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Micro-propagation via shoot tip and nodal segment in ethnomedicinal plant– *Woodfordia fruticosa* (L.) Kurz.

Vimla Meena[1] and Satish Kumar[2]
[1](Department of Biotechnology, Mewar University, Chittorgarh, Rajasthan, India)
[2](Mata Basanti Devi School of Biosciences and Biotechnology, Agra, (U. P.) India-282 007)
Corresponding Author: Satish Kumar, e-mail; kumarsragi@gmail.com

Abstract: *Experiments were conducted for the standardization of in vitro culture technique for the mass propagation of Woodfordia fruticosa, a medicinally important, pungent, acrid, cooling, toxic, alexteric, uterine, sedative, anthelmintic plant. MS media was used in the present studies. While such media have been moderately to highly successful in terms of multiplication yields, it has become increasingly important to improve productivity and reduce the time taken to multiply commercially important plant material. In the present study, nodal segment was used as explants and cultured on MS medium supplemented with various concentrations of different hormones. Among all these combination best results were obtained from the explants cultured on MS medium supplemented with 2, 4- D (1.0 mg/l) combined with BAP (0.5mg/l). Satisfactory results were also obtained on MS medium containing BAP or NAA (1.0 mg/l).*

Keywords: *Alexteric, in vitro culture technique, Sedative, Woodfordia fruticosa*

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I. Introduction

Woodfordia fruticosa (L.) Kurz., the fire-flame-bush, is a woody shrub which is in regular demand amongst traditional medicine practitioners. In India, this plant is extensively used in Ayurvedic and Unani systems of medicine [1]. Although all parts of the plant possess valuable medicinal properties, there is a high demand of its flowers both in domestic and international market especially in the preparation of herbal medicines. Medicinal properties of *Dhataki* are well known and well documented. It is an important ingredient of preparations used to increase female fertility [2,3,4]. Many commercial drugs contain flowers, fruits, leaves and buds mixed with pedicels and thinner twigs of this plant [4]. The importance of tannin- Woodfordin I, extracted from *W. fruticosa*, has been documented as a potential lead compound against myelogenous leukemia [5].

Propagation via seed in this species is difficult as seedlings are highly susceptible to wilt caused by fungus-*Fusarium* spp. [6]. Moreover, seeds lose their viability within 6 months of collection [7]. Vegetative propagation is difficult as this plant is considered by many workers to be a hard-to-root species irrespective of season and hormone treatment [6,8,9]. Therefore, measures required to conserve it using tissue culture techniques. Over past several years, tissue culture has rapidly evolved one of the major tools for propagating true to type plants in a very short time. A large number of medicinal plants have been successfully regenerated and marketed via tissue culture techniques.

The aim of the present study is to establish a highly efficient *in vitro* regeneration protocol for *W. fruticosa* using nodal explants.

II. Material and methods

2.1. Collection of plant material and sterilization

Shoot cuttings with the youngest three leaves were collected from the 5-6 year old flowering plants of *Woodfordia fruticosa* (fam. Lythraceae) growing in the forest of Shahabad near Baran district in Rajasthan. The plants were grown into the pots with the help of grafting method. In present investigation, source plant material for explants was collected from pot grown plants.

2.2. In vitro culture technique

Juvenile shoot cuttings were carefully collected from pot grown plants with the help of surgicals. After excision, shoot tips were washed in running tap water for 10-15 min. and then washed with 0.1% Labolene (Merck, India) detergent solution for 2-3 min. 0.1% Cabendazim (Bavistin; Bhaskar Agro Chemicals) treatment was given to the plants in order to protect them from the fungal attack in the near future and 0.03% Streptomycin treatment for antimicrobial treatment. Further, plant material was sterilized with ethanol for 30-60 sec. and 4-5

times rinsed with distilled water. Plant material was further surface sterilized by treating with freshly prepared 0.1% solution of $HgCl_2$, for 3-5 min and rinsed 6-8 times with autoclaved distilled water inside the laminar air flow chamber. Nodal segments (with a single axillary bud) measuring about 5-8 mm long were excised aseptically and transferred vertically on Murashige & Skoog (hereafter MS) [10] medium supplemented with specific concentrations of BAP, Kn, 2, 4-D, NAA (0.5, 1, 1.5, 2.5, 4 mg/l), singly or in combinations.

All cultures were incubated at 26 ± 1^0C with a relative humidity of 50-60% and a photoperiod of 12h per day at 50-60 μW m^2 S^{-1} from daylight fluorescent tubes. For each experiment a minimum of 7 replicates were taken and experiments were repeated thrice. Observations were recorded after an interval of 3 week. After 30 days, shoot tips with differentiated shoot buds were sub-cultured on fresh medium. Same experiment was repeated for shoot multiplication.

III. Results & discussion

After 30 days of culture the frequency, number and length of shoots regenerated from nodal segments were found to be optimum on MS medium supplemented with BAP (0.5mg/l) and 2, 4-D (1 mg/l). Other hormonal combinations of 2, 4-D, BAP, NAA and Kn in different concentrations, were also evaluated for their effect on *in vitro* morphogenesis.

Optimum response (4.71±0.51) of shoot proliferation from nodal segments was observed on NAA (1 mg/l). Effects of various hormones added singly or in combinations were also investigated with the help of raising cultures, significantly good response was obtained on MS medium supplemented with NAA (2 mg/l) + BAP (1.5 mg/l), NAA (3 mg/l), + Kn (0.5 mg/l) and 2,4-D (1mg/l) + Kn (0.5 mg/l) (Table2).

In an earlier study, [11] used SH culture initiation medium for micropropagation of many monocot and dicot plants. Earlier in *Woodfordia fruticosa* [12] developed a very successful protocol of micropropagation. Their results indicated that the highest multiplication of *W. fruticosa* (26-35 shoots/shoot tip) on SH culture initiation media with 2.22 μM of BA and 2.60 μM of NAA followed by subculture on medium supplemented with 0.88 μM BA. The shoot multiplication rate was further accelerated by re-culturing 0.4-0.6 cm nodal segments of regenerated shoots in media with 4.40 μM BA. In a study, effect of BA had been investigated and it has been reported that 1.9 shoots/explant using basal sprouts of *Lagerstroemia parviflora* cultured on MS medium enriched with 2.22 μM BA [13].

In vitro regenerated *Woodfordia* plantlets could be multiplied by sub-culturing at regular 3-week intervals. This mode of multiplication ensured the continuous production of shoots without a decline in growth performance. In some studies, half MS medium supplemented with IBA (4.90 μL) for *in vitro* rooting of *Humulus lupulus* had been found to be very effective [14]. Similar results were reported in *Saussurea obvallata* by the addition of MS medium with 2.50 μL IBA for *in vitro* rooting [15].

Induction of multiple shoots in fire flamed bush from nodal segments was studied by addition of cytokinins in the nutrient medium. BAP was found to be highly essential and most efficient. The effect of Kn was found to be moderate in comparison to BAP for the induction, proliferation and subsequent growth of multiple shoots. Similar effect was also reported in *Syzygium cumini* [16]; and *Gmelina arborea* [17].

Maximum number of shoots (166.2±3.89) from nodal segments on MS medium supplemented with 0.5 mg/l BAP with 95% shoot regeneration response had earlier been reported in *Woodfordia fruticosa* [18]. Shoot elongation was best in media with BAP 0.2 mg/l. When BAP concentration was increased from 0.5 to 1.0 mg/l or even more (2.5 mg/l), it reduced the average number of multiple shoots produced. It also resulted in shorter shoots with lesser number of leaves.

Highest regeneration efficiency had been reported from nodal segment with 35±1.65 multiple shoots/explant on MS medium supplemented with BA (17.7 μM) in *Woodfordia fruticosa* [19]. The *in vitro* regenerated shoots attained average shoot length of 4.5±0.25 cm within 5 weeks of culture. About 90% of which could root on medium containing half-strength MS salts fortified with 4.90 μM IBA. The regenerated plantlets were established in a greenhouse with good survival response.

Root induction from regenerated shoots was also studied by addition of IBA (0.2-1.5 mg/l) and IAA (0.2-1.5 mg/l). IBA (1.0 mg/l) had been proved to be the most suitable for root induction with a network of 30-35 roots per shoot and the average root length being 6.5±0.41 cm; similar effects of IBA were also observed in *Pluchea lanceolata* [20], and in *Centella asiatica* [21].

Table: 1. Response of shoot proliferation from nodal segments of *Woodfordia fruticosa* cultured on MS medium supplemented with auxins and cytokinins.

PGR	Concentration (mg/l)	No. of explants cultured	No. of explants responded	Percentage response	No. of shoots proliferated (M±SE)
2,4-D	0.5	21	17	80.95	2.85±0.50
	1.0	21	19	90.47	4.28±0.56
	1.5	21	18	85.71	3.42±0.65
	2.5	21	16	76.19	2.57±0.57
	4.0	21	15	71.42	2.42±0.42

NAA	0.5	21	19	90.47	4.42±0.48
	1.0	21	20	95.23	4.71±0.42
	1.5	21	18	85.71	3.57±0.57
	2.5	21	16	76.19	2.42±0.37
	4.0	21	14	66.66	2.28±0.42
BAP	0.5	21	19	90.47	4.57±0.53
	1.0	21	20	95.23	4.71±0.51
	1.5	21	18	85.71	3.71±0.64
	2.5	21	17	80.95	2.71±0.42
	4.0	21	16	76.19	2.42±0.48
Kn	0.5	21	15	71.42	2.14±0.34
	1.0	21	16	76.19	2.42±0.37
	1.5	21	17	80.95	2.57±0.75
	2.5	21	19	90.47	3.42±0.53
	4.0	21	18	85.71	3.28±0.42

(M±SE)= Mean ± Standard Error
2,4-D= 2,4-dichlorophenoxy acetic acid
NAA= *α*-naphthalene acetic acid
BAP= *6*-benzylamino purine
Kn= Kinetin

Table-2: Interactive effect of Auxins and Cytokinins on shoot multiplication by subculture of shoot clumps in *Woodfordia fruticosa*

PGRs	Concentration (mg/l)	No. of explants cultured	No. of explants responded	Percentage response	No. of shoots proliferated (M±SE)
2,4-D+BAP	1+0.5	21	20	95.23	4.85±0.51
	1+1.5	21	19	90.47	4.57±0.68
	2+0.5	21	18	85.71	4.42±0.65
	2+1.5	21	17	80.95	4.14±0.67
	3+0.5	21	18	85.71	4.28±0.56
	3+1.5	21	17	80.95	4.14±0.74
	4+0.5	21	16	76.19	3.85±0.59
	4+1.5	21	16	76.19	3.85±0.74
	5+0.5	21	15	71.42	3.57±0.78
2,4-D+Kn	1+0.5	21	17	80.95	4.14±0.74
	1+1.5	21	18	85.71	4.28±0.65
	2+0.5	21	17	80.95	4.00±082
	2+1.5	21	16	76.19	3.71±0.75
	3+0.5	21	15	71.42	3.42±0.57
	3+1.5	21	16	76.19	3.85±0.68
	4+0.5	21	15	71.42	3.14±0.67
	4+1.5	21	14	66.66	2.85±0.59
	5+0.5	21	13	61.90	2.28±0.68
NAA+BAP	1+0.5	21	17	80.95	4.14±0.74
	1+1.5	21	16	76.19	3.28±0.75
	2+0.5	21	15	71.42	3.14±0.80
	2+1.5	21	14	66.66	2.85±0.86
	3+0.5	21	14	66.66	2.71±0.56
	3+1.5	21	13	61.90	2.57±0.65
	4+0.5	21	14	66.66	2.42±0.841
	4+1.5	21	12	57.14	2.28±0.746
	5+0.5	21	11	52.38	1.85±0.594
NAA+Kn	1+0.5	21	15	71.42	3.42±0.611
	1+1.5	21	16	76.19	3.71±0.473
	2+0.5	21	17	80.95	3.85±0.508
	2+1.5	21	18	85.71	4.14±0.670
	3+0.5	21	19	90.47	4.42±0.670
	3+1.5	21	18	85.71	4.00±0.723
	4+0.5	21	13	61.90	2.71±0.420
	4+1.5	21	14	66.66	3.14±0.594
	5+0.5	21	15	71.42	3.42±0.782

(M±SE)= Mean ± Standard Error
2,4-D= 2,4-dichlorophenoxy acetic acid
NAA= *α*-naphthalene acetic acid
BAP= *6*-benzylamino purine
Kn= Kinetin

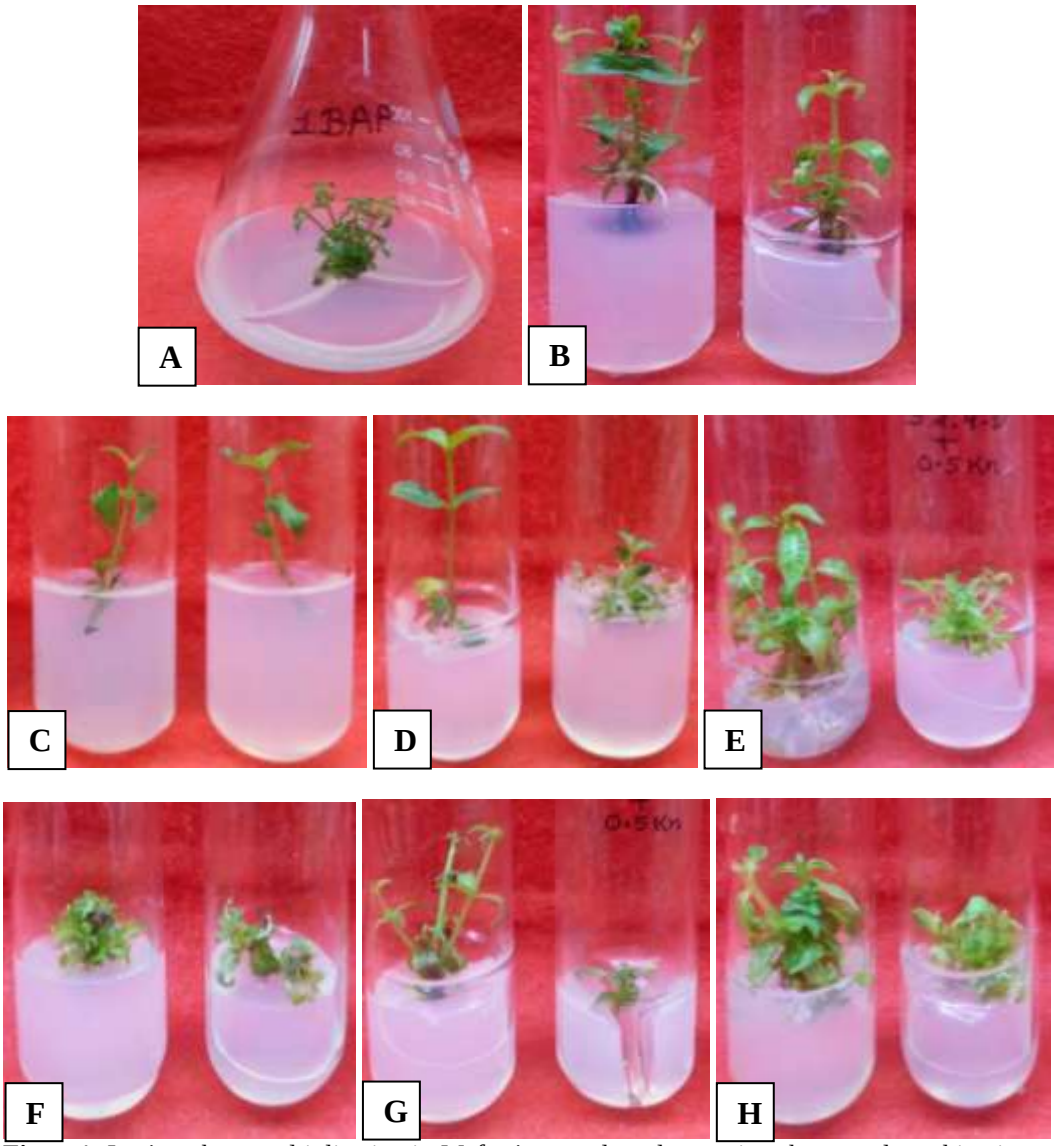

Figure1. *In vitro* shoot multiplication in *W. fruticosa* cultured on various hormonal combinations-

(**A-D**) *from shoot tips;* **A**. on MS + BAP (1 mg/l); **B**. on MS + Kn (2.5 mg/l); **C**. on MS+ 2,4-D (1 mg/l); **D**. on MS+ NAA (1 mg/l)
(**E-H**) *from nodal segments*, **E.** on MS+ 2, 4- D (1 mg/l) + Kn (0.5 mg/l); **F**. on MS+ BAP (1 mg/l)+ NAA+ (0.5 mg/l); **G**. on MS + NAA (1 mg/l + Kn (1.5 mg/l) and **H**. on MS+ 2, 4-D (1 mg/l) + BAP (0.5mg/l)

IV. Conclusion

Shoot tips and nodal segments have been used for the fast propagation of plant *in-vitro*. In *W. fruticosa* MS medium for exploring the effect of auxins alone; among various auxins tested, NAA proved to be most suitable for shoot multiplication (maximum no. of shoots 4.71±0.42). However, effect of various hormonal combinations was also studied; it was found that a combination of 2, 4-d and BAP was found to be optimal for shoot growth with maximum number of shoots 4.85±0.51.

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