

Effects of Sugars on *in vitro* Culture of *Bauhinia purpurea* L.

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Abstract

Bauhinia purpurea L. is a moderate sized tree with multipurpose value. It is distributed in sub-Himalayan tracts. Propagation of this plant was tested on different sugars, maltose, sucrose, glucose, galactose, lactose and fructose each with MS medium. Multiplication rate of plants were recorded after 8 weeks of culture. Sucrose produced 2.20 nodes and 10.05 mm shoot length the other sugars did not induce shoot length elongation and node developments.

Key words: micropropagation, nodal explants, Maltose, sucrose, glucose, galactose, lactose and fructose.

Introduction

Bauhinia purpurea L. is a leguminous plant of moderate sized possessing ornamental and fodder value. It is distributed in sub-Himalayan tracts. It has been cultivated in the plain regions up to the elevation of 1350 m. The tree also yields gum and the ethnobotanical reports made by Manandhar (2000) showed that its fruits are cooked and also pickle. The wood is used for agricultural implements and is suitable for scanting and rafters in inferior construction work. The long flat pods are best until February or March. Pettit *et al.* (2006) have isolated new and very remarkable (dibenzan L b, floxipens) cancer cell growth inhibitors and have designated them as Bauhiniastatins 1-4. Upon evaluation of anticancer activity Bauhiniastatins 1 exhibited significant growth inhibition of P 388 cancer cell line.

Micropropagation of *Bauhinia purpurea* L. has successfully been developed by Kumar (1992) using Murashige and Skoog (1962) (MS) medium with 5.0 μM kinetin. Similarly, *in vitro* regeneration of *B. vahlii* has been developed by Dhar and Upreti (1999) using MS medium supplemented with 2.5 μM kinetin plus 100 mg/l adenine sulphate. Murthur and Mukunthakumar (1992) developed *in vitro* propagation protocols for two leguminous trees, *B. variegata* and *Parkinsonia aculeata* from nodal

explants of mature trees using MS medium with 13.3 μM and 8.9 μM BAP respectively. Shukla *et al.* (2010) observed maximum multiple shoots on MS medium supplemented with 1.0 mg/l kinetin from nodal explants of *Withania somnifera*. Whereas Pant and Swar (2011) carried out micropropagation of *Cymbidium iriodoides* on MS medium supplemented with 1.0 mg/l BAP and 1.0 mg/l NAA was found best for germination. Here, the experiments are mainly emphasized for the sugar test.

Methodology

The seeds of *B. purpurea* were procured from district Aforestation Division, Hattisar, Kathmandu, Nepal and were carried to the Institute of Pharmacognosy, Vienna, Austria and were preserved at 4°C refrigerator until experimental use. The healthy seeds were washed with few drops of Teepol detergent solution. They were soaked in distilled water for an hour prior to sterilization. The soaked seeds were washed with distilled water for 5 times and sterilized with 10 % sodium hypochlorite solution for 10 minutes and removed the traces of sodium hypochlorite by washing thoroughly with sterilized distilled water five times inside laminar flow hood chamber. Finally the seeds were again sterilized in 70 % alcohol for one minute and washed with sterilized distilled water for 5 times to remove the alcohol. The seeds

were inoculated on 8% (bacteriological) agar medium containing 3% sucrose and the pH was adjusted to 5.8 before autoclaving and then sterilized at 15 lb./sq. inch pressure for 15 minutes in autoclave. Cultures were maintained in the culture room at 25°C (±2°C). Cool white fluorescent light of an intensity of approx. 40 μmol.m⁻² s⁻¹ was supplied through OSRAM BIOLUX tubes at a 16 hr light period. Nodal explants obtained from germinated seedlings were cultured on MS medium containing 0.5 μM BAP produced multiple shoots which were used for experimental purposes. Maltose, sucrose, glucose, galactose, lactose and fructose each with 30 mg/l was augmented with MS medium.

The nodal explants obtained from MS medium 0.5 μM BAP were cut into 2cm pieces and were placed on MS medium each with different sugars in baby food jar 200 ml capacity with 40 ml medium. In each of the vessels 4 explants were inoculated. All the results obtained were worked out statistically with SPSS, a system of analytical procedure.

Results and Discussion

Most of the sugars in the culture of nodal explants showed poor response of nodal multiplication and shoot length elongation. On MS medium supplemented with maltose produced 1.55 nodes and 9.0 mm shoot length elongation. In presence of sucrose produced 2.20 nodes and 10.05 mm shoot length was formed. Whereas, glucose, galactose, lactose and fructose did not induce shoot length elongation and node developments (Fig.1, 2). All the sugars except maltose did not lead to the formation of calli. The nodal explants grown on MS with glucose, galactose, lactose and fructose could not response well. The explants turned yellow after two weeks of culture (Table 1).

Table 1. Effects of sugars in *Bauhinia purpurea*

Treatments/ Media (μM)	Number of Nodes/culture Mean ± SE	Shoot length(mm) Mean ± SE	Q Calli (mm) Mean ± SE
Sugars (30 mg/l)			
Maltose	1.55 ± 0.1	9.00 ± 0.9	0.25 ± 0.25
Sucrose	2.20 ± 0.2	10.05 ± 0.8	0.00 ± 0.0
Glucose	1.25 ± 0.1	6.75 ± 0.5	0.00 ± 0.0
Galactose	1.25 ± 0.2	7.55 ± 0.5	0.00 ± 0.0
Lactose	1.00 ± 0.0	5.30 ± 0.6	0.00 ± 0.0
Fructose	1.00 ± 0.0	6.90 ± 0.5	0.00 ± 0.0



Fig.1. Plants growing on fructose, galactose and glucose

Relatively few scientists have tested different sugars. The effect of sugars on *B. purpurea* was apparently seen that sucrose was found the most effective followed by maltose, glucose, galactose, lactose and fructose.



Fig.2. Plants growing on lactose, maltose and sucrose

Mataui (1998) found fructose, galactose and sucrose to be callogenesis sugars, whereas maltose, lactose and galactose were found less callus stimulatory sugars. In present experiment only maltose showed few calli

formations. Premkumar *et al.* (2011) developed a protocol for micropropagation of *Scoparia dulcis* L. on MS medium supplemented with 22.32 μ M KI and 4.44 μ M BAP. Goyal *et al.* (1984) found sucrose was the best among all the carbohydrates tested for proliferations and elongation of shoots on *Prosopis cineraria* L. So, present work also corroborates with them.

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