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MICROPROPAGATION AND MASS MULTIPLICATION OF HIGHLY MEDICINAL PLANT BACOPA MONNIERI (L.) WETTST.

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Abstract: *Bacopa monnieri* (L.) WETTST. commonly known as Brahmi is an important medicinal herb. It is widely used as a brain tonic to enhance memory development, learning and concentration and to provide relief to patients with anxiety or epileptic disorders. The bioactive components like bacosides present in this herb make it highly valuable in pharma industries. Plant tissue culture is an important tool for mass production of any plant species from a single individual in a relatively short time. It is also cheap source to get secondary metabolites in bulk quantity. In present study, different growth regulators were tried with MS medium to standardized protocol for shoot initiation, shoot multiplication, root initiation and callus induction of *Bacopa* from different explant.

Key words: *Bacopa monnieri*, bacosides, *in vitro*, Micropropagation, MS medium.

I. INTRODUCTION

Bacopa monnieri (L.) WETTST (Family-Scrophulariaceae), commonly known as Brahmi is used in indigenous system (s) of medicine in India. The plant is a prostate, creeping, juicy, succulent, glabrous herb that branches profusely, found in wet places, damp or marshy areas near the border of the ponds, water cannels, wells, irrigated fields etc (Chopra *et al*., 1992). In the traditional system of Indian medicine (Ayurveda), 'Brahmi' is classified as medhya rasayana, that is, a drug that is supposed to counteract the effects of mental stress and improve intelligence and memory function (Sharma *et al*., 2010). It possesses anti-inflammatory, analgesic and antipyretic activity (Vohra *et al*., 1997). It is also used to treat asthma, insanity, epilepsy, hoarseness, enlargement of spleen, snake bite, rheumatism, leprosy, eczema and ring worm, it is also used as a diuretic, appetitive and cardio tonic (Basu and Walia, 1994). The plant is reported to contain tetracyclic triterpenoids saponins, bacosides A and B, phytosterols , hersaponin , flavonoids viz. luteolin-7-glucoside, glucoronyl-7-apigenin (Alam *et al*., 2011). It is used for controlling asthma, rheumatism, hoarseness and fever. Also it is used in generalized weakness, lethargy, fatigue and exhaustion (Jayaram *et al*., 1993).

The plant possesses a wide variety of pharmacologically active principles. There are mainly 52 phytochemicals namely Nicotine, D-Mannitol, Bacoside A, Bacopasaponin A, Bacopasaponin B, Bacopasaponin C, Bacopasaponin D, Bacopasaponin E, Bacopasaponin F, Bacopasaponin G, Bacopaside I, Bacopaside II, Bacopaside III, Bacopaside IV, Bacopaside V, Bacopaside VI, Bacopaside VII, Bacopaside VIII, Bacopaside XII, Plantainoside B, Betulinic acid, Cucurbitacin A, Cucurbitacin B, Cucurbitacin C, Cucurbitacin D, Cucurbitacin E, Stearic acid, Rosavin, 3,4Dimethoxycinnamic acid, Ascorbic acid, Asiatic acid, Brahmic acid, Wogonin, Oroxindin, Loliolide, Stigmasterol, β-sitosterol, Ebelin, lactone, Stigmastanol, Bacosterol, Bacosine, Heptacosane, Octacosane, Nonacosane, Triacontane, Hentriacontane,

Dotriacontane, Apigenin, Quercetin, Ursolic acid, Luteolin, Asiaticoside are responsible for the cognitive and therapeutic effects (Jeyasri et al., 2020).

Plant tissue culture is the process of small pieces of any living meristematic tissues (explants) isolated from a plant and grown aseptically on a semi defined or defined nutrient medium (Ignacimuthu, 1997). It is considered in wide sense which comprises the various culture methods of plant organs, tissues which facilitates experimental approach with a large objective of developmental biology and crop modification. It provides new possibilities for in vitro propagation and manipulation of plants and also recognized as an efficient tool for rapid clonal propagation (Negrutiu et al., 1984). In present investigation, different concentrations of growth regulators like auxin and cytokinin were tried with Murashige and Skoog's medium to standardized protocol for shoot initiation, shoot multiplication, root initiation and callus induction of *Bacopa* from different explant.

II. MATERIALS AND METHODS

2.1 Preparation of explant and sterilization

The explant like stem node and leaf segment were collected from young healthy plantlets of *Bacopa monnieri*, from different localities in the campus of Dr. Babasaheb Ambedkar Marathwada University, Chh. Sambhajinagar. All these explant were washed twice with running tap water for 5 minutes, followed by 70% ethanol for 2 minute and finally with double distilled water for 5 minutes. Surface sterilization of explant was carried out by washing with sterile distilled water for 5 minutes followed by various concentration of mercuric chloride ($HgCl_2$), leaf explant sterilized with 0.02% whereas stem node with 0.1% of $HgCl_2$. It was followed by two subsequent rinses with sterilized double distill water in laminar airflow. All these explant were cuts in to small pieces with the help of scalpel and inoculated on suitable concentration of MS-media.

2.2 Culture medium

All experiments of present investigation were tried on MS media (Murashige and Skoog, 1962) supplemented with various concentration of growth regulators. MS medium was supplied with 30 gm/L sucrose as a carbon source and 3 gm/L clerigar for solidification respectively and pH was adjusted to 5.8. The media were steam sterilized in an autoclave under 15 psi pressure and 121°C.

2.3 Culture condition

After the inoculation culture bottles were transfers to culture room under a 16 hrs. photoperiod supplied by cool white fluorescent cool tubes light and temperature at 25± 2°C. At least 10 replicates raised for each treatment and data were recorded in table.

III. RESULTS AND DISCUSSION

Table 1 Effect of different concentrations of 2, 4-D and IBA along with BAP and KIN in MS medium on callus induction of *Bacopa monnieri* (L.) WETTST

Growth hormones (mg/L)				Leaf segment		Stem node	
2,4-D	**IBA**	**BAP**	**KIN**	**Frequency of callus induction**	**Texture of callus**	**Frequency of callus induction**	**Texture of callus**
0.5	--	0.5	--	+++	**Friable, Greenish**	+++	**Friable, Greenish**
0.5	--	1.0	--	+++	**Friable, Greenish**	++	Friable, Greenish
0.5	--	1.5	--	++	Yellow green	++	Yellow green
0.5	--	2.0	--	++	Yellowish	+	Yellowish
0.5	--	2.5	--	+	Swelling of explants	+	Swelling of explants
0.5	--	3.0	--	+	Swelling of explants	+	Swelling of explants
0.5	--	--	0.5	+++	**Friable, Greenish**	++	Yellow green
0.5	--	--	1.0	+++	**Friable, Greenish**	++	Yellow green
0.5	--	--	1.5	++	Yellowish	+	Yellowish
0.5	--	--	2.0	+	Compact, Greenish	+	Compact, Greenish
0.5	--	--	2.5	+	Compact, Greenish	+	Compact, Greenish
0.5	--	--	3.0	+	Swelling of explants	+	Swelling of explants
--	0.5	0.5	--	++	Yellow green	++	Yellow green
--	0.5	1.0	--	++	Yellowish	++	Yellowish
--	0.5	1.5	--	+++	**Friable, Greenish**	+++	**Friable, Greenish**
--	0.5	2.0	--	++	Yellow green	+	brown
--	0.5	2.5	--	+	Compact, Greenish	+	Compact, Greenish
--	0.5	3.0	--	+	Swelling of explants	+	Swelling of explants
--	0.5	--	0.5	++	Yellow green	++	Yellow green
--	0.5	--	1.0	+++	**Friable, Greenish**	++	Yellow green
--	0.5	--	1.5	++	Yellow green	+++	**Friable, Greenish**
--	0.5	--	2.0	++	Yellowish	++	Yellowish
--	0.5	--	2.5	+	Compact, brown	+	Compact, Greenish
--	0.5	--	3.0	+	Swelling of explants	+	Swelling of explants

Where, + Poor Callus, ++ Moderate Callus, +++ Massive Callus.

For induction of callus in *Bacopa monnieri* different concentration of auxin and cytokinin were used. All combination of growth regulators were found more or less potent for induction of callus. It could be revealed that, as compared to other concentration of auxin, 2, 4-D has more potential to induce profuse callus. Callus induced with these concentrations was greenish colour with friable nature. Maximum rate of callus induction were recorded on 0.5 mg/L of 2, 4-D and 0.5 mg/L of BAP using both explant. However it was found maximum at 1.5 mg/L of BAP and 1.0 mg/L of KIN along with 0.5 mg/L of IBA using leaf and stem node as explant respectively (Table 1).

Table 2 Effects of different concentration of auxin and cytokinin on shoot multiplication of *Bacopa monnieri* (L.) WETTST

Explant	Growth hormones (mg/L)				Average Shoot length (cm)	No. of shoot/Explant
	IAA	IBA	BAP	KIN		
Leaf segment	0.5	--	0.5	--	1.7	2.4
	0.5	--	1.0	--	2.3	2.5
	0.5	--	1.5	--	2.8	3.2
	0.5	--	2.0	--	**3.2**	7.5
	0.5	--	2.5	--	2.8	4.1
	0.5	--	3.0	--	2.7	4.0
	0.5	--	--	0.5	1.1	2.6
	0.5	--	--	1.0	1.4	2.9
	0.5	--	--	1.5	**3.6**	5.2
	0.5	--	--	2.0	2.4	4.1
	0.5	--	--	2.5	2.6	3.2
	0.5	--	--	3.0	1.4	3.0
Stem node	--	0.5	0.5	--	1.8	2.6
	--	0.5	1.0	--	1.9	2.7
	--	0.5	1.5	--	2.4	2.6
	--	0.5	2.0	--	**4.5**	8.1
	--	0.5	2.5	--	3.1	3.1
	--	0.5	3.0	--	2.0	3.2
	--	0.5	--	0.5	1.6	2.0
	--	0.5	--	1.0	1.9	2.1
	--	0.5	--	1.5	**3.9**	4.6
	--	0.5	--	2.0	2.4	2.8
	--	0.5	--	2.5	2.1	3.2
	--	0.5	--	3.0	2.7	3.1

After 21 days average shoot length of 10 replicates.

Shoot regeneration was achieved from both explant from BAP and KIN in combination of 0.5 mg/L of IAA and IBA. Higher concentration of BAP was found effective to induced shoot regeneration however lower and excessive concentration revealed poor result of shoot regeneration. The maximum shoot induction percentage along with shoot length was recorded from 0.5 mg/L of IAA in combination with 2.0 mg/L of BAP and 0.5 mg/L of IBA with 2.0 mg/L of BAP using leaf and node as explant respectively(Table 2). Similar kinds of result were reported by Fuke *et al*., (2020).

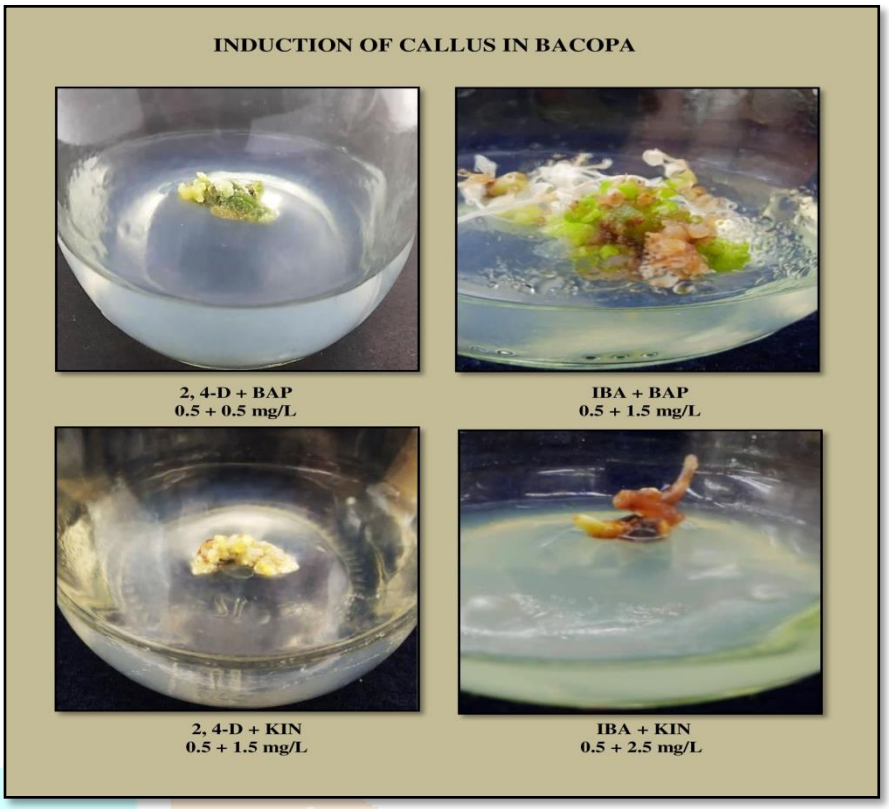

Figure 1 Effect of different concentrations of auxin and cytokinin on callus induction

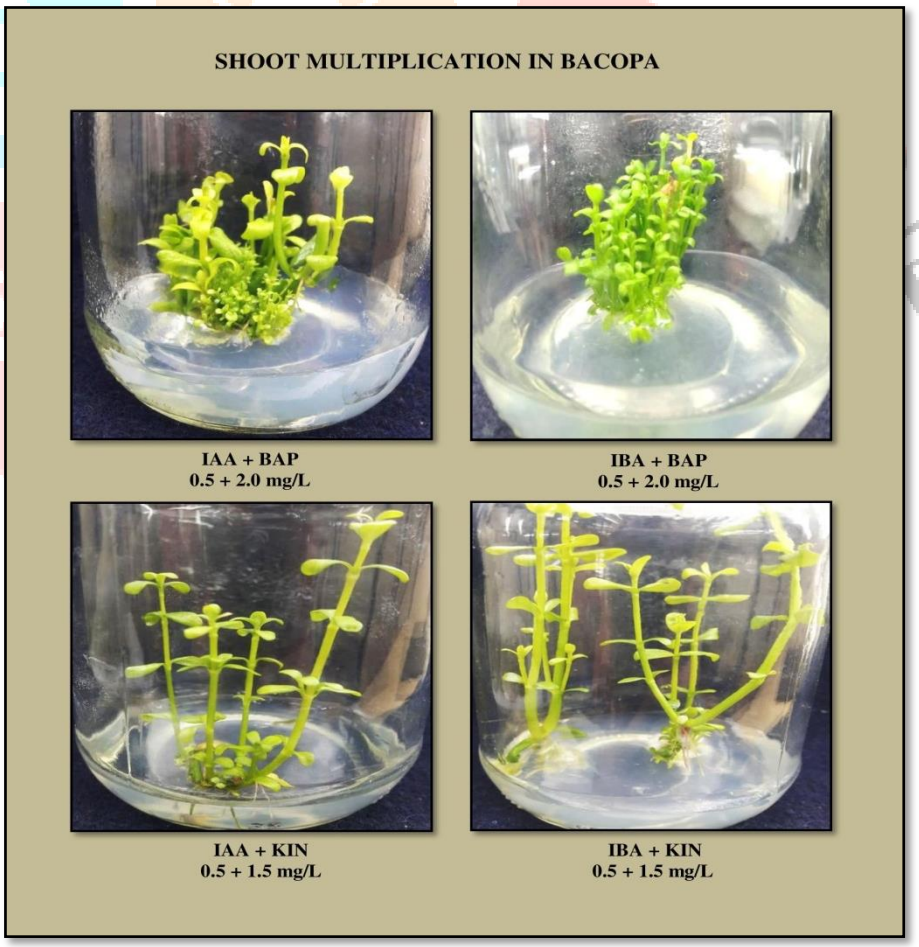

Figure 2 Effects of different concentration of auxin and cytokinin on shoot multiplication of *Bacopa monnieri*

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