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ORIGINAL ARTICLE

Micropropagation of Brahmi for large scale production and conservation in BA and IBA supplemented media

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ABSTRACT

Brahmi (Bacopa monnieri (L) Penn.) is a commercially important high value medicinal plant. Due to high level of exploitation for medicinal industry its conservation strategy is very important. This paper is an attempt to develop an in vitro technique for conservation and large scale production of this plant. BA and IBA were taken as PGR supplements and two types of explants node with axillary bud and whole leaf were taken as explants. MS and B_5 media were tested for their comparative study. Both media gave direct regeneration with BA and IBA in a ratio of 10:1. But results were superior in MS medium with 1.0+0.1mg l^{-1} of BA plus IBA and nodal explants with axillary bud were better explant giving an average of 157.00 plantlets transferable to soil in 120 days.

***Key words**: Regeneration, MS medium, B_5 medium, 6-Benzyle Adenine (BA), Indol-3-butyric Acid (IBA)*

INTRODUCTION

Brahmi (*Bacopa monnieri* (L) Pennall) is a small creeper of Scrophulariaceae family, having lathery, light green opposite leaves of 1-1.5 cm length and white solitary flowers; the plant extracts has a bitter taste.

Brahmi with a number of traditional medicinal uses, over 3,000 years old Ayurveda mentions its uses, specially as memory vitalizer. Traditionally it is being used in bronchitis, chronic coughs, asthma, hoarseness, arthritis, rheumatism, back-ache, inflammatory condition and fluid retention, epileptic fits, depression etc. In 1963, Central Drug Research Institute (CDRI) of India extracted two active molecules Bacoside A and Bacoside B from it. Later on brahmine, herpestaine ($C_{34}H_{46}N_2O_6$) and mixture of 3 bases were discovered. All these discoveries have increased the importance of the plant in pharmaceutical Industries. But the whole requirement of *Bacopa* is mainly made from natural population, moreover it is a bad competitor so that it can colonies only in space free from other more aggressive species (Shah 1965). This has lead to its fast decrease in population, creating the necessity of its conservation as well as large scale production to meet the high demand. In this regard plant tissue culture can prove as a very important tool.

For successful tissue culture, type of explants is an important aspect. Different plant organs like seed, seedling parts, axillary and apical meristem, leaf etc. are used for the purpose. Many medicinal plants are propagated through *in vitro* organogenesis from nodal axillary bud meristem eg. *Withania somnifera* (Sen & Sharma 1991), *Simmondsia chinensis* (Sardana & Batra 1998), *Rauvolfia serpentina* (Deka *et al.*, 2000), *Adenophylla triphylla* (Chen *et al.*, 2001). Successful regeneration from leaf explant has been reported in many cases viz. *Glycine max* (Wright *et al.*, 1987), *Arachis hypogea* (Eapen *et al.*, 1993), *Vigna aconitifolia* (Malabadi 2002a), *Clitoria ternatea* L (Malabadi 2002b), *Withania somnifera* L. (Fatima & Anis 2012), *Labisia pumila* (Ling *et al.*, 2013). For this paper both these explants were tried.

There are many reports of callus mediated *in vitro* regeneration of medicinal plants (Sarasan *et al.*, 1994; Lusia & Rojas 1996; Ahroni 1997; Castillo & Jordan 1997; Devi & Sarma 2004; Devi & Sarma 2009; Devi & Sarma 2013). But in callus mediated organogenesis the genotype may get changed as polyploidy occurs in callus regeneration. This paper is an attempt to evaluate best protocol for the direct regeneration of *Bacopa* in *in vitro* culture without formation of intermediate callus.

MATERIAL AND METHOD

Two media, Murashige & Skoog medium (1962) and Gamborg B_5 medium (1968) were tested supplemented with different concentrations of 6-Benzyle Adenine (BA), Indol-3-butyric Acid (IBA). Two explants, node with axillary bud and whole leaf were used for the purpose. 1cm. long node with axillary bud and whole leaf were taken as explants. Materials were first cleaned under tap water. Then surface sterilized first with 70 per cent alcohol followed by 0.01 per cent $HgCl_2$ solution. Single explant, one in each flask was inoculated on sterilized media under aseptic condition. Media were supplemented with different concentrations and combinations of BA and IBA together. The pH of media was adjusted to 5.7 and media were sterilized under 16 lb pressure at 121°C. Culture tubes were incubated at the temperature of 25±1°C under 12hr. photoperiods /day (2500-3000 lux). During the whole procedure aseptic condition was maintained. The three repetitions were done and mean and standard error calculated by standard formula.

RESULTS AND DISCUSSION

The node with axillary bud explants in MS medium with of BA plus IBA at the two combinations of concentrations exhibited spectacular results at 0.1mgl^{-1}+0.01mgl^{-1} and 1.0 mgl^{-1}+0.1mgl^{-1} producing 100.33 and 156.67 number of shoots respectively in 120days. In this medium multiple shoots directly developed from the nodal explants in 60 days with 0.1mg l^{-1} of BA and 0.01mg l^{-1} of IBA.

In 120 days 100 numbers of shoots transferable to soil were obtained. Same explants in same medium started direct regeneration of multiple shoots in 15days with 1.0 mg l^{-1} of BA and 0.1mg l^{-1} of IBA. On 120 days this culture gave on an average 157 number of plantlets transferable to soil. Other combinations did not yield such positive results (Table 1). The regenerated plantlets in MS medium with 0.1mg l^{-1} +0.01mg l^{-1} of BA plus IBA shoots, internodes, leaves and roots attained average length of 6.10, 1.03, 0.73 and 4.90 cm respectively where as in 1.0mg l^{-1} +0.1mg l^{-1} of BA plus IBA the same parameters showed average length of 9.67, 1.20, 1.13 and 3.10 cm respectively (Table 2).

In B_5 medium also node with BA plus IBA, above two concentrations gave satisfactory results with axillary bud explants. Multiple shoots developed in 13 days. In the combination of 0.1mg l^{-1} +0.01mg l^{-1} and 1.0mg l^{-1}+0.1mg l^{-1} of BA plus IBA, in an average 48.67 and 73.67 number of plantlets were regenerated in 120 days. In all other combinations single shoot developed only (Table 1).

In terms of plantlet quality in B_5 medium with 0.1mg l^{-1} +0.01mg l^{-1} of BA plus IBA shoots, internodes, leaves and roots attained average length of 2.93, 0.43, 0.73 and 3.60 cm respectively where as in 1.0 mgl^{-1}+0.1mg l^{-1} of BA plus IBA the same parameters showed average length of 5.70, 1.07, 0.90 and 2.93 cm respectively (Table 2).

The leaf explants gave 40 plantlets by direct regeneration in MS medium supplemented with 0.1 mgl^{-1} BA and 0.01 mg l^{-1} IBA in 120 days. Multiple shoot formation started in 48 days. Shoots, internodes, leaves and roots attained average length of 4.73, 0.90, 0.83 and 4.73cm respectively. In the same medium supplemented with 1.0 mg l^{-1} BA and 0.1 mg l^{-1} of IBA leaf explants regenerated multiple shoots in 15 days and in 120 days 72 plantlets transferable to soil were obtained. Shoots, internodes, leaves and roots attained in an average length of 8.60, 1.07, 1.03 and 3.13 cm respectively (Table 1 & 2).

In B_5 medium with 0.1 mgl^{-1} BA and 0.01 mgl^{-1} IBA resulted in formation of 12.67 number of plantlets directly from the leaf explants. Shoots, internodes, leaves and roots attained average length of 4.07, 0.77, 0.67 and 4.10cm respectively. With same medium and explants in 1.0 mgl^{-1} BA and 0.1 mgl^{-1} IBA, 23 number of plantlets were formed in 120 days. Shoots, internodes, leaves and roots attained a length of 3.67, 0.67, 0.77 and 3.15cm respectively in an average (Table 1 & 2).

If the rooting parameters were seen then roots developed in high amounts from both the explants in both the medium. The rooting was observed in the same regenerating medium and quite favourable in this PGR supplements in all the concentrations tried. In node with axillary bud explants the rooting was very profuse and they were counted as root per plantlet as it was counted at the time of transfer of plantlets to the soil. In MS media with 0.1 mg l^{-1} + 0.01 mg l^{-1} of BA plus IBA rooting was in an average 14.67 per plantlet, where as with 1.0 mg l^{-1} +0.1mg l^{-1} of BA plus IBA, it

was 5.33. In B_5 medium, same explants with same concentration of PGRs formed 12.33 and 6.33 roots per plantlet. From leaf explants root developed in bunch on the lower side of explants and shoot as a bunch on the upper side. In MS medium in an average 51.67 and 69.33 roots were formed per leaf explants with 0.1 mg l^{-1} +0.01mg l^{-1} and 1.0 mg l^{-1} +0.1mg l^{-1} of BA plus IBA. In B_5 medium same concentrations of PGRs leaf explants formed 13.67 and 15.33 roots per explant. At the time of separating the plantlets care was taken that each plantlet gets separated with few roots. Number of roots increased with increase in IBA concentrations. Best results were observed in MS medium supplemented with 1.0 mg l^{-1} each of BA and IBA (an average 15.00) and 0.1+1.0 mg l^{-1} of BA plus IBA (an average of 27.67 roots), although no multiple shoots were seen in these combinations (Table 3).

Table 1: Mean number of shoots regenerated in from two explants in two media with different concentrations of BA plus IBA

PGRs (mg l^{-1})		Mean no. of shoots per explant			
		Observation after 120 days			
		Node with axillary bud explant		Leaf explant	
BA	IBA	MS medium	B_5 medium	MS medium	B_5 medium
0.1	0.01	100.33 ± 3.38	48.67 ± 0.88	39.67 ± 2.90	12.67 ± 0.88
0.1	0.1	1.33 ± 0.33	1.33 ± 0.33	0.00 ± 0.00	0.00 ± 0.00
0.1	1.0	1.67 ± 0.33	1.33 ± 0.33	0.00 ± 0.00	0.00 ± 0.00
1.0	1.0	3.33 ± 0.33	1.33 ± 0.33	0.00 ± 0.00	0.00 ± 0.00
1.0	0.1	156.67 ± 4.41	73.67 ± 2.33	71.67 ± 2.04	23.00 ± 2.04

Table 2: Variation in different parameters of the plantlet regenerated in BA plus IBA

PGRs (mg l^{-1})	Length in cm measured at the time of transfer to the pot							
	Observation after 120 days							
	Node with axillary bud explant				Leaf explant			
	MS medium		B_5 medium		MS medium		B_5 medium	
BA plus IBA	0.1+0.01 mg l^{-1}	1.0+ 0.1 mg l^{-1}	0.1+ 0.01 mg l^{-1}	1.0+ 0.1 mg l^{-1}	0.1+ 0.01 mg l^{-1}	1.0+ 0.1 mg l^{-1}	0.1+ 0.01 mg l^{-1}	1.0+ 0.1 mg l^{-1}
Length of Shoot (in cm.)	6.10±0.20	9.67±0.44	2.93±0.08	5.70±0.12	4.73±0.12	8.60±0.20	4.07±0.21	3.67±0.20
Internode Length (in cm.)	1.03±0.03	1.20±0.05	0.43±0.03	1.07±0.03	0.90±0.06	1.07±0.03	0.77±0.03	0.67±0.06
Length of leaf (in cm.)	0.73±0.03	1.13±0.03	0.73±0.03	0.90±0.06	0.83±0.03	1.03±0.06	0.67±0.03	0.77±0.03
Length of root (in cm.)	4.90±0.05	3.10±0.12	3.60±0.15	2.93±0.23	4.73±1.02	3.13±0.14	4.10±0.20	3.13±0.14

(Mean of three repetitions taken and Standard Error calculated)

Table 3: Mean number of roots regenerated in from two explants in two media with different concentrations of BA plus IBA

PGRs (mg l^{-1})		Mean no. of roots per explant			
		Observation after 120 days			
		Node with axillary bud explant		Leaf explant	
BA	IBA	MS medium	B_5 medium	MS medium	B_5 medium
0.1	0.01	14.67 ± 1.45*	12.33 ± 1.45*	51.67 ± 1.28	13.67 ± 0.88
0.1	0.1	5.33 ± 0.33	5.33 ± 0.33	0.00 ± 0.00	0.00 ± 0.00
0.1	1.0	27.67 ± 1.45	11.67 ± 1.66	0.00 ± 0.00	0.00 ± 0.00
1.0	1.0	15.00 ± 1.15	8.33 ± 0.88	0.00 ± 0.00	0.00 ± 0.00
1.0	0.1	5.33 ± 0.88*	6.33 ± 1.28*	69.33 ± 1.20	15.33 ± 1.20

(* roots per plantlets)

The direct multiple regeneration was observed from both the explants in both the media supplemented with BA plus IBA at the concentration of 0.1 mgl^{-1}+0.01mgl^{-1} and 1.0 mgl^{-1}+0.1mgl^{-1}.

In all other concentration tried no multiple shoot developed. In terms of number of regenerated plantlets as well as quality of the regenerated plantlets the 10:1 ratio of BA and IBA is very much fruitful but higher concentration of the PGRs at 1.0 mg l^{-1}+0.1 mg l^{-1} was more useful. Of the two media tried MS proved to be better medium as in all regenerative concentrations the results were superior MS medium with both the explants. Of the two explants tried node with axillary bud proved to be better explant then leaf explants for regeneration of *Bacopa*.

Fig. 1: Graphical comparison of shoot regeneration from two explants in two media

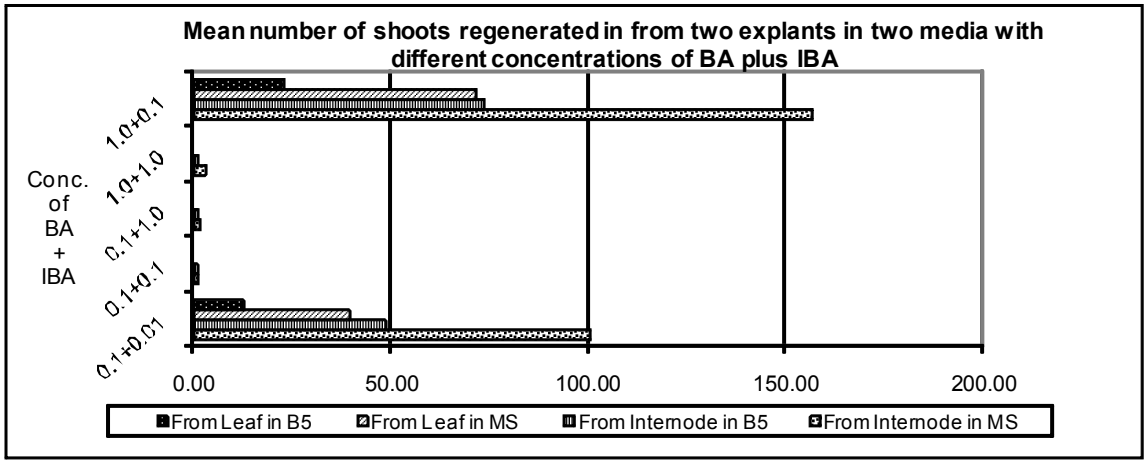
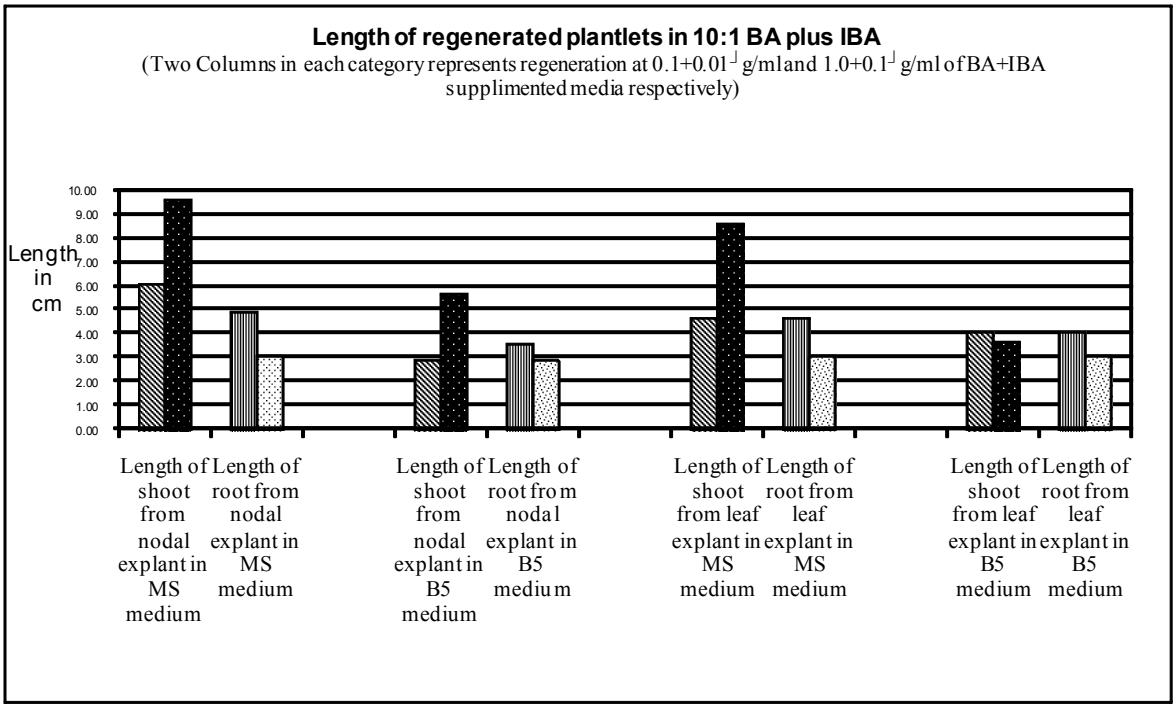


Fig. 2: Graphical comparison of plantlets generated from two explants in two media



The results indicate that 10:1 ratio of BA: IBA as an effective concentration for direct multiple shoot formation from both the explants in both the media. This result are in conformity with nodal culture of *Momordica dioica* Roxb., where a particular ratio of BA and IBA (2:1) resulted multiple shoots and number of regeneration increased in multiplication of hormone (Pawar *et al.*, 2004).

The leaf of *Bacopa* was used successfully as explant for *in vitro* regeneration. For leaf explants 10:1 ratio of these PGRs were fruitful combination for regeneration in both the media and no regeneration occurred in any other concentrations tried. An increase in number of regenerated

shoots and quality of plantlets in terms of size of shoot, leaf and root was observed with increase in concentration of BA and IBA provided ratio remains 10:1. Increase in concentration of BA improved results in sweet potato (Bordoloi & Sarma 1997). Success of leaf explants in regeneration has been demonstrated in many cases viz. in *Ascocenda* (Vij & Kaur 1999), *Albizzia* (Ramamurthy & Savithramma 2003) and strawberry cultivars (Singh & Dubey 2003). But this result of direct regeneration was in contrary to BA-IBA effect of leaf of sweet potato where callusing has been extensive (Bordoloi 1994). In this study, MS was better medium than B_5 in terms of plantlet quality, number of regenerated plantlets and time required for regeneration. Similar results in MS medium with BA and auxin (NAA) as proved optimum for direct organogenesis from leaf explants in the form of shoots and microshoots, on *Beta* sp. (Detrez *et al.*, 1988), sweet potato (Bordoloi & Sarma 1997) and Indian spinach (Mitra & Mukharjee 2001).

But the result from node with axillary bud explant was far better than the results leaf explants. Best results were obtained in both the media supplemented with of 0.1 mg l^{-1}+0.01mg l^{-1} and 1.0 mg l^{-1}+ 0.1 mg l^{-1} of BA plus IBA, a ratio of 10:1 of cytokinin and auxin.

On comparing the results from both the explants and in both the media in the two fruitful concentrations of BA+IBA, it is observed that nodal explant in 1.0+0.1mgl^{-1} of BA+IBA in MS medium show best results, even in B_5 medium this concentration with the same explant show batter results then any regeneration from leaf explants. The next best regeneration result from same explant in MS media is at 0.1+0.01 mg l^{-1} of BA +IBA. From the comparison it is clear that node with axillary bud is batter explant than whole leaf of *Bacopa*. Similar superiority of stem explants over the leaf explants was observed in *Labisia pumila* (Ling *et al.*, 2013). Ling *et al.*, (2013) observed that it may be due to the fact that, in plants, cytokinin is usually translocated from root to shoot via xylem vessels (Kuroha & Satoh 2007). Hence, the presence of endogenous cytokinin in the stem tissues (Nandi *et al*., 1990) with the addition of auxin into the medium might have eventually promoted the formation of shoot from the explants. This was shown by Su *et al*., (2011) who found that a low concentration of auxin combined with cytokinin might aid in shoot initiation. One of the advantages of adding auxin at lower concentration on the culture media is to nullify the effect of the higher concentration of cytokinin on axillary shoot elongation (Hu & Wang 1983). Hence, the ratio of cytokinin to auxin is a critical determinant of organogenesis in plant tissue culture (Xu *et al*., 2008). A lower concentration of auxin along with a higher concentration of cytokinins was most promising for the induction and multiplication of shoots in *W. somnifera* from both the explants (Fatima & Anis 2012). Auxin exerts an effect on DNA replication, while cytokinin seems to exert some control over the events leading to mitosis (Pasternak *et al*., 2000). Therefore, auxins might be considered as 'inducers' of the all cycle (Fatima & Anis 2012) while cytokinins might behave more as its 'promoter' (Wood *et al*., 1990). The presence of axillary bud, the high concentration of cytokinin and lower ratio of auxin in the media might have induced formation of more shoot primordial instead of growth of single axillary bud in node with axillary bud explants and the xylem transport of the cytokinin helps in quick transport of PGRs in the explants to get quicker results.

CONCLUSUION

Hence, from the above discussion we can conclude that nodal explants cultured in MS medium in a ratio of 10:1 of BA plus IBA, specially at 1.0+0.1 mg l^{-1} concentration can be fruitful for successful direct regeneration of *Bacopa* for large scale production and conservation.

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