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In vitro the various concentrations of BAP (6-*Benzyleaminopurine*) supplemented in different culture media for shoot proliferation of brahmi (*Bacopa monnieri*)

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Abstract
Brahmi is medicinal herb growing in muddy and subtropical latitudes with very good economic potential. It has been used as botanical medicines in many countries for thousands of years, they are used for preparing food, feed, and beverages, formulating ethno medicinal and ethno veterinary remedies and preparing traditional and modern cosmetic products. An efficient protocol for rapid *in vitro* propagation of valuable medicinal plant brahmi by using shoot tip and nodal cutting as explants were done. Brahmi is likely to become a major source of a number of medicinal products of high value in the coming future. This research work was carried out in Tissue Culture Laboratory, College of Horticulture of Chandra Shekhar Azad University of Agriculture and Technology, at Kanpur. Different experiments were conducted in order to achieve the objectives of the present investigation. The present work deals with the establishment of a viable protocol for this valued medicinal plant. Out of ten different treatments regime tried for induction and multiplication on shoots explants^{-1}. Taken two types of media *i.e.* MS media and B_5 media supplemented with different doses of plant growth regulators *i.e.* 1 mg/L of BAP, 2 mg/L BAP, 3 mg/L BAP, 4 mg/L BAP and 5 mg/L BAP for shoot induction and data to be recorded at weekly interval from first week to six weeks. Resulted that media MS was given best response at weekly interval, supplemented with different doses of plant growth regulators (*i.e.* 1 mg/L of BAP, 2 mg/L BAP, 3 mg/L BAP, 4 mg/L BAP and 5 mg/L BAP) for shoot induction per explants of brahmi as compare to B_5 media. Doses 3.0 mg/L BAP supplemented in MS media and B5 media was found best to induce maximum number of shoots per explants followed by 4.0 mg/L BAP in brahmi. Explants nodal cutting was done highly shoot proliferation with MS media and doses of plant growth regulators as compare to shoot tip explants of Brahmi.

Keywords: Shoot tip, nodal cutting, media MS, B_5, doses of BAP

Introduction
Brahmi is a creeping, glabrous, succulent herb, rooting at nodes whose habitat includes wetlands and muddy shores. Commonly known as Brahmi, the plant is 10-30 cm long with 1-2 mm thick, soft, glabrous stem having ascending branches. The leaves are 0.6-2.5 cm long and 3-8 mm broad. Flowers of brahmi are white or pale-bluish in colour, axillary, solitary, arranged on long slender pedicels. The fruits are ovoid, acute, 2-celled, 2valved capsules and tipped with style base. It is an ancient and renowned medicinal plant with a legendary reputation as memory vitalizer (Anonymous, 1998) [2].

Brahmi has been extensively used in the traditional system of medicine for centuries including Ayurveda. It was earlier used as a brain tonic to enhance memory development, learning, and concentration and to provide relief to patients with anxiety or epileptic disorders. In India, brahmi has been used for treating dermatosis, anaemia, diabetes and infertility since ancient times. With recent biotechnological advances, several therapeutic properties of brahmi have been clinically tested and established**.**

Brahmi placed second in a priority list of most important Indian medicinal plants on the basis of its medicinal properties, commercial value and potential for further research and development (Mohapatra and Rath, 2005; Sharma *et al*., 2007) [11, 12]. *In vitro* techniques such as tissue and organ culture offer plant breeders new openings for clonally propagation, genetic manipulation and production of inbred lines supplementing the routine vegetative production rare plant species. Using the *in vitro* method, a million – fold increase per year in clonal

multiplication over conventional methods is possible.

The rapidity of multiplication of true-to-type plants and efficient transplantation of *B. monnieri* can be useful in conservation and propagation of elite plants for commercial exploitation. So a suitable *in vitro* regeneration protocol is needed for the mass multiplication under various conditions and its conservation. In addition, the production of secondary metabolites through field cultivation of plants has various disadvantages such as low yields and variations in the amounts produced due to geographical, seasonal, and environmental conditions **(**Jain *et al*. 2013) [9].

In India, Tissue culture research began nearly four decades ago with the first report on production of test tube fertilization. Plant cell and tissue culture is defined as the capability to regenerate and propagate plants from single cells, tissues and organs under sterile and controlled environmental conditions. Tissue culture methods have also been employed to study the basic aspects of plant growth, metabolism, differentiation and morphogenesis and provide ideal opportunity to manipulate these processes. Advancement in tissue cultural methodology led many recalcitrant plants amenable to *in vitro* regeneration and to the development of haploids, somatic hybrids and pathogen free plants.

The multiple uses of this important medicinal plant have led to its indiscriminate collecting and use. It is estimated that about 100 000 tonnes of brahmi material is collected from the wild every year for commercial use in India (Ahmad 1993) [1]. Though the plant is found abundantly in wetlands, the drug content of the plant is very low (about 0.2%), and hence large amounts of plant material are required for drug extraction. Germplasm of vegetatively propagated crops is traditionally conserved ex situ in clonal field repositories, which is quite expensive, labour intensive and leaves the material vulnerable to natural calamities. Therefore the need was felt to develop alternative methods of storage for safe conservation and utilization of germplasm of *B. monnieri*, which has very specific growing conditions.

In vitro plant regeneration techniques have been established as a useful concept for ex situ conservation of rare, endangered and threatened plant species. On the basis of its vast medicinal importance and present status of this herb an attempt was made to develop an efficient plant regeneration protocol for *in vitro* culture using nodal explants.

Earlier *in vitro* propagation of *Bacopa monnieri* was attempted through organogenesis from different explants such as shoot tips, axillary nodes, leaves and callus (Binita *et al*., 2005; Debnath, 2008; Escandon *et al*., 2006) [4, 7, 8]. The present study was aimed at developing a simple, rapid and an efficient protocol of plant regeneration from leaf explants of *Bacopa monnieri.* At the present research combinations of 2, 4-D and Kinetin in Murashige-Skoog (MS) medium basal salts were applied to optimize PGRs concentration for callus induction and growth.

These factors optimizing growth criteria the constituents, concentration and combination of plant growth regulators (PGRs), play an important role in callus induction and growth, generally the medium contained 1-2 mg/L of 2, 4-dichlorophenoxy acetic acid (2, 4-D) or its combination with other PGRs. Naphthalene acetic acid (NAA), picloram and Kinetin were also essential in some cases, but the most effective auxin compound and concentration have varied with respect to explants tissue type.

Material and Method

Plant material

Juvenile shoots and node was isolated from three month old mature plants of *Bacopa monnieri* grown in the Herbal and Medicinal Garden, Collage of Agriculture, Chandra Shekhar Azad university of agriculture and technology, Kanpur. Stem cuttings and shoot tip were collected from the field grown superior plants. Roots of the collected accessions were removed and explant namely leaves and stem sections were subjected to sterilization process, after thorough washing under the running tap water.

Choice and sizing of explants

For micro propagation explants of Brahmi (*Bacopa monnieri*) were used:

1. Shoot tip: Apical shoots were used as explants after removing the surrounding leaves.
2. Nodal cutting explants: explants were used after removing the apical shoot.

Sterilization of culture media

Sterilization is an important requirement to ensure absence of microbial contamination. Glass bottles containing media were sterilized in an autoclave at 121 °C temperature and 15lbs / sq. inch pressure for 16 minutes. Double distilled water, glassware, cotton wools, brown papers and other instruments were also autoclaved at 121 °C temperature and 15lbs / sq. inch pressure for one hour.

Sterilization of glassware and other equipment:

All glassware were sterilized either in autoclave at 15 psi for 30 min or sterilized in hot air oven at 60 °C using autoclave begs. The sterilized glassware was cooled down before use.

Growth Regulator and Instruments

Two types of plant growth regulators were used for this experiment. The growth regulator was 6- benzyl amino purine (BAP) for shooting proliferation. For plant nutrients, MS medium and B5 medium were used which contain macronutrients, micronutrients and vitamins. The culture vessels such as test tube (150×25 mm), culture bottle (12×5 cm), conical flask (250 ml, 100 ml), measuring cylinders, glass rods, beakers, pipette pumps, Para film, cotton plug, rubber bands, filter paper, aluminum foils, forceps, firebox, marker pen, spirit lamp, needle, sharp blade, stereomicroscope, electronic balance, autoclave, pH meter, magnetic stirrer, laminar airflow hood etc. were used at the present investigation.

Culture Medium and Culture Procedure

The sterilized culture bottle, test-tubes, and conical flask containing MS medium with various concentrations of BAP at different combination for shoot induction. For proliferation, the best responding explants were transferred to MS media containing different concentration of BAP (0, 1, 2, 3, 4, 5 mg/L). MS media containing 3% sucrose and gelled with 0.70% agar (about) was employed throughout the experiments. The pH of the medium was adjusted to 5.78 before autoclaving. Sterilized explants were inoculated on previously prepared culture medium through laminar airflow.

Result and Discussion

Number of shoots explants^{-1} at 1st week

Table 1 reveal that number of shoots explants^{-1} of brahmi in 1st at week 1st experiment shows significant differences among number of shoots explants^{-1} both shoot tip (ST) and nodal cutting (NC), media MS and B_5 at different doses of BAP (*Benzyl amino purine*). Explants at nodal cutting (1.135) showed higher no. of shoots in brahmi significant difference to shoot tip (0.729). Media MS exhibit higher number of shoots in media *i.e*. (1.220) of brahmi showed significant difference to media B_5 (0.644). Does 3 mg/L BAP (*Benzyl amino purine*) were showed higher number of shoots given significant difference (1.196) followed by dose 4 mg/L BAP (1.152) among other doses in brahmi explants^{-1}.

In interaction of media and dose media MS and dose, 3 mg/L BAP (1.339) showed higher significant difference among other interaction of media and doses followed by media MS and dose 4 mg/L BAP (1.296). Media B_5 and dose 1mg/L BAP (0.0006) was recorded lower number of shoots explants^{-1} in brahmi. In interaction between explants and doses, explants nodal cutting and dose 3 mg/L BAP were recorded higher number of shoots explants^{-1} in brahmi *i.e.* (1.353) followed by explants nodal cutting and dose 4 mg/L BAP showed number of shoots in explants^{-1} (1.303). In interaction level of explants and media, explants nodal cutting and media MS were recorded higher number of shoots explants^{-1} in brahmi (1.300) followed by explants shoot tip and media MS i.e. (1.41). Minimum no of shoot explants^{-1} were found to be in explant shoot tip and media B_5 (0.318).

Table 1 reveal that number of shoots explants^{-1} of brahmi in 1st at week 2nd experiment shows significant differences among number of shoots explants^{-1}both shoot tip (ST) and nodal cutting (NC), media MS and B_5 at different doses of BAP (*Benzyl amino purine*). Explants at nodal cutting (1.1023) showed higher no. of shoots in brahmi significant difference to shoot tip (1.225). Media MS exhibit higher number of shoots in media *i.e*. (1.329) of brahmi showed significant difference to media B_5 (0.999). Does 3 mg/L BAP (*Benzyl amino purine*) were showed higher number of shoots given significant difference (1.351) followed by dose 4 mg/L BAP (1.272 among other doses in brahmi. explants^{-1}

In interaction of media and dose media MS and dose, 3 mg/L BAP (1.501) showed higher significant difference among other interaction of media and doses followed by media MS and dose 4 mg/L BAP (1.403). Media B_5 and dose 1 mg/L BAP (0.796) was recorded lower

Number of shoots explants^{-1} at 2nd week

Data presented in the table 2 number of shoots explants^{-1} of brahmi in 2nd week at 1st experiment shows significant differences among number of shoots explants^{-1} both shoot tip (ST) and nodal cutting (NC), media MS and B_5 at different doses of BAP (*Benzyl amino purine*). Explants at nodal cutting (1.719) showed higher no. of shoots in brahmi significant difference to shoot tip (1.291). Media MS exhibit higher number of shoots in media *i.e*. (1.732) of brahmi showed significant difference to media B_5 (1.277). Does 3 mg/L BAP (*Benzyl amino purine*) were showed higher number of shoots given significant difference (1.832) followed by dose 4 mg/L BAP (1.697) among other doses in brahmi explants^{-1}.

In interaction of media and dose media MS and dose, 3 mg/L BAP (2.014) showed higher significant difference among other interaction of media and doses followed by media MS and dose 4 mg/ L BAP (1.909). Media B_5 and dose 1mg/L BAP (0.900) was recorded lower number of shoots explants^{-1} in brahmi. In interaction between explants and doses, explants nodal cutting and dose 3 mg/L BAP were recorded higher number of shoots explants^{-1} in brahmi *i.e.* (1.961) followed by explants nodal cutting and dose 4 mg/L BAP showed number of shoots in explants^{-1} (1.895). In interaction level of explants and media, explants nodal cutting and media MS were showed statistically non-significant at 5% number of shoots explants^{-1} in brahmi (1.964) followed by explants shoot tip and media MS i.e. (1.500). Minimum no of shoot explants^{-1} were found to be in explant shoot tip and media B_5 (1.081).

Table 2 reveal that number of shoots explants^{-1} of brahmi in 2nd week at 2nd experiment shows significant differences among explants both shoot tip (ST) and nodal cutting (NC), media MS and B_5 at different doses of BAP (*Benzyl amino purine*). Explants at nodal cutting (1.926) showed higher no. of shoots in brahmi significant difference to shoot tip (1.482). Media MS exhibit higher number of shoots in media *i.e*. (1.918) of brahmi showed significant difference to media B_5 (1.490). Does 3 mg/L BAP (*Benzyl amino purine*) were showed higher number of shoots given significant difference (2.046) followed by dose 4 mg/L BAP (1.895) among other doses in brahmi explants^{-1}.

Table 1: Number of shoots explants^{-1} of Brahmi in 1st and 2nd experiments. (Week I)

Treatments	Number of shoots explants^{-1} (Experiment-1st)					Number of shoots explants^{-1} (Experiment-2nd)				
	Shoot tip (ST)	Nodal cutting (NC)		Mean		Shoot tip (ST)	Nodal cutting (NC)		Mean	
M_1xD_1	1.000	1.206		1.103		1.102	1.206		1.154	
M_1xD_2	1.010	1.2087		1.109		1.202	1.293		1.248	
M_1xD_3	1.2873	1.391		1.339		1.399	1.601		1.500	
M_1xD_4	1.200	1.392		1.296		1.289	1.518		1.4034	
M_1xD_5	1.206	1.303		1.254		1.286	1.393		1.340	
M_2xD_1	0.00105	0.00015		0.0006		0.991	0.6003		0.796	
M_2xD_2	0.00025	1.106		0.5530		1.207	0.6052		0.906	
M_2xD_3	0.7904	1.316		1.0532		1.390	1.012		1.201	
M_2xD_4	0.8004	1.215		1.007		1.287	0.994		1.141	
M_2xD_5	0.00025	1.206		0.6031		1.100	0.800		0.9498	
Mean	0.729	1.135		0.9319		1.225	1.1023		1.164	
	M_1 (MS)	M_2 (B_5)	Mean	ST	NC	M_1 (MS)	M_2 (B_5)	Mean	ST	NC
D_1 (1 mg/L BAP)	1.103	0.0006	0.5518	0.5005	0.603	1.154	0.796	0.975	1.047	0.903
D_2 (2 mg/L BAP)	1.109	0.5530	0.8312	0.5051	1.157	1.248	0.9061	1.0770	1.204	0.949
D_3 (3 mg/L BAP)	1.339	1.053	1.196	1.0389	1.353	1.501	1.201	1.351	1.394	1.306
D_4 (4 mg/L BAP)	1.296	1.007	1.152	1.0002	1.303	1.403	1.141	1.272	1.288	1.256
D_5 (5 mg/L BAP)	1.254	0.603	0.929	0.6030	1.254	1.340	0.950	1.145	1.193	1.096

Mean	1.220	0.644	0.9320	0.7295	1.134	1.329	0.999	1.164	1.225	1.102
	ST	NC		Mean		ST	NC		Mean	
Media (M_1)	1.141	1.300		1.220		1.256	1.4023		1.329	
Media (M_2)	0.318	0.968		0.644		1.195	0.802		0.999	
Mean	0.729	1.134		0.932		1.225	1.103		1.164	
	S.E. (diff)	**C.D. (0.05)**				**S.E. (diff.)**	**C.D. (0.05)**			
Media (M)	0.0023	0.0048		M		0.0025	0.0052			
Dose (D)	0.0058	0.0121		D		0.0062	0.0129			
Explants(E)	0.0023	0.0048		P		0.0025	0.0052			
M x D	0.0116	0.0241		M x D		0.0124	0.0258			
D x E	0.0046	0.0096		D x P		0.0050	0.0103			
M x E	0.0046	0.0096		M x P		0.0050	0.0103			
M x E x D	0.0231	0.0482		M x P x D		0.0248	0.0516			

Table 2: Number of shoots explants^{-1} of Brahmi in 1st and 2nd experiments. (Week II)

Treatments	**Number of shoots explants^{-1} (Experiment-1st)**					**Number of shoots explants^{-1} (Experiment-2nd)**				
	Shoot tip (ST)	**Nodal cutting (NC)**		**Mean**		**Shoot tip (ST)**	**Nodal cutting (NC)**		**Mean**	
M_1xD_1	1.196	1.583		1.390		1.291	1.794		1.542	
M_1xD_2	1.296	1.791		1.544		1.405	2.001		1.703	
M_1xD_3	1.805	2.222		2.014		2.001	2.470		2.235	
M_1xD_4	1.610	2.209		1.910		1.819	2.371		2.095	
M_1xD_5	1.592	2.018		1.805		1.713	2.314		2.013	
M_2xD_1	0.605	1.195		0.900		0.795	1.395		1.095	
M_2xD_2	0.805	1.402		1.103		0.999	1.611		1.305	
M_2xD_3	1.601	1.700		1.650		1.808	1.905		1.856	
M_2xD_4	1.387	1.581		1.486		1.589	1.800		1.695	
M_2xD_5	1.006	1.487		1.246		1.340	1.603		1.501	
Mean	1.291	1.719		1.504		1.482	1.926		1.704	
	M_1 (MS)	**M_2 (B_5)**	**Mean**	**ST**	**NC**	**M_1 (MS)**	**M_2 (B_5)**	**Mean**	**ST**	**NC**
D_1 (1 mg/L BAP)	1.390	0.900	1.145	0.901	1.389	1.542	1.095	1.319	1.043	1.594
D_2 (2 mg/L BAP)	1.544	1.103	1.323	1.051	1.596	1.703	1.305	1.503	1.202	1.806
D_3 (3 mg/L BAP)	2.014	1.650	1.832	1.703	1.961	2.235	1.856	2.046	1.904	2.187
D_4 (4 mg/L BAP)	1.909	1.484	1.697	1.498	1.895	2.095	1.695	1.895	1.704	2.085
D_5 (5 mg/L BAP)	1.805	1.246	1.525	1.299	1.753	2.013	1.501	1.757	1.556	1.959
Mean	1.732	1.277	1.504	1.290	1.719	1.918	1.490	1.704	1.482	1.926
	ST	NC		Mean		ST	NC		Mean	
Media (M_1)	1.500	1.964		1.732		1.646	2.190		1.918	
Media (M_2)	1.081	1.473		1.277		1.318	1.663		1.490	
Mean	1.290	1.719		1.504		1.482	1.926		1.704	
	S.E. (diff)	**C.D. (0.05)**				**S.E. (diff.)**	**C.D. (0.05)**			
Media (M)	0.0032	0.0067		Media (M)		0.0037	0.0077			
Dose (D)	0.0081	0.0169		Dose (D)		0.0092	0.0193			
Explants(E)	0.0032	0.0067		Explants(E)		0.0037	0.0077			
M x D	0.0162	0.0337		M x D		0.0185	0.0386			
D x E	0.0065	0.0135		D x E		0.0074	0.0154			
M x E	0.0065	NS		M x E		0.0074	0.0154			
M x E x D	0.0323	0.0675		M x E x D		0.0370	0.0771			

In interaction of media and dose media MS and dose, 3 mg/L BAP (2.235) showed higher significant difference among other interaction of media and doses followed by media MS and dose 4 mg/L BAP (2.095). Media B_5 and dose 1mg/L BAP (1.095) was recorded lower number of shoots explants^{-1} in brahmi. In interaction between explants and doses, explants nodal cutting and dose 3 mg/L BAP were recorded higher number of shoots explants^{-1} in brahmi *i.e.* (2.187) followed by explants nodal cutting and dose 4 mg/L BAP showed number of shoots in explants^{-1} (2.085). In interaction level of explants and media, explants nodal cutting and media MS were recorded higher number of shoots explants^{-1} in brahmi (2.190) followed by explants shoot tip and media MS i.e. (1.646). Minimum no of shoot explants^{-1} were found to be in explant shoot tip and media B_5 (1.318).

In both experiments, explants, media and doses showed significant response, explants nodal cutting, media MS and doses 3 mg/L BAP given best response to increase higher number of shoots in brahmi and In first experiment interaction between media and explants showed that statistically non-significant difference at 5.0% level of significance.

Number of shoots explants^{-1} at 3rd week

Table 3 reveal that number of shoots explants^{-1} of brahmi in 3rd week at 1st experiment shows significant differences among explants both shoot tip (ST) and nodal cutting (NC), media MS and B_5 at different doses of BAP (*Benzyl amino purine*). Explants at nodal cutting (2.532) showed higher no. of shoots in brahmi significant difference to shoot tip (1.884). Media MS exhibit higher number of shoots in media *i.e.* (2.569) of brahmi showed significant difference to media B_5 (1.847). Does 3 mg/L BAP (*Benzyl amino purine*) were showed higher higher number of shoots given significant difference (2.687) followed by dose 4 mg/L BAP (2.498) among other doses in brahmi explants^{-1}.

In interaction of media and dose media MS and dose, 3 mg/L BAP (3.081) showed higher significant difference among other interaction of media and doses followed by media MS and dose 4 mg/L BAP (2.875). Media B_5 and dose 1 mg/L BAP (1.343) was recorded lower number of shoots explants^{-1} in brahmi. In interaction between explants and doses, explants nodal cutting and dose 3 mg/L BAP were recorded higher number of shoots explants^{-1} in brahmi *i.e.* (3.037) followed by explants nodal cutting and dose 4 mg/L BAP showed number of shoots in explants^{-1} (2.894). In interaction level of explants and media, explants nodal cutting and media MS were recorded higher number of shoots explants^{-1} in brahmi (3.086) followed by explants shoot tip and media MS i.e. (2.052). Minimum no of shoot explants^{-1} were found to be in explant shoot tip and media B_5 (1.715).

Table 3 reveal that number of shoots explants^{-1} of brahmi in 3rd week at 2nd experiment shows significant differences among explants both shoot tip (ST) and nodal cutting (NC), media MS and B_5 at different doses of BAP (*Benzyl amino purine*). Explants at nodal cutting (2.790) showed higher no. of shoots in brahmi significant difference to shoot tip (2.697). Media MS exhibit higher number of shoots *i.e*. (3.318) of brahmi showed significant difference to media B_5 (2.168). Does 3 mg/L BAP (*Benzyl amino purine*) were showed higher number of shoots given significant difference media (3.301) followed by dose 4 mg/L BAP (3.025) among other doses in brahmi explants^{-1}.

Table 3: Number of shoots explants^{-1} of Brahmi in 1st and 2nd experiments. (Week III)

Treatments	Number of shoots explants^{-1} (Experiment-1st)					Number of shoots explants^{-1} (Experiment-2nd)				
	Shoot tip (ST)	Nodal cutting (NC)		Mean		Shoot tip (ST)	Nodal cutting (NC)		Mean	
M_1xD_1	1.599	2.216		1.907		2.272	2.965		2.619	
M_1xD_2	1.805	2.804		2.305		2.929	3.215		3.072	
M_1xD_3	2.486	3.677		3.081		3.677	4.246		3.961	
M_1xD_4	2.185	3.565		2.875		3.471	3.818		3.644	
M_1xD_5	2.186	3.169		2.677		3.015	3.576		3.295	
M_2xD_1	1.186	1.501		1.343		1.807	1.588		1.698	
M_2xD_2	1.400	1.786		1.593		1.988	1.782		1.885	
M_2xD_3	2.189	2.397		2.293		2.778	2.501		2.640	
M_2xD_4	2.018	2.224		2.121		2.614	2.196		2.405	
M_2xD_5	1.783	1.991		1.887		2.417	2.012		2.214	
Mean	1.884	2.533		2.208		2.697	2.790		2.743	
	M_1 (MS)	M_2 (B_5)	Mean	ST	NC	M_1 (MS)	M_2 (B_5)	Mean	ST	NC
D_1 (1mg/l BAP)	1.907	1.343	1.625	1.393	1.858	2.619	1.698	2.158	2.039	2.277
D_2 (2mg/l BAP)	2.305	1.593	1.949	1.602	2.295	3.072	1.885	2.478	2.458	2.499
D_3 (3mg/l BAP)	3.081	2.293	2.687	2.338	3.037	3.961	2.640	3.301	3.227	3.374
D_4 (4mg/l BAP)	2.875	2.121	2.498	2.101	2.894	3.644	2.405	3.025	3.043	3.007
D_5 (5mg/l BAP)	2.677	1.887	2.282	1.984	2.580	3.295	2.214	2.755	2.716	2.794
Mean	2.569	1.847	2.208	1.884	2.533	3.318	2.168	2.743	2.697	2.790
	ST	NC		Mean		ST	NC		Mean	
Media (M_1)	2.052	3.086		2.569		3.073	3.564		3.318	
Media (M_2)	1.715	1.980		1.847		2.321	2.016		2.168	
Mean	1.883745	2.533		2.208		2.697	2.790		2.743	
	S.E. (diff)	C.D. (0.05)				S.E. (diff.)	C.D. (0.05)			
Media (M)	0.0050	0.0103		Media (M)		0.0065	0.0136			
Dose (D)	0.0124	0.0259		Dose (D)		0.0163	0.0341			
Explants(E)	0.0050	0.0103		Explants(E)		0.0065	0.0136			
M x D	0.0248	0.0517		M x D		0.0327	0.0681			
D x E	0.0099	0.0207		D x E		0.0131	0.0272			
M x E	0.0099	0.0207		M x E		0.0131	0.0272			
M x E x D	0.0496	0.1035		M x E x D		0.0653	NS			

In interaction of media and dose media MS and dose, 3 mg/L BAP (3.961) showed higher significant difference among other interaction of media and doses followed by media MS and dose 4 mg/L BAP (3.644). Media B_5 and dose 1mg/L BAP (1.698) was recorded lower number of shoots explants^{-1} in brahmi. In interaction between explants and doses, explants nodal cutting and dose 3 mg/L BAP were recorded higher number of shoots explants^{-1} in brahmi *i.e.* (3.227) followed by explants nodal cutting and dose 4 mg/L BAP showed number of shoots in explants^{-1} (3.043). In interaction level of explants and media, explants nodal cutting and media MS were recorded higher number of shoots explants^{-1} in brahmi (3.073) followed by explants shoot tip and media MS i.e. (3.564). Minimum no of shoot explants^{-1} were found to be in explant shoot tip and media B_5 (2.016).

In both experiments, explants, media and doses showed significant response, explants nodal cutting, media MS and doses 3 mg/L BAP given best response to increase higher number of shoots in brahmi and interactions between media and doses, media and explants, doses and explants were also recorded significant response at 5.0% level of significance.

Number of shoots explants^{-1} at 4th week

Table 4 reveal that number of shoots explants^{-1} of brahmi in at 4th week of 1st experiment shows significant differences among explants both shoot tip (ST) and nodal cutting (NC), media MS and B_5 at different doses of BAP (*Benzyl amino purine*). Explants at nodal cutting (4.051) showed higher no. of shoots in brahmi significant difference to shoot tip (2.759). Media MS exhibit higher number of shoots *i.e*. (4.279) of brahmi showed significant difference to media B_5 (2.531). Does 3 mg/L BAP (*Benzyl amino purine*) were showed higher higher number of shoots given significant difference (4.249) followed by dose 4 mg/L BAP (3.953) among other doses in

brahmi explants^{-1}.

In interaction of media and dose, media MS and dose, 3 mg/L BAP (5.402) showed higher significant difference among other interaction of media and doses followed by media MS and dose 4 mg/L BAP (5.006). Media B_5 and dose 1 mg/L BAP (1.785) was recorded lower number of shoots explants^{-1} in brahmi. In interaction between explants and doses, explants nodal cutting and dose 3 mg/L BAP were recorded higher number of shoots explants^{-1} in brahmi *i.e.* (5.102) followed by explants nodal cutting and dose 4 mg/L BAP showed number of shoots in explants^{-1} (4.792). In interaction level of explants and media, explants nodal cutting and media MS were recorded higher number of shoots explants^{-1} in brahmi (5.326) followed by explants shoot tip and media MS *i.e.* (3.231). Minimum no of shoot explants^{-1} were found to be in explant shoot tip and media B_5 (2.286).

Table 4 reveal that number of shoots explants^{-1} of brahmi in 4rth week at 2nd experiment shows significant differences among explants both shoot tip (ST) and nodal cutting (NC), media MS and B_5 at different doses of BAP. Explantsat nodal cutting (4.443) showed higher no. of shoots in brahmi significant difference to shoot tip cutting (3.568). Media MS exhibit higher number of shoots *i.e.* (5.198) of brahmi showed significant difference to media B_5 (2.813). Does 3 mg/L BAP (*Benzyl amino purine*) were showed higher number of shoots given significant difference (4.746) followed by dose 4 mg/L BAP (4.357) among other doses in brahmi explants^{-1}.

Table 4: Number of shoots explants^{-1} of Brahmi in 1st and 2nd experiments. (Week IV)

Treatments	Number of shoots explants^{-1} (Experiment-1st)					Number of shoots explants^{-1} (Experiment-2nd)				
	Shoot tip (ST)	Nodal cutting (NC)		Mean		Shoot tip (ST)	Nodal cutting (NC)		Mean	
M_1xD_1	2.374	3.431		2.902		3.576	5.036		4.306	
M_1xD_2	2.787	4.653		3.720		3.812	5.161		4.487	
M_1xD_3	3.981	6.824		5.402		4.859	7.530		6.194	
M_1xD_4	3.631	6.381		5.006		4.204	7.035		5.620	
M_1xD_5	3.384	5.342		4.363		4.031	6.732		5.381	
M_2xD_1	1.594	1.976		1.785		1.981	2.391		2.186	
M_2xD_2	2.016	2.495		2.255		2.525	2.807		2.666	
M_2xD_3	2.812	3.380		3.096		2.995	3.599		3.297	
M_2xD_4	2.597	3.203		2.900		2.816	3.374		3.095	
M_2xD_5	2.414	2.826		2.620		2.616	3.026		2.821	
Mean	2.759	4.051		3.405		3.568	4.443		4.005	
	M_1 (MS)	M_2 (B_5)	Mean	ST	NC	M_1 (MS)	M_2 (B_5)	Mean	ST	NC
D_1 (1 mg/L BAP)	2.902	1.785	2.344	1.984	2.703	4.306	2.186	3.246	2.776	3.509
D_2 (2 mg/L BAP)	3.720	2.255	2.988	2.401	3.574	4.487	2.666	3.576	3.154	3.843
D_3 (3 mg/L BAP)	5.402	3.096	4.249	3.396	5.102	6.194	3.297	4.746	4.120	5.263
D_4 (4 mg/L BAP)	5.006	2.900	3.953	3.114	4.792	5.620	3.095	4.357	3.704	4.925
D_5 (5 mg/L BAP)	4.363	2.620	3.491	2.899	4.084	5.381	2.821	4.101	3.428	4.674
Mean	4.279	2.531	3.405	2.759	4.051	5.198	2.813	4.005	3.436	4.443
	ST	NC		Mean		ST	NC		Mean	
Media (M_1)	3.231	5.326		4.279		4.096	6.299		5.198	
Media (M_2)	2.286	2.776		2.531		2.776	2.587		2.681	
Mean	2.759	4.051		3.405		3.436	4.443		3.939	
	S.E. (diff)	C.D. (0.05)				S.E. (diff.)	C.D. (0.05)			
Media (M)	0.0076	0.0158		Media (M)		0.0099	0.0207			
Dose (D)	0.0190	0.0396		Dose (D)		0.0249	0.0518			
Explants(E)	0.0076	0.0158		Explants(E)		0.0099	0.0207			
M x D	0.0379	0.0792		M x D		0.0497	0.1037			
D x E	0.0152	0.0317		D x E		0.0199	0.0415			
M x E	0.0152	0.0317		M x E		0.0199	0.0415			
M x E x D	0.0759	0.1583		M x E x D		0.0994	0.2074			

In interaction of media and dose media MS and dose, 3 mg/L BAP (6.194) showed higher significant difference among other interaction of media and doses followed by media MS and dose 4 mg/L BAP (5.620). Media B_5 and dose 1 mg/L BAP (2.186) was recorded lower number of shoots explants^{-1} in brahmi. In interaction between explants and doses, explants nodal cutting and dose 3 mg/L BAP were recorded higher number of shoots explants^{-1} in brahmi *i.e.* (4.120) followed by explants nodal cutting and dose 4 mg/L BAP showed number of shoots in explants^{-1} (3.704). In interaction level of explants and media, explants nodal cutting and media MS were recorded significant effects to higher number of shoots explants^{-1} in brahmi (4.096) followed by explants shoot tip and media MS i.e. (6.299). Minimum no of shoot explants^{-1} were found to be in explant shoot tip and media B_5 (2.587).

In both experiments, explants, media and doses showed significant response, explants nodal cutting, media MS and doses 3 mg/L BAP given best response to increase higher number of shoots in brahmi and interactions between media and doses, media and explants, doses and explants were also recorded significant response at 5.0% level of significance.

Number of shoots explants^{-1} at 5th week

Table 5 reveal that number of shoots explants^{-1} of brahmi in at 5th week of 1st experiment shows significant differences among explants both shoot tip (ST) and nodal cutting (NC), media MS and B_5 at different doses of BAP (*Benzyl amino purine*). Explants at nodal cutting (6.689) showed higher no. of shoots in brahmi significant difference to shoot tip (4.638). Media MS exhibit higher number of shoots *i.e.* (7.556) of brahmi showed significant difference to media B_5 (3.771). Does 3 mg/L BAP (*Benzyl amino purine*) were showed higher higher number of shoots given significant difference (7.252) followed by dose 4 mg/L BAP (6.563) among other doses in

brahmi explants^{-1}.

In interaction of media and dose, media MS and dose, 3 mg/L BAP (9.853) showed higher significant difference among other interaction of media and doses followed by media MS and dose 4 mg/L BAP (8.903). Media B_5 and dose 1 mg/L BAP (2.810) was recorded lower number of shoots explants^{-1} in brahmi. In interaction between explants and doses, explants nodal cutting and dose 3 mg/L BAP were recorded higher number of shoots explants^{-1} in brahmi *i.e.* (8.797) followed by explants nodal cutting and dose 4 mg/L BAP showed number of shoots in explants^{-1} (7.922). In interaction level of explants and media, explants nodal cutting and media MS were recorded higher number of shoots explants^{-1} in brahmi (9.120) followed by explants shoot tip and media MS *i.e.* (5.992). Minimum no of shoot explants^{-1} were found to be in explant shoot tip and media B_5 (3.285).

Table 5 reveal that number of shoots explants^{-1} of brahmi in 5th week at 2nd experiment shows significant differences among explants both shoot tip (ST) and nodal cutting (NC), media MS and B_5 at different doses of BAP (*Benzyl amino purine*). Explants at nodal cutting (6.373) showed higher no. of shoots in brahmi significant difference to shoot tip (6.223). Media MS exhibit higher number of shoots *i.e.* (8.638) of brahmi showed significant difference to media B_5 (3.958). Does 3 mg/L BAP (*Benzyl amino purine*) were showed higher number of shoots given significant difference (7.968) followed by dose 4 mg/L BAP (7.259) among other doses in brahmi explants^{-1}.

Table 5: Number of shoots explants^{-1} of Brahmi in 1st and 2nd experiments. (Week V)

Treatments	Number of shoots explants^{-1} (Experiment-1st)					Number of shoots explants^{-1} (Experiment-2nd)				
	Shoot tip (ST)	Nodal cutting (NC)		Mean		Shoot tip (ST)	Nodal cutting (NC)		Mean	
M_1xD_1	4.190	5.562		4.876		5.058	5.833		5.946	
M_1xD_2	5.031	7.745		6.388		6.204	6.342		6.773	
M_1xD_3	7.628	10.08		9.853		8.099	11.001		11.050	
M_1xD_4	6.781	9.025		8.903		7.877	10.555		10.216	
M_1xD_5	6.326	8.191		7.759		6.607	8.800		9.203	
M_2xD_1	2.610	3.010		2.810		2.965	3.419		3.192	
M_2xD_2	2.977	3.988		3.482		3.214	3.812		3.513	
M_2xD_3	3.787	5.516		4.652		3.967	5.804		4.886	
M_2xD_4	3.628	4.819		4.223		3.822	4.781		4.301	
M_2xD_5	3.422	3.952		3.687		3.414	4.3791		3.896	
Mean	4.638	6.689		5.663		6.223	6.373		6.298	
	M_1 (MS)	M_2 (B_5)	Mean	ST	NC	M_1 (MS)	M_2 (B_5)	Mean	ST	NC
D_1 (1 mg/L BAP)	4.876	2.810	3.843	3.400	4.286	5.946	3.192	4.569	4.512	4.626
D_2 (2 mg/L BAP)	6.388	3.482	4.935	4.004	5.867	6.773	3.513	5.143	5.209	5.077
D_3 (3 mg/L BAP)	9.853	4.652	7.252	5.708	8.797	11.050	4.886	7.968	8.033	7.903
D_4 (4 mg/L BAP)	8.903	4.223	6.563	5.205	7.922	10.216	4.301	7.259	6.850	7.668
D_5 (5 mg/L BAP)	7.759	3.687	5.723	4.874	6.571	9.203	3.896	6.550	6.510	6.589
Mean	7.556	3.771	5.663	4.638	6.689	8.638	3.958	6.298	6.223	6.373
	ST	NC		Mean		ST	NC		Mean	
Media (M_1)	5.992	9.120		7.556		8.969	8.306		8.639	
Media (M_2)	3.285	4.257		3.771		3.476	4.439		3.958	
Mean	4.638	6.689		5.663		6.223	6.373		6.298	
	S.E. (diff)	C.D. (0.05)				S.E. (diff.)	C.D. (0.05)			
Media (M)	0.0104	0.0217		Media (M)		0.0165	0.0344			
Dose (D)	0.0260	0.0543		Dose (D)		0.0413	0.0861			
Explants(E)	0.0104	0.0217		Explants(E)		0.0165	0.0344			
M x D	0.0521	0.1086		M x D		0.0825	0.1722			
D x E	0.0208	0.0435		D x E		0.0330	0.0689			
M x E	0.0208	0.0435		M x E		0.0330	0.0689			
M x E x D	0.1042	0.2173		M x E x D		0.1651	0.3444			

In interaction of media and dose media MS and dose, 3 mg/L BAP (11.050) showed higher significant difference among other interaction of media and doses followed by media MS and dose 4 mg/L BAP (10.216). Media B_5 and dose 1mg/L BAP (3.192) was recorded lower number of shoots explants^{-1} in brahmi. In interaction between explants and doses, explants nodal cutting and dose 3 mg/L BAP were recorded higher number of shoots explants^{-1} in brahmi *i.e.* (7.903) followed by explants nodal cutting and dose 4 mg/L BAP showed number of shoots in explants^{-1} (7.668). In interaction level of explants and media, explants nodal cutting and media MS were recorded significant effects to higher number of shoots explants^{-1} in brahmi (8.306) followed by explants shoot tip and media MS i.e. (8.969). Minimum no of shoot explants^{-1} were found to be in explant shoot tip and media B_5 (3.476).

In both experiments, explants, media and doses showed significant response, explants nodal cutting, media MS and doses 3 mg/L BAP given best response to increase higher number of shoots in brahmi and interactions between media and doses, media and explants, doses and explants were also recorded significant response at 5.0% level of significance.

Number of shoots explants^{-1} at 6th week

Table 6 reveal that number of shoots explants^{-1} of brahmi in at 6th week of 1st experiment shows significant differences among explants both shoot tip (ST) and nodal cutting (NC), media MS and B_5 at different doses of BAP (*Benzyl amino purine*). Explants at nodal cutting (8.967) showed higher no. of shoots in brahmi significant difference to shoot tip (6.645). Media MS exhibit higher number of shoots *i.e.* (10.401) of brahmi showed significant difference to media B_5 (5.210). Does 3 mg/L BAP (*Benzyl amino purine*) were showed higher higher number of shoots given significant difference (9.854) followed by dose 4 mg/L BAP (8.632) among other doses in

brahmi explants^{-1}.

In interaction of media and dose, media MS and dose, 3 mg/L BAP (13.288) showed higher significant difference among other interaction of media and doses followed by media MS and dose 4 mg/L BAP (11.805). Media B_5 and dose 1 mg/L BAP (4.088) was recorded lower number of shoots explants^{-1} in brahmi. In interaction between explants and doses, explants nodal cutting and dose 3 mg/L BAP were recorded higher number of shoots explants^{-1} in brahmi *i.e.* (11.586) followed by explants nodal cutting and dose 4 mg/L BAP showed number of shoots in explants^{-1} (9.886). In interaction level of explants and media, explants nodal cutting and media MS were recorded higher number of shoots explants^{-1} in brahmi (12.355) followed by explants shoot tip and media MS *i.e.* (8.445). Minimum no of shoot explants^{-1} were found to be in explant shoot tip and media B_5 (4.842).

Table 6: Number of shoots explants^{-1} of Brahmi in 1st and 2nd experiments. (Week VI)

Treatments	Number of shoots explants^{-1} (Experiment-1st)					Number of shoots explants^{-1} (Experiment-2nd)				
	Shoot tip (ST)	Nodal cutting (NC)		Mean		Shoot tip (ST)	Nodal cutting (NC)		Mean	
M_1xD_1	5.816	8.636		7.226		8.096	8.899		8.497	
M_1xD_2	6.974	10.167		8.570		9.251	10.797		10.024	
M_1xD_3	10.898	15.678		13.288		14.115	15.622		14.869	
M_1xD_4	9.499	14.111		11.805		13.133	14.919		14.026	
M_1xD_5	9.040	13.187		11.114		12.858	13.263		13.060	
M_2xD_1	3.963	4.213		4.088		4.366	5.030		4.698	
M_2xD_2	4.590	5.174		4.882		4.843	5.505		5.174	
M_2xD_3	5.345	7.494		6.419		6.072	7.712		6.892	
M_2xD_4	5.257	5.660		5.459		5.752	6.064		5.908	
M_2xD_5	5.054	5.354		5.204		5.582	5.856		5.719	
Mean	6.643	8.967		7.805		8.407	9.367		8.887	
	M_1 (MS)	M_2 (B_5)	Mean	ST	NC	M_1 (MS)	M_2 (B_5)	Mean	ST	NC
D_1 (1 mg/L BAP)	7.226	4.088	5.657	4.889	6.424	8.497	4.698	6.598	6.231	6.964
D_2 (2 mg/L BAP)	8.570	4.882	6.726	5.782	7.670	10.024	5.174	7.599	7.047	8.151
D_3 (3 mg/L BAP)	13.288	6.419	9.854	8.121	11.586	14.869	6.892	10.880	10.094	11.667
D_4 (4 mg/L BAP)	11.805	5.459	8.632	7.378	9.886	14.026	5.908	9.967	9.443	10.491
D_5 (5 mg/L BAP)	11.114	5.204	8.159	7.047	9.270	13.060	5.719	9.390	9.220	9.560
Mean	10.401	5.210	7.805	6.643	8.967	12.095	5.678	8.887	8.407	9.367
	ST	NC		Mean		ST	NC		Mean	
Media (M_1)	8.445	12.356		10.401		11.490	12.700		12.095	
Media (M_2)	4.842	5.579		5.210		5.323	6.034		5.678	
Mean	6.643	8.967		7.805		8.407	9.367		8.887	
	S.E. (diff)	C.D. (0.05)				S.E. (diff.)	C.D. (0.05)			
Media (M)	0.0193	0.0402		Media (M)		0.0245	0.0512			
Dose (D)	0.0482	0.1006		Dose (D)		0.0613	0.1279			
Explants(E)	0.0193	0.0402		Explants(E)		0.0245	0.0512			
M x D	0.0964	0.2012		M x D		0.1226	0.2558			
D x E	0.0386	0.0805		D x E		0.0490	0.1023			
M x E	0.0386	0.0805		M x E		0.0490	NS			
M x E x D	0.1929	0.4023		M x E x D		0.2452	NS			

Table 6 reveal that number of shoots explants^{-1} of brahmi in 6th week at 2nd experiment shows significant differences among explants both shoot tip (ST) and nodal cutting (NC), media MS and B_5 at different doses of BAP (*Benzyl amino purine*). Explants at nodal cutting (9.367) showed higher no. of shoots in brahmi significant difference to shoot tip (8.407). Media MS exhibit higher number of shoots *i.e.* (12.095) of brahmi showed significant difference to media B_5 (5.678). Does 3 mg/L BAP (*Benzyl amino purine*) were showed higher higher number of shoots given significant difference (10.880) followed by dose 4 mg/L BAP (9.442) among other doses in brahmi explants^{-1}.

In interaction of media and dose media MS and dose, 3 mg/L BAP (14.869) showed higher significant difference among other interaction of media and doses followed by media MS and dose 4 mg/L BAP (14.026). Media B_5 and dose 1 mg/L BAP (4.698) was recorded lower number of shoots explants^{-1} in brahmi. In interaction between explants and doses, explants nodal cutting and dose 3 mg/L BAP were recorded significant higher number of shoots explants^{-1} in brahmi *i.e.* (11.667) followed by explants nodal cutting and dose 4 mg/L BAP showed number of shoots in explants^{-1} (10.491). In interaction level of explants and media, explants nodal cutting and media MS were recorded non-significant effects to higher number of shoots explants^{-1} in brahmi (12.700) followed by explants shoot tip and media MS i.e. (11.490). Minimum no of shoot explants^{-1} were found to be in explant shoot tip and media B_5 (5.323).

In both experiments, explants, media and doses showed significant response, explants nodal cutting, media MS and doses 3mg /L BAP given best response to increase higher number of shoots in brahmi. All interactions showed significant effects in both trials. Except interaction between media and explants in second trials were recorded non-significant at 5.0% level of significance.

Tissue culture is the cultivation of the plant cells, tissue and organs on specially formulated nutrients media. Number of shoots explants^{-1} of brahmi in 1st week reveal that (Figure 1 & 2) significant difference varied among explants both shoots tip and nodal cutting. Media and different doses of BAP explants nodal cutting showed significant highest and MS media took significantly maximum number of shoots seems to be in dose 3 mg/L BAP among other doses. During both experiments a similar finding was also corroborated by (Chaplot *et al.*, 2005)

Effect of plant growth regulators on shoot proliferation explants in brahmi at 1st at week interaction effect of explants and media, media and doses showed significant effect over number of shoots in brahmi. Interaction between explants and doses was also showed significant. Interaction effect it has been presented in result earlier during both experiments. Demand of brahmi has increased many fold in result past due to its highly priced medicinal properties.

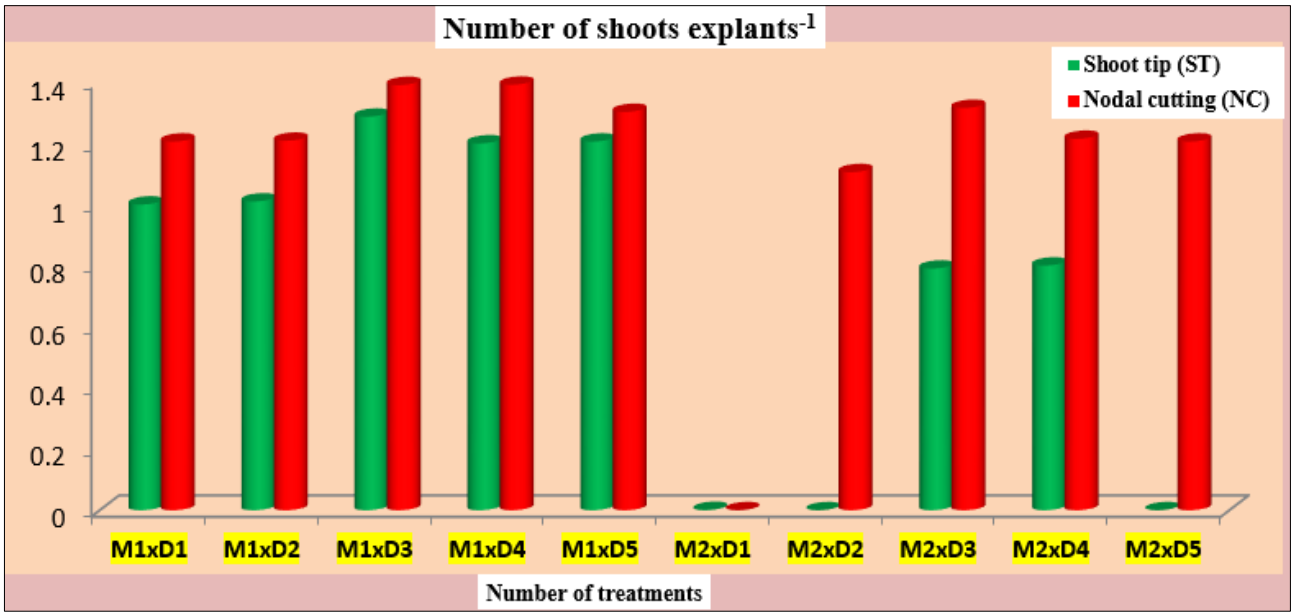

Fig 1: Number of shoots explants-1 of Brahmi at 1st week of 1st experiment

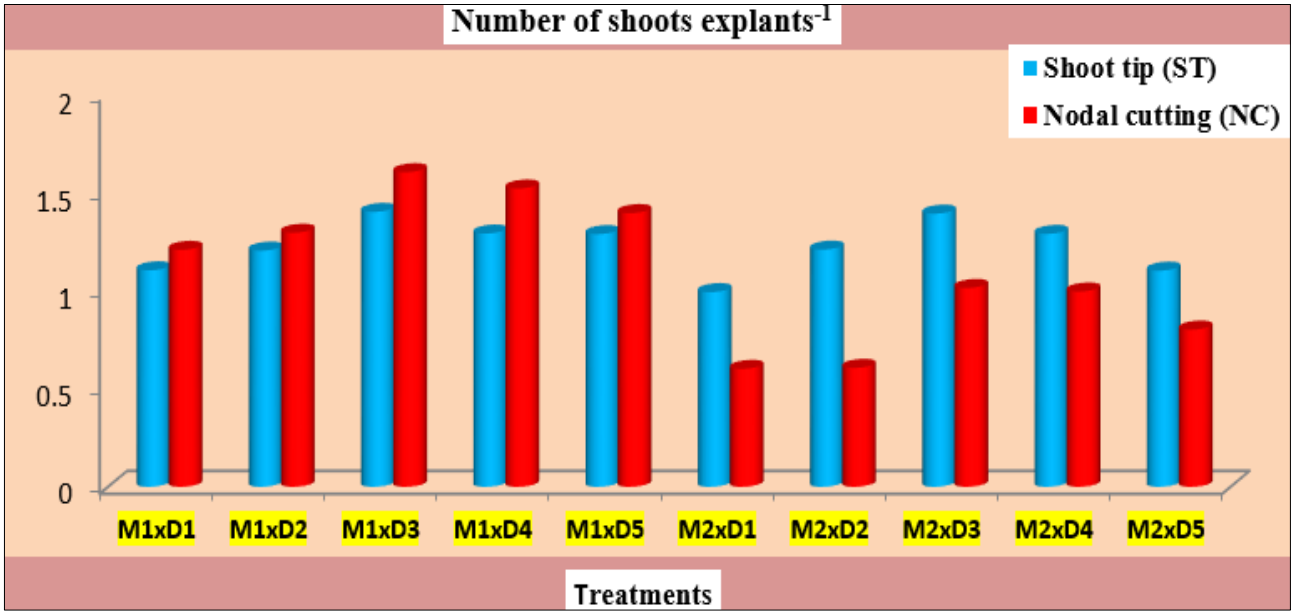

Fig 2: Number of shoots explants-1 of Brahmi at 1st week of 2nd experiment

M1	MS media	D1	1 mg/L BAP
M2	B5 media	D2	2 mg/L BAP
D	Doses	D3	3 mg/L BAP
		D4	4 mg/L BAP
		D5	5 mg/L BAP

Number of shoots explants-1 of brahmi a in 2nd week reveal that (Figure 3 & 4) significant difference varied among explants both shoots tip and nodal cutting. Media and different doses of BAP explants nodal cutting showed significant highest and MS media took significantly maximum number of shoots seems to be in dose 3mg/l BAP among other doses. During both experiments a similar finding was also corroborated by (Ceasar *et al*., 2010) [5]

Effect of plant growth regulators on shoot proliferation explants in brahmi at 2nd week interaction effect of explants and media, media and doses showed significant effect over number of shoots in brahmi. Interaction between explants and media was showed non-significant effect at 5%. Interaction effect it has been presented in result earlier during both experiments.

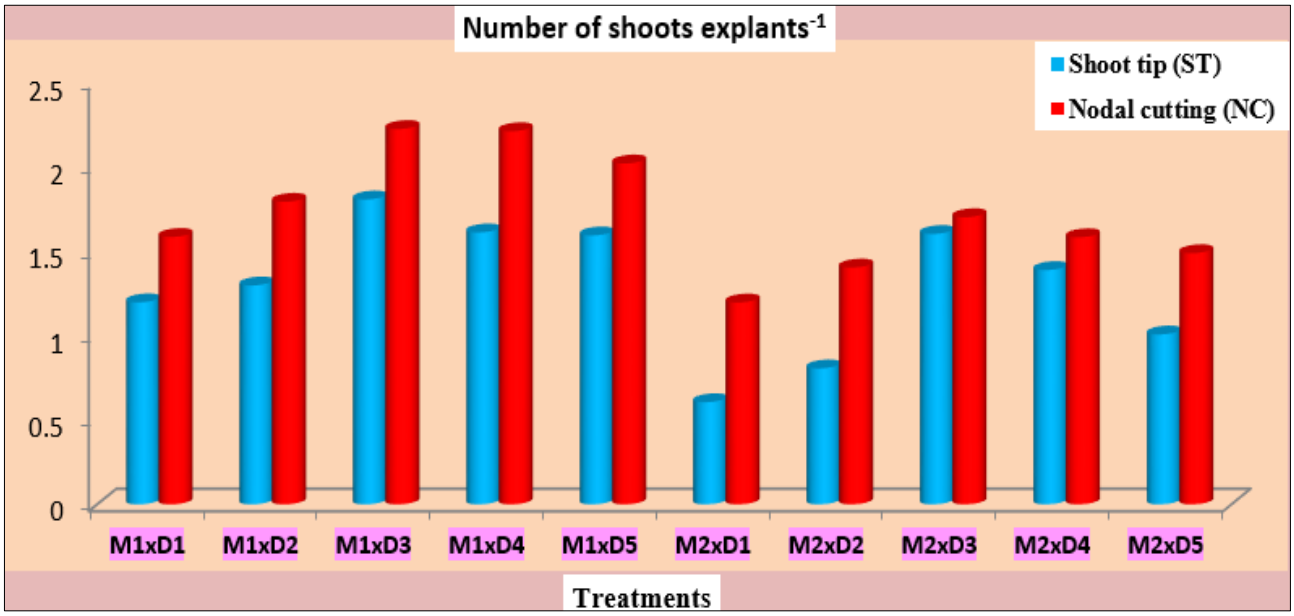

Fig 3: Number of shoots explants^{-1} of Brahmi at 2nd week of 1st experiment

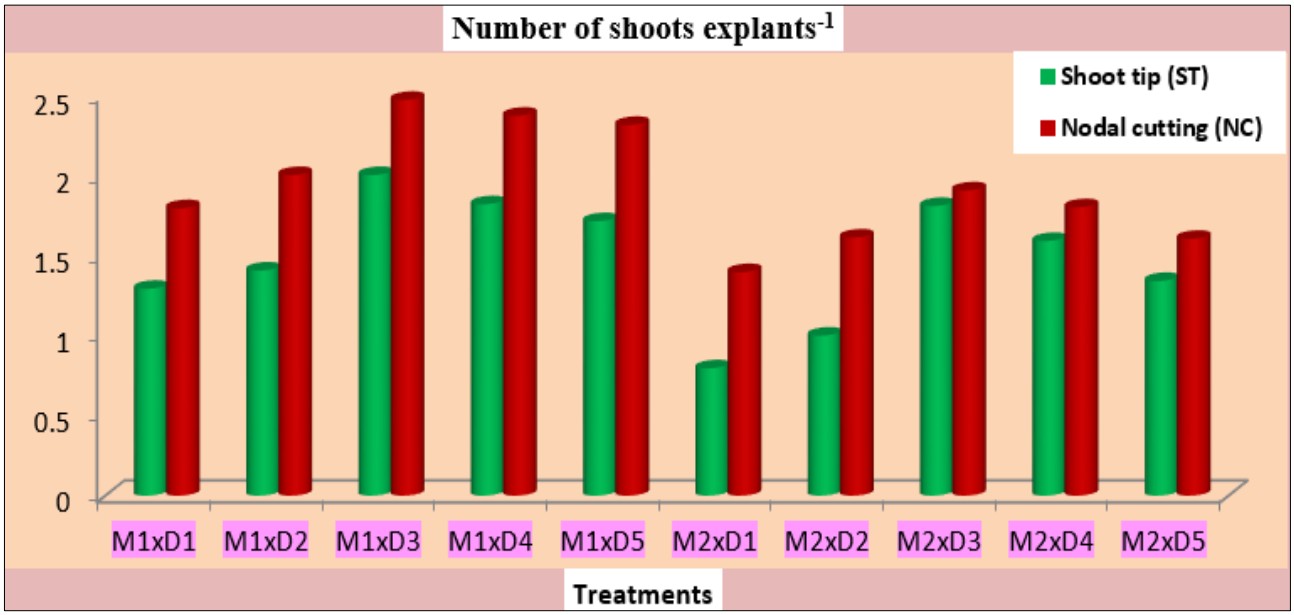

Fig 4: Number of shoots explants^{-1} of Brahmi at 2nd week of 2nd experiment

M1	MS media	D1	1 mg/L BAP
M2	B5 media	D2	2 mg/L BAP
D	Doses	D3	3 mg/L BAP
		D4	4 mg/L BAP
		D5	5 mg/L BAP

Data pertaining to number of shoots explants^{-1} of brahmi a in 3rd week reveal that (Figure 5 & 6) significant difference varied among explants both shoots tip and nodal cutting. Media and different doses of BAP explants nodal cutting showed significant highest and MS media took significantly maximum number of shoots seems to be in dose 3mg/l BAP among other doses. During both experiments a similar finding was also corroborated by (Yusuf *et al.*, 2011) [14]

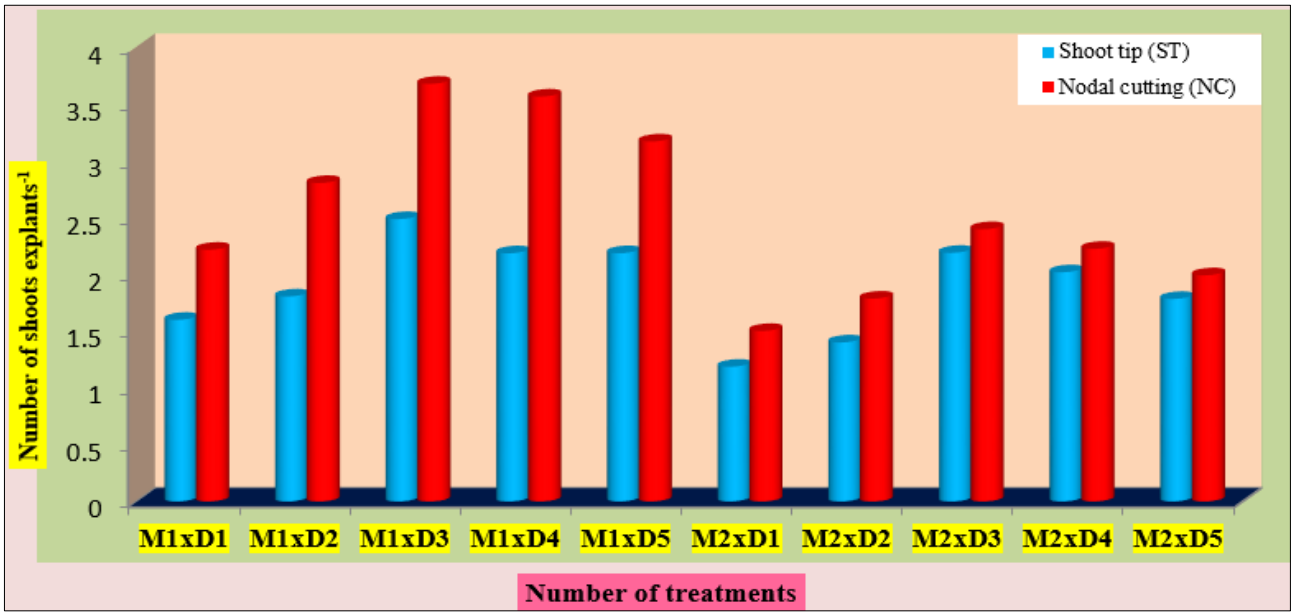

Fig 5: Number of shoots explants^{-1} of Brahmi at 3rd Week of 1st experiments

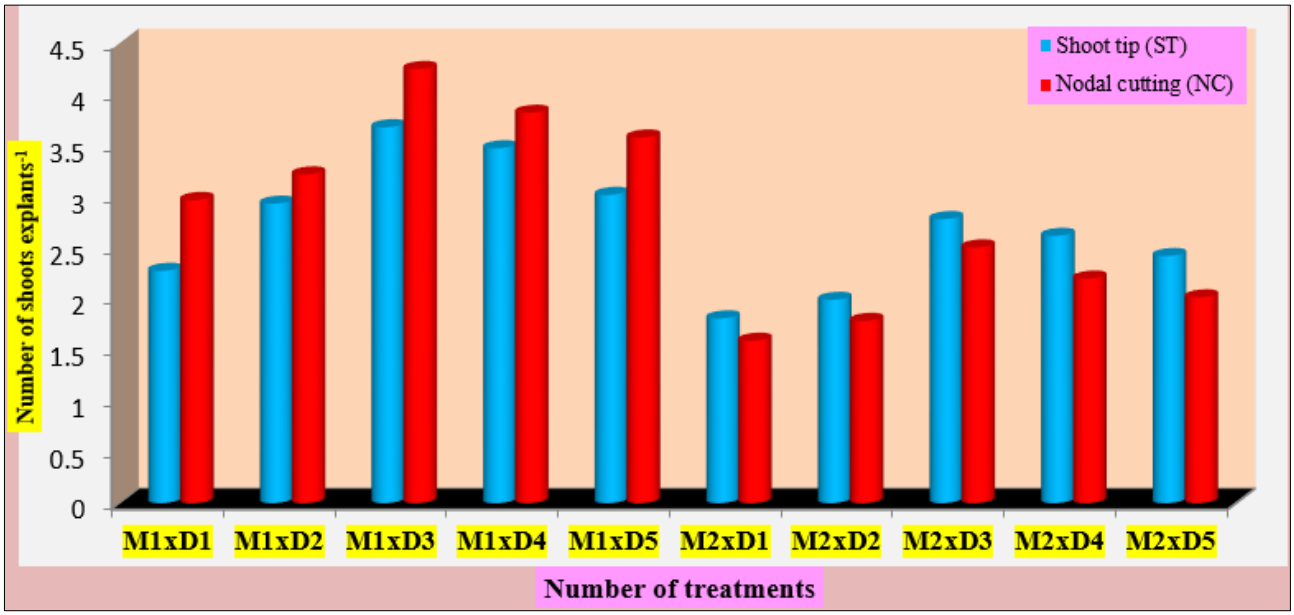

Fig 6: Number of shoots explants^{-1} of Brahmi at 3rd Week of 2nd experiments

M1	MS media	D1	1 mg/L BAP
M2	B5 media	D2	2 mg/L BAP
D	Doses	D3	3 mg/L BAP
		D4	4 mg/L BAP
		D5	5 mg/L BAP

Effect of plant growth regulators on shoot proliferation explants in brahmi at 3rd week interaction effect of explants and media, media and doses showed significant effect over number of shoots in brahmi. Interaction between explants and doses was also showed significant. Interaction effect it has been presented in result earlier during both experiments.

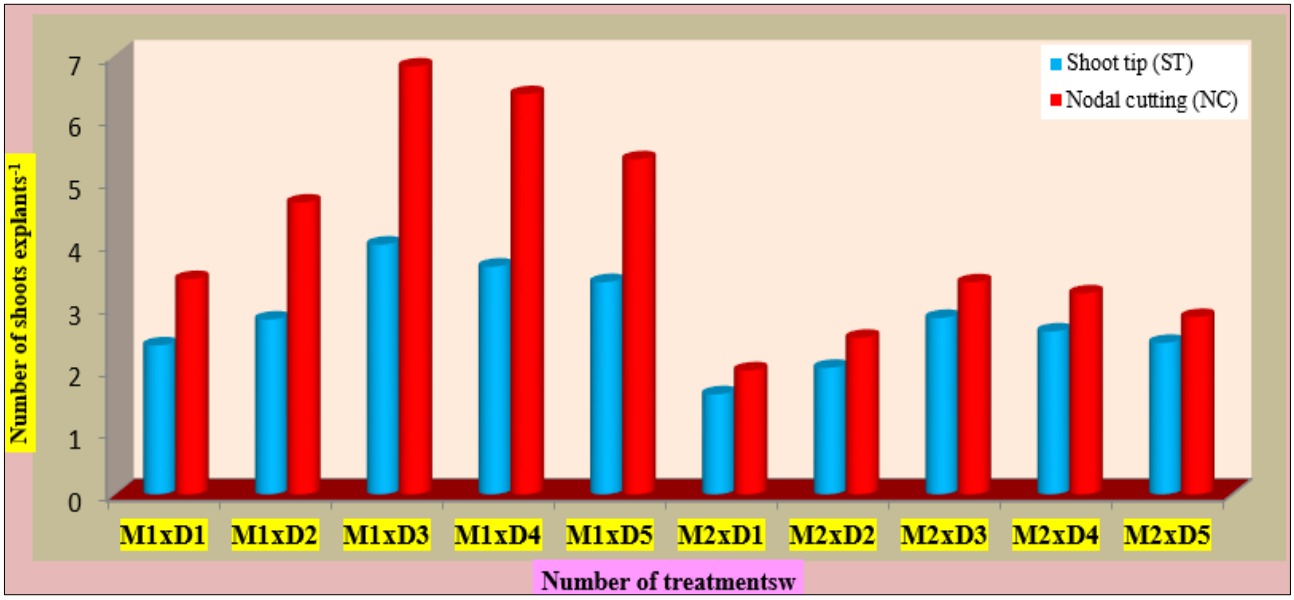

Fig 7: Number of shoots explants^{-1} of Brahmi at 4th Week of 1st experiments

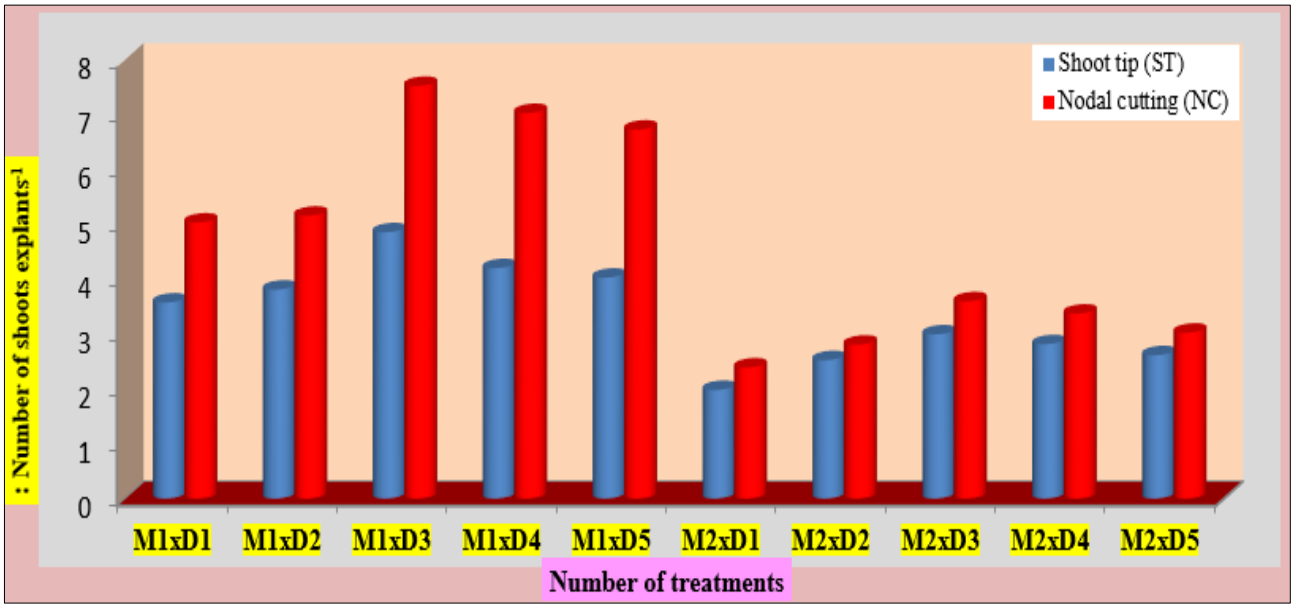

Fig 8: Number of shoots explants^{-1} of Brahmi at 4th Week of 2nd experiments

M1	MS media	D1	1 mg/L BAP
M2	B5 media	D2	2 mg/L BAP
D	Doses	D3	3 mg/L BAP
		D4	4 mg/L BAP
		D5	5 mg/L BAP

Number of shoots explants^{-1} of brahmi in 4th week reveal that (Figure 7 & 8) significant difference varied among explants both shoots tip and nodal cutting. Media and different doses of BAP explants nodal cutting showed significant highest and MS media took significantly maximum number of shoots seems to be in dose 3mg/l BAP among other doses. During both experiments a similar finding was also corroborated by (Kumari *et al*., 2010) [10]

Effect of plant growth regulators on shoot proliferation explants in brahmi at 4th week interaction effect of explants and media, media and doses showed significant effect over number of shoots in brahmi. Interaction between explants and doses was also showed significant. Interaction effect it has been presented in result earlier during both experiments.

Demand of brahmi has increased many fold in result past due to its highly priced medicinal properties. Number of shoots explants^{-1} of brahmi a in 5th week reveal that (Figure 9 & 10) significant difference varied among explants both shoots tip and nodal cutting. Media and different doses of BAP explants nodal cutting showed significant highest and MS media took significantly maximum number of shoots seems to be in dose 3mg/l BAP among other doses. During both experiments a similar finding was also corroborated by (Behera *et al*.2015)

Effect of plant growth regulators on shoot proliferation explants in brahmi at 5th week interaction effect of explants and media, media and doses showed significant effect over number of shoots in brahmi. Interaction between explants and media was showed non-significant effect at 5%. Interaction effect it has been presented in result earlier during both experiments.

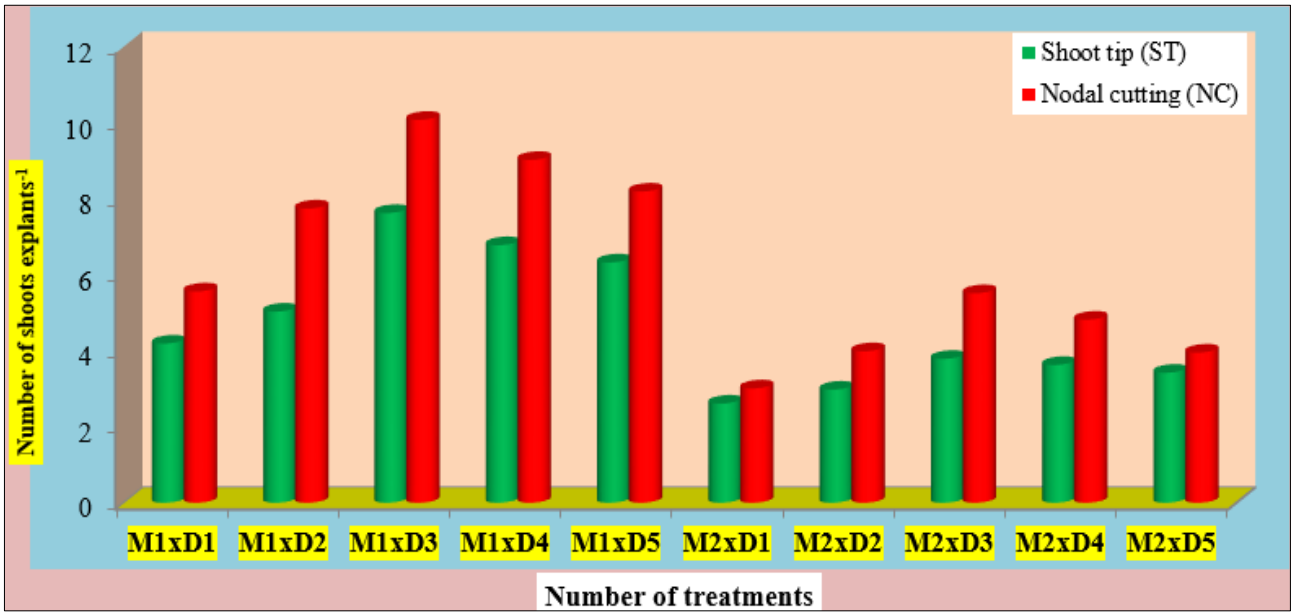

Fig 9: Number of shoots explants^{-1} of Brahmi at 5th Week of 1st experiments

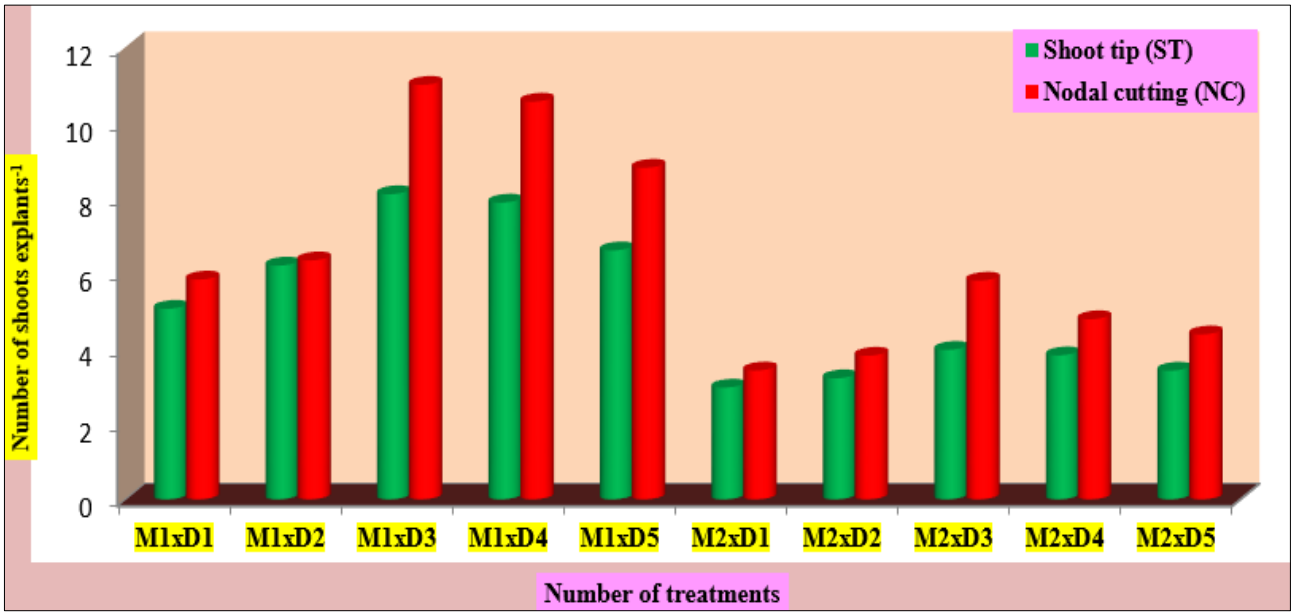

Fig 10: Number of shoots explants^{-1} of Brahmi at 5th Week of 2nd experiments

M1	MS media	D1	1 mg/L BAP
M2	B5 media	D2	2 mg/L BAP
D	Doses	D3	3 mg/L BAP
		D4	4 mg/L BAP
		D5	5 mg/L BAP

Data pertaining to number of shoots explants^{-1} of brahmi in 6th week reveal that (11 and 12) significant difference varied among explants both shoots tip and nodal cutting. Media and different doses of BAP explants nodal cutting showed significant highest and MS media took significantly maximum number of shoots seems to be in dose 3 mg/L BAP among other doses. During both experiments a similar finding was also corroborated by (Tiwari, K.N *et al.*, 2012) [13]

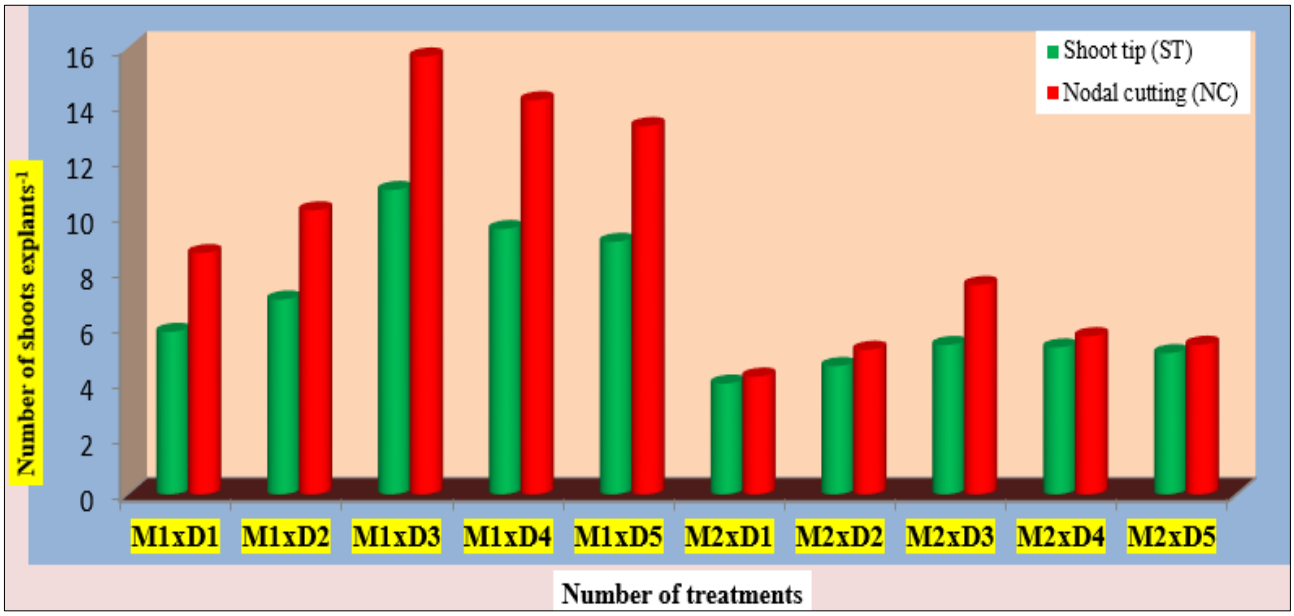

Fig 11: Number of shoots explants^{-1} of Brahmi at 6th Week of 1st experiments

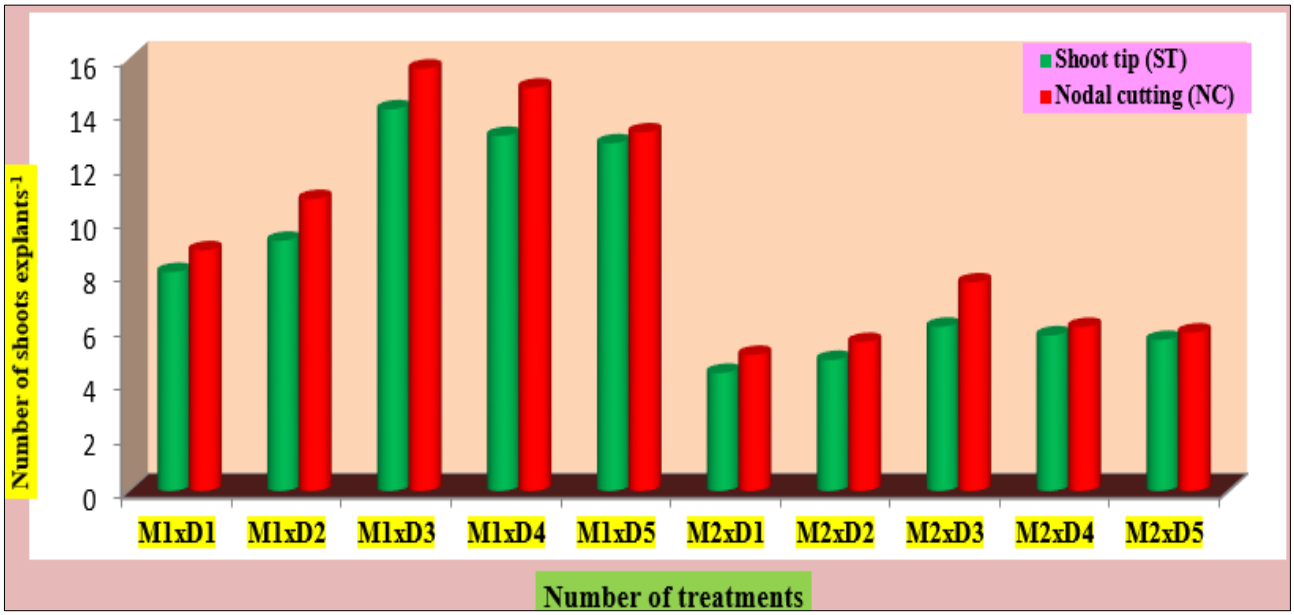

Fig 12: Number of shoots explants^{-1} of Brahmi at 6th Week of 2nd experiments

M1	MS media	D1	1 mg/L BAP
M2	B5 media	D2	2 mg/L BAP
D	Doses	D3	3 mg/L BAP
		D4	4 mg/L BAP
		D5	5 mg/L BAP

Effect of plant growth regulators on shoot proliferation explants in brahmi at 6th week interaction effect of explants and media, media and doses showed significant effect over number of shoots in brahmi. Interaction between explants and doses was also showed significant. Interaction effect it has been presented in result earlier during both experiments.

Conclusion

Explant nodal cutting (NC) as compare to shoot tip (ST) was found most suitable and increased the proliferation of Brahmi. MS medium supplemented with doses of 3mg /L BAP among other doses given best response for shooting as compare to B_5 media of Brahmi.

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