pm 0.10g and 3.43 \pm 0.11g respectively. While table sugar at 2% level showed 4.81 \pm 0.12g. There was a steady increase in the fresh weight of shoot as the

concentration of glucose and fructose was increased in the medium from 1% -3%. Higher concentrations of carbon sources resulted in callus formation (Table 1).

Chlorophyll content

Plants cultured on media supplemented with sugarcane juice at 20% level had the highest photosynthetic activity, and the plants cultured on fructose and glucose the lowest (Table 2). The leaves of the plants cultured on sugarcane juice (20%) had the highest chlorophyll content (0.81 \pm 2.0mg/g tissue) followed by those of the plants cultured on 2% sucrose (0.63 \pm 2.0mg/g tissue). The leaves of the plants cultured on glucose and fructose had the lowest chlorophyll content.

Table 2: Effect of carbon sources on the physiology of *in vitro* grown plants of *Pogostemon cablin* Benth. after 30 days in MS medium supplemented with 0.5mg/L BA and 0.5mg/L KN.

Carbon sources		Chlorophyll content (mg/g) \pm SD ^a	Total protein content (mg/ml) \pm SD ^a	Carbohydrate content (mg/ml) \pm SD ^a
Sucrose	1%	0.42 \pm 1.4	12.8 \pm 0.21	15.2 \pm 0.11
	2%	0.63 \pm 2.0	18.5 \pm 0.25	17.4 \pm 0.34
	3%	0.33 \pm 1.5	12.4 \pm 0.23	18.2 \pm 0.30
Glucose	1%	0.33 \pm 2.1	13.6 \pm 0.26	11.6 \pm 0.53
	2%	0.31 \pm 1.8	12.9 \pm 0.10	12.3 \pm 0.36
	3%	0.22 \pm 2.1	12.3 \pm 0.15	13.0 \pm 0.36
Fructose	1%	0.33 \pm 2.5	12.2 \pm 0.20	12.9 \pm 0.20
	2%	0.32 \pm 1.5	13.2 \pm 0.25	12.9 \pm 0.26
	3%	0.26 \pm 2.0	12.2 \pm 0.32	13.5 \pm 0.20
Table sugar	1%	0.46 \pm 2.8	14.5 \pm 0.35	14.5 \pm 0.26
	2%	0.52 \pm 2.5	15.6 \pm 0.15	14.7 \pm 0.10
	3%	0.43 \pm 2.1	12.3 \pm 0.25	15.3 \pm 0.15
Sugarcane juice	10%	0.73 \pm 1.5	16.1 \pm 0.23	16.2 \pm 0.12
	20%	0.81 \pm 2.0	18.8 \pm 0.24	17.5 \pm 0.25
	30%	0.79 \pm 2.5	15.3 \pm 0.21	17.9 \pm 0.13
Control		—	—	—
F- value		256*	245.4 *	265.6*

CI at 95%.

*Significant at 5% level

^a Data indicate mean \pm standard deviation. Ten replicates were used per treatments and experiment was repeated twice.

Total protein content

The protein content of the plants differed significantly with the type and concentration of the carbon sources used in the treatments (Table 2). The maximum protein content was observed on media supplemented with 20% sugarcane juice (18.8 \pm 0.24mg/ml) followed by 2% sucrose (18.5 \pm 0.25mg/ml). The least content was observed on 3% fructose containing media (12.2 \pm 0.20mg/ml). Table sugar also showed a better result which can be comparable with the use of sucrose in the medium.

Total carbohydrate content

The highest carbohydrate content was observed on the medium supplemented with 3% carbon sources. Sucrose at 3% produced highest protein content of 18.2 \pm 0.30mg/ml. This was followed by 30% sugarcane juice (17.9 \pm 0.13mg/ml). The lower content was observed when glucose and fructose were used in the medium. From the table 2, it is evident that irrespective of the carbon sources used, increase in the concentration of the carbon sources resulted in increasing the total protein content.

4. Discussion

Different types and levels of carbon sources were tried to study their effect on *in vitro* growth of patchouli. Previous report by ILL- Whan and Korban [8] indicated that the type of carbon source used in the culture medium affects the growth of *in vitro* plants in various ways. In our study also, growth of patchouli is strongly influenced by different carbon sources supplemented in the media. Even though carbohydrates are of prime importance for cell growth, maintenance and differentiation *in vitro*, the fundamental aspects of carbon utilization and metabolism in cell and tissue cultures have yet to be fully understood [25, 26].

Normally analytical grade sucrose is used for tissue culture studies. In plant tissue culture, sucrose serves as a carbohydrate supply to provide energy for cell. In order to reduce the cost of the culture medium, commercially available table sugar and sugarcane juice at different levels were studied. Many authors have reported that various sources of carbon such as glucose, fructose, mannitol and sorbitol play an important role in tissue culture of asparagus [27],

cucumber [28]. For the first time sugar cane extract is supplemented to MS medium, as a source of carbon on which the growth and multiplication of shoots is vigorous. The use of sugarcane juice at 20% showed better response towards multiple shoot formation (61), shoot elongation (4.8cm), increased chlorophyll content (0.8mg/g tissue) and total protein content (18.1mg/ml). This might be due to the fact that sugarcane juice is one of the best sources of energy. Also it contains 15% of natural sugar and is a good source of riboflavin, calcium, magnesium and potassium. These additional factors would have possibly affected overall response of patchouli multiplication *in vitro*. Similar results were obtained in other studies related to addition of plant extracts/juice of coconut, tomato, potato, onion, banana, orange, apple, pineapple and yeast to the culture medium [17, 18, 19, 20 and 29].

Sucrose has been reported to be the best source of carbon and energy [10]. However in the present study, the use of sugarcane juice has shown better results than the use of sucrose. The results of commercial table sugars and sucrose in the media have shown comparable results. This suggests that sucrose can be replaced by table sugar for patchouli tissue culture. Many laboratories have reported the use of table sugar in plant propagation medium [30, 31]. Zapata [32] has successfully reduced the cost of banana tissue culture by 90% by replacing the tissue culture sucrose grade with a commercial sugar. The use of sugarcane juice can further reduce the cost of the media since commercial sugars are processed from sugarcane. It is therefore recommended that sugarcane juice can be considered as low cost substitute for patchouli micropropagation.

The plants cultured on glucose and fructose had poor growth compared to other carbon sources. Similar results are reported in *Pinus sylvestris* by ILL- Whan and Korban [8]. Bouza et al. [15] reported that the addition of fructose to the medium results in hyperhydricity which leads to low cellulose and chlorophyll contents, less ethylene production and abnormal nitrogen and sugar metabolism.

The decrease in shoot multiplication at higher concentration of carbon sources may be due to the inhibition of organogenesis and induction of callus proliferation. The differences

in shoot length, multiple shoots, fresh weight, total protein and carbohydrate content could be due to the differences in their photosynthetic activities (chlorophyll content). The substantial increase in the total carbohydrate content at higher concentration of carbon sources could be attributed to sugar accumulation.

5. Conclusion

It can be concluded that various carbon sources used in our experiment affected the growth of patchouli plants. Furthermore, 20% sugarcane juice and 2% table sugar can be used totally as a replacement of 2% sucrose which was the carbon source used in most of the plant tissue culture including patchouli. Since sucrose is expensive, the present investigation suggests a new source of carbon in the form of sugarcane juice and table sugar which are not expensive and available easily. However, further research is required to explore the possible growth promoting factors in sugarcane juice.

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