RESEARCH ARTICLE

High frequency of multiple shoot induction and genistein and daidzein in *Desmodium gangeticum* (L.) Dc. by using different concentrations of BAP

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Chavhan Arvind

Cite this article as:
Patil VN, Somkuwar SR, Shambharkar RB, Kabnoorkar PS and Deokule SS (2016) Comparative Pharmacognostic Study of *Chlorophytum glaucum* Dalz. and *Chlorophytum breviscapum* Dalz, *Int. J. of Life Sciences*, A6: 101-104.

Acknowledgement:
The first author would like to thank to Maulana Azad National Fellowship Scheme, University Grant Commission for providing the financial assistance during the research work..

ABSTRACT

Nodal explants were inoculated with basal cut surface down on medium MS with BAP. The different concentrations BAP ranging from 0.5, 1, 1.5, and 2 mg/lit were used for obtaining multiple shoots. After 28 days maximum number of multiple shoots were obtained on medium containing 0.5mg/ lit of BAP which was approximately 100 / culture. In the present study 0.5mg/ lit of BAP concentration was found to be the ideal concentration for high frequency of multiple shoots induction. This is the first report of such high frequency of multiple shoot induction. Maximum Genistein 6.273 µg/g DW and Daidzein 8.224 µg/g DW content was found at 0.5 and 0. 52 mg/lit BAP respectively in in stem derived from shoot biomass. Minimum Genistein and Daidzein content was found in controlled (1.805 and 2.300µg/g DW respectively). We found that difference in content of Genistein and Daidzein was also affected by concentration of BAP i.e. increased concentration of BAP and increased number of multiple shoots showed correlation with increased concentration of Genistein and Daidzein.

Key words: BAP, genistein, daidzein, *Desmodium gangeticum*.

INTRODUCTION

Desmodium gangeticum (L.) DC belongs to family Fabaceae (Leguminoceae). It is known as Salparni in Sanskrit. It is a sub-erect, under-shrub 0.6–1.2m high with irregular angled, branched woody stem. Leaves are unifoliate or trifoliate. Flowers are small, pink to purple in color (Chopra et al., 1956). It is found in India, China, Africa, Australia, Ceylon, Burma, Malay Peninsula, Islands, Philippines and Tropical Africa (Anonymous, 1952; Cook, 1967; Hooker, 1973).Whole plant or mainly the roots are used in medicines. In Ayurveda, it is used to treat the various conditions such as snakebite, ulcer and diabetes (Dharmani et al., 2001), in asthma, bronchitis, dysentery, fever (Dharmani and Palit, 2006), in heart diseases (Kirtikar and Basu, 1935). It is used in Ayurvedic preparations like Dashmoola-Kwatha and Dashamoola-rishta (Kirtikar and Basu, 1935; Chopra et al., 1956). In the Ayurvedic system

of medicines, it is used as an analgesic, antiarthritis, against cough, rheumatism, astringent, in diarrhea, tonic, diuretic, fever, biliousness, cough, vomiting, asthma, snake-bite, scorpion- sting (Anonymous, 1992). *D. gangeticum* is reported to contain flavones and isoflavonoid glycoside and it is known to contain genistein and daidzein (Grynkiewicz et al., 2005). Genistein and daidzein are isoflavone that form the part of a diverse group of natural constituents of foods (Ruiz Larrea *et al*., 1997).

The drug *D. gangeticum* is mostly collected from wild sources to meet the requirement of pharmaceutical industries. Department of Indian Systems of Medicine and Homeopathy, Ministry of Health and Family Welfare, Government of India has formulated a Central Scheme for Cultivation and Development of Medicinal Plants. *D. gangeticum* is one of the species identified for promoting the cultivation in order to reduce the pressure on natural habitat and to meet the shortage against the demand of the industry (Rawat and Sharma, 1998). It is identified as a promising plant which is in great demand and of a high commercial potential. An estimated domestic demand for *D. gangeticum* is about 678.4 tones/year (Anonymous, 2001). The main aim of this study was to see the effect of different concentration of BAP with MS medium on *in vitro* shoot regeneration of *D. gangeticum* using the nodal explants and the genistein and daidzein content in them.

MATERIALS AND METHODS

Collection of plant materials

The plant material was collected from Western Ghats of Maharashtra and from Tadoba National Park, Chandrapur, Maharashtra, India. Efforts were made to collect the plant material in flowering and fruiting condition for the correct botanical identification and authentication. It was identified with help of Flora of Presidency of Bombay (Cook, 1967). Herbarium specimens were prepared and it was from Botanical survey of India, Western Circle, Pune. The herbariums are deposited in both the places. The voucher specimen number is BSI/WRC/Tech/2011/PAVNDGI. The plants were grown in Botanical garden of Department of Botany, Savitribai Phule University of Pune.

Preparation of Seeds

The fresh mature legumes of *D. gangeticum* were harvested for the germination. The outer covering of the legumes were removed by hand and keep in dark.

Sterilization of Seeds

The fresh mature seeds were treated with concentrated H_2SO_4 for 10±1 minute. Two washes given with distilled water for 2 minutes (without sterilized). Then, the seeds were taken under aseptic condition and two washes were given with sterilized distilled water for 2 minutes each which is followed by the treatments with 0.1% $HgCl_2$ for 1minute. The procedure terminated with four washes of sterilized distilled water for 1minute each (Patil and Deokule, 2012).

Seeds germination and Medium used

The seeds were inoculated on half (½) strength Murashige and Skoog (MS) medium. The pH of the medium was adjusted to 5.7 with 1N NaOH/ 1N HCl before addition of 0.8% agar and autoclaved at 15 lb/Inch ^{2} pressures and 121^{o}C temperature for 20 minutes. In the initial stage of seed germination, the cultures were kept in dark at 25^{o}C and 90% humidity, in Environmental test chamber, for 4-5 days. Then, the cultures were transferred to culture room, where they were maintained at 25^o±2^{o}C and 16/8 hours (light/dark) photoperiod provided through white fluorescent tubes with light intensity of 3000 lux. The mediums used for seedling development was ½ MS medium. The culture vessels were maintained in the same culture room of the seeds germination point. The growth responses of seedling were observed. The plant materials were used after attaining the height about 15 -20 cm for shoot regeneration.

The explants were inoculated on Murashige and Skoog's medium (Murashige and Skoog, 1962). The pH of the medium was adjusted to 5.8 with 0.1 N NaOH / HCl before addition of 0.8% agar. Medium was autoclaved at 121ºC at 15 lbs for 20 min. The cultures were incubated at 25 ± 2ºC under photoperiod 16/8 h (light/dark). The light source used was cool white florescent tubes providing an illumination of 2000/lux /m^2 /s.

Inoculation of Nodal Explants

Nodal explants were inoculated with basal cut surface down on MS medium with BA. The different concentrations of BA ranging from 0.25, 0.5, 0.75 and

1mg/lit were used for obtaining the multiple shoots. After four weeks multiple shoots was on high number and were sub-cultured on the new medium of same combinations.

Extraction of Genistein and daidzein

The obtained multiple shoots were checked for the genistein and daidzein content. The dried samples of the cultures were accurately weighed and macerated in 80% aqueous methanol. The Isoflavones were dissolved into the methanol using 10min of sonication to break up cellular materials, followed by overnight soaking in the solvent at room temperature. The insoluble materials were removed by filtration through a double layer of filter paper (Whatman No. 4 and then No. 1) and filtrate was collected as a sample for genistein and daidzein quantification (Liggins et al., 1998).

Quantitative method for estimation of Genistein

Quantification of genistein and daidzein content was done by High Performance Thin Layer chromatography (HPTLC) analysis. The genistein and daidzein extraction was done as described previously. The Standard genistein and daidzein was purchased from Hi-Media, Mumbai were used at different concentrations and quantification of experimental samples was carried out using std. graph prepared by using standard of genistein and daidzein.

RESULTS AND DISCUSSIONS

In order to induce the multiple shoots in *D. gangeticum*, the nodal sectors were cultured on medium containing different concentrations of BAP (0.25, 0.5, 0.75 and 1mg/lit). After 45 days maximum number of multiple shoots were observed on medium containing 0.5mg /lit of BAP which was approximately 84.28±0.7 per culture (Table 1, Figure 1). In the present study 0.5mg/lit of BAP concentration was found to be an ideal concentration for high frequency of multiple shoots induction (Table 1 and Fig. 1). This is the first report of such high frequency of multiple shoot induction. Maximum genistein 6.273±0.01µg/g DW content was found at 0.5mg/lit BAP and minimum genistein content was found at 1mg/lit BAP (5.251±0.02µg/g DW) while maximum daidzein 8.224±0.05µg/g DW content was found at 0.25mg/lit BAP and minimum daidzein content was found at 1mg/lit BA (6.974±0.07µg/g DW) (Table 1). Moreover, the size and physical appearance of shoots formed on each medium did not show any difference except the number of multiple shoots and genistein and daidzein concentration. The number of multiple shoots formed after 45 days differed for all concentrations, suggesting that during this period increasing time period and increasing amount of BAP up to 0.5 mg/lit has effective upon shoot multiplication. As the concentration increased, the numbers of shoot multiplication were found to be decreased.

Fig 1: Multiple shoots induction on MS medium containing 0.25mg and 0.5mg/ lit BAP.

Table 1: Multiple shoot induction and genistein and daidzein concentration in *D. gangeticum* (L.) DC on different concentration of BAP

Sr. No.	Concentration of BAP (mg/ lit)	Explants forming shoots (%)	Number of multiple shoots induced	Genistein (µg/g DW)	Daidzein (mg/g DW)
1	Control	9±0.05	19±0.06	1.805 ± 0.06	2.300 ±0.04
2	0.25	100±0.0	76.71±0.8	6.051 ± 0.02	8.224 ±0.05
3	0.5	100±0.0	84.28±0.7	6.273± 0.01	7.731 ±0.09
4	0.75	85.71±0.9	71.42±0.9	5.754±0.07	7.027 ±0.06
5	1	71.42±1.1	68.85±1.0	5.251±0.02	6.974 ±0.07

- Data scored after 4 weeks of culture incubation.
-All the results are mean of 3 observations ± S.D

In this study, the maximum shoots obtained on 0.5mg/lit is as 84.28±0.7 shoots (Table 1) (Figure 1). The highest genistein content on 0.5mg/lit is 6.273± 0.01µg/g DW (Table-1) while the highest daidzein content is 8.224±0.05µg/g DW at 0.25mg/lit BAP. We found that, the difference in contents of genistein and daidzein was also affected by the concentration of BAP i.e. increased the concentration of BAP up to 0.5mg/lit increased the number of multiple shoots. This is the first report of high number of multiple shoots induction and genistein and daidzein production in *D. gangeticum*.

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