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Shoot induction and daidzein production in *Desmodium gangeticum* (L.) DC by using different Concentrations of Kinetin

Patil VN* and Deokule SS

*Vidyabharti College, Seloo Dist. Wardha - 442104 (MS) India.
Professor, Department of Botany, University of Pune, Pune- 411 007.
*Corresponding author Email : vnpatil85@gmail.com

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Abstract

Nodal explants were inoculated with basal cut surface down on MS medium with Kinetin. The different concentrations of Kinetin ranging from 0.25, 0.5, 0.75 and 1mg/lit were used for obtaining multiple shoots. After 40 days, maximum number of multiple shoots were obtained on medium containing 0.5mg/lit of Kinetin which was approximately 34.28±0.1 per culture. In the present study, 0.5mg/lit of Kinetin concentration was found to be an ideal concentration for high frequency of multiple shoots induction. This is the first report of such high frequency of multiple shoot induction in *D. gangeticum*. Maximum daidzein 7.991±0.02µg/g D.W. content was found at 0.25mg/lit kinetin. Minimum daidzein content was found at 1mg/lit Kinetin (5.504±0.02µg/g D.W.). We found that, the difference in the content of daidzein was also affected by concentration of Kinetin i.e. increased the concentration of Kinetin up to 0.5mg/lit, increased number of multiple shoots but decreasing concentration of daidzein.

Keywords: Kinetin, daidzein, multiple shoot, *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 [1]. It is found in India, China, Africa, Australia, Ceylon, Burma, Malay Peninsula, Islands, Philippines and Tropical Africa [2, 3, 4].

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 [5], in asthma, bronchitis, dysentery, fever [6], in heart diseases [7]. It is used in Ayurvedic preparations like Dashmoola-Kwatha and Dashamoola-rishta [1,7]. 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 [8]. *D. gangeticum* is reported to contain flavones and isoflavonoid glycoside and it is known to contain genistein, daidzein [9]. Daidzein and genistein are isoflavone that form the part of a diverse group of natural constituents of foods [10].

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 [11]. 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 [12]. *In vitro* plant regeneration from various explants has been reported in *D. gangeticum* [13, 14, 15] as well as callus induction from stem explant [16] and leaf explant [17]. The main aim of this study was to see the effect of different concentration of Kinetin with MS medium on *in vitro* shoot regeneration of *D. gangeticum* using the nodal explants and the daidzein content in them.

Methodology

Collection and Identification of Plant Material

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 [3]. Herbarium specimens were prepared and it was first authenticated from Dr. S. S. Deokule, Professor and Head, Department Botany, University of Pune, Pune and also 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.

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 1 minute. The procedure terminated with four washes of sterilized distilled water for 1 minute each [13, 14].

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 [18]. 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 121°C at 15 lbs for 20 min. The cultures were incubated at 25 ± 2°C 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 kinetin. The different concentrations of kinetin 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 Daidzein

The obtained multiple shoots were checked for the 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 daidzein quantification [19].

Quantitative method for estimation of Daidzein

Quantification of daidzein content was done by High Performance Thin Layer chromatography (HPTLC) analysis. The daidzein extraction was done as described previously. The Standard 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 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 kinetin (0.25, 0.5, 0.75 and 1mg/lit). After 40 days maximum number of multiple shoots were observed on medium containing 0.5mg /lit of kinetin which was approximately 34.28±0.1 per culture (Table 1, Figure 1). In the present study 0.5mg/lit of kinetin concentration was found to be an ideal concentration for high frequency of multiple shoots induction. This is the first report of such high frequency of multiple shoot induction cultured on MS medium with different concentration of kinetin. Maximum daidzein 7.991±0.02µg/g DW content was found at 0.25mg/lit kinetin and minimum daidzein content was found at 1mg/lit kinetin (5.504±0.02µg/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 daidzein concentration. The number of multiple shoots formed after 30 days differed for all concentrations, suggesting that during this period increasing time period and increasing amount of kinetin 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 0.5mg/ lit Kinetin.

Table 1: Multiple shoot induction and Daidzein content in *D. gangeticum* on different concentration of Kinetin

Sr. No.	Concentration of Kinetin (mg/ lit)	Explants forming shoots (%)	Number of multiple shoots induced	Daidzein in stem derived from shoot biomass (µg/g DW)
1	Control	28%	19±0.02	1.805 ± 0.01
2	0.25	78.57%	26.71±0.2	**7.991±0.02**
3	**0.5**	**100%**	**34.28±0.1**	5.724±0.01
4	0.75	64.28%	21.42±0.3	6.691±0.03
5	1	64.28%	20.85±1.0	5.504±0.02

- Data scored after 4 weeks of culture incubation.
- All the results are mean of 14 replicates observations ± S.D.

In this study, the maximum shoots obtained on 0.5mg/lit is as 34.28±0.1 shoots (Table 1) (Figure 1). The highest daidzein content on 0.25mg/lit is 7.991±0.02 µg/g DW (Table-1). We found that, the difference in contents of daidzein was also affected by the concentration of kinetin i.e. increased the concentration of kinetin up to 0.5mg/lit increased the number of multiple shoots. This is the first report of high number of multiple shoots induction and daidzein production in *D. gangeticum* from kinetin.

Conflicts of interest: The authors stated that no conflicts of interest.

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