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RESEARCH ARTICLE

EFFECT OF MEDIUM IN SHOOT TIPS AND NODAL SEGMENTS OF *Andrographis paniculata*

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Selection of an appropriate culture medium and the use of correct growth regulators were critical for the optimum growth response of the explants. Three different media formulations Murashige and Skoog (MS), Gamborg (B_5) and Nitsch were tested to find out the best nutrient composition for successful micropropagation of *A. paniculata*. In MS medium initial bud break was observed within 5 days of culture with a maximum average of 2.2 shoots after 30 days of culture. In Gamborg's medium slight greening was observed but multiple shoot formation was entirely absent after 30 days of culture whereas, no shoot induction was found in the Nitsch medium. After selecting the basal medium the response of establishment of shoot tips and nodal segments in ½ and full strength MS medium was investigated. The rate of shoot proliferation was found higher in ½ MS as compared to full MS medium. In full strength MS the rate of survivability of the explants was poor moreover, browning of the explants and stunted growths were observed. Shoots tips were found to produce lesser number of multiple shoots than the nodal explants. Rooting was achieved in 0.5mg/l IAA or IBA. The regenerated plants were successfully acclimatized in the greenhouse and transferred to field with 98% survival rate.

Key words: *Andrographis paniculata*, Murashige and Skoog, Gamborg, Nitsch, Micropropagation.

INTRODUCTION

Andrographis paniculata (Burm. f) Nees, also called as Kalmegh or "King of Bitters" belongs to the family *Acanthaceae*. It is an annual, branched, herbaceous plant erecting to a height of 30-110 cm in moist shady places (Mishra *et al.*, 2007). With reference to trade an estimated consumption of *A. paniculata* aerial parts is 250 tones (Sharma *et al*., 2008; Kataky and Handique, 2010a). The demand of Kalmegh is increasing day by day (Chauhan et al., 2009) as high demand for andrographolide by the pharmaceutical industries is largely met by extraction of the compound from wild populations; however, the commercial exploitation of this compound is hampered due to its limited availability (Kanjilal *et al.*, 2002; Purkayastha *et al*., 2008; Kataky and Handique, 2010b). The heavy demand of andrographolide in Indian as well as international markets has motivated Indian farmers to start commercial cultivation of this medicinal plant (Kanjilal *et al*., 2002). Conventional propagation of this species is limited to vegetative means, which is difficult and slow in meeting the commercial quantities required (Martin, 2004; Purkayastha *et al*., 2008; Kataky and Handique, 2010b). Variability among the seed-derived progenies and scanty and delayed rooting of seedlings curbs its propagation via seeds (Martin, 2004; Purkayastha *et al*., 2008; Kataky and Handique, 2010b). Thus, micropropagation is the proven method for efficient *in vitro* propagation of medicinal and aromatic plants and for commercial exploitation of valuable plant-derived pharmaceuticals (Bajaj *et al*., 1988; Purohit *et al*., 1994; Pattnaik and Chand, 1996; Rout 2002; Faisal *et al.*, 2005; Purkayastha *et al*., 2008). Although *in vitro* propagation of *A. paniculata* has been published previously by Prathanthurarag *et al*., 1996; Martin, 2004; Natarajan *et al*., 2006; Purkayastha *et al*., 2008 and Kataky and Handique 2010b. The present investigation deals with the impact of different basal medium and their strength on the *in vitro* propagation of *A. paniculata* from shoot tips and nodal segments. Moreover, to overcome the drawbacks associated with the earlier reports and to improve and develop new *in vitro* regeneration systems for *Andrographis paniculata* using nodal and shoot tip explants to meet the needs of the pharmaceutical industries and annual consumption, in limited time and minimum cost.

MATERIALS AND METHODS

The success of micropropagation depends on a number of factors, which affect directly or indirectly on proper establishment of explants in the medium**.** Selection of an appropriate culture medium and the use of correct growth regulators were critical for the optimum growth response of the explants. Three different media formulations MS (Murashige and Skoogs, 1962), B_5 (Gamborg, 1968) and Nitsch, 1967 were tested to find out the best nutrient composition for successful micropropagation of *A. paniculata*. Nodes and shoot tip explants were inoculated in all the three different media with 2mg/l BAP and on the basis of number of shoots developed after 30 days of inoculation; the best medium was selected and used for further studies. After selecting the basal medium the response of establishment of shoot tips and nodal

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segments in ½ and full strength MS medium was investigated.

Statistical analysis: All tissue culture experiments were laid out in a Randomized Block Design (RBD) with 5 replicates of 25 explants and data were analyzed as a two way multivariable analysis of variance and the significance of differences between means were estimated at 5% level of significance.

RESULT AND DISCUSSION

Standardization of an appropriate culture medium with the use of correct growth regulators is critical for the optimum growth response of the explants. Performance of different tissue culture media viz., MS, B_5 and Nitsch's was studied on the nodal and shoot tip explants. On the basis of these results the basal medium was selected and further experiments were designed to achieve the goal. Results revealed that out of the three media formulations tried, in the present study MS gave the best results, requiring the minimum days to initiate maximum number of shoot buds per explant compared to the other two viz., B_5 and Nitsch's (Figure 1 and Table 1). It has been observed that several medicinal plants perform well in MS medium eg. *Adhatoda beddomei* (Sudha and Seeni, 1994) and *Clerodendron colebrookianum* (Mao *et al.*, 1995). Several species of *Limonium* belonging to the plumbaginaceae family were micropropagated in MS medium with different concentrations of growth regulators (Ruffoni *et al*., 2001; Chetia and Handique, 2003).

Although both the composition and amount of growth regulators supplemented in the three media and the physiological state of the explants at the time of inoculation were identical, they showed differences in their establishment and growth which must be due to the differences in salt composition of the original media. Thus, it is difficult to establish by visual observation that why a particular medium produces better shoot growth compared to another. It is likely to be the complex interactions of mineral nutrients, plant growth regulators, gelling agents, and the plant nutritional requirements, uptake mechanisms and other growth factors like light and temperature (Williams, 1999; Borchetia and Handique, 2008). The strength of the MS medium was also studied for multiple shoot induction with nodal segments and shoot tip explants. Full MS medium shows browning of the explant and absence of shoot elongation. Similar results were also reported in *A. paniculata* by Prathanturarug *et al*., 1996 and Purkayastha *et al*., 2008 in full MS medium. However, Prathanturarug *et al*., 1996 reported addition on NAA reduce the number of explants with browing symptoms however it decrease the effect of cytokinin on shoot proliferation in *A. paniculata*. ½ MS medium shows better response compared to full MS in terms of multiple shooting; shoot elongation and proliferation moreover, the symptoms like browning of the explants and stunted growth was also overcome (Kataky and Handique, 2010b) (Figure 2 and Table 2). Suitability of ½ MS basal medium in tea shoot multiplication was reported by earlier workers Phukan and Mitra, 1984; Banerjee and Agarwal, 1990; Pandidurai *et al*., 1996; Borchetia and Handique, 2008 which is in

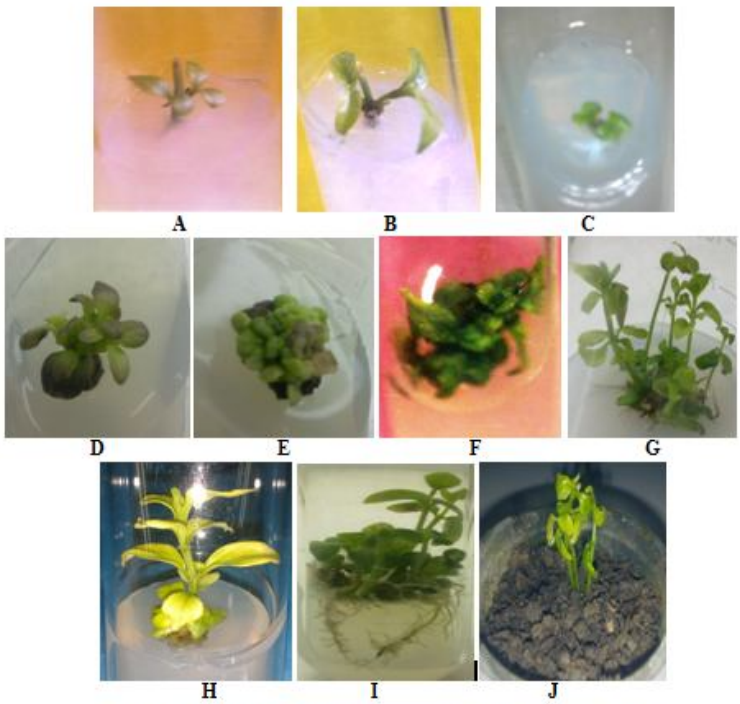

Plate 1: Effect of medium in nodes and shoot tips of *A. paniculata*

A. *Andrographis* in full strength MS medium; **B.** *Andrographis* in ½ strength MS medium; **C.** *Andrographis* in Gamborg's medium after 30 days of culture; **D. & E.** Browning and stunted growth in full MS medium; **F.** Response of shoot tip in ½ MS medium; **G.** Response of nodes in ½ MS medium; **H.** Basal callus in auxin and cytokinin combination; **I.** *In vitro* rooting; **J.** Acclimatization in greenhouse condition.

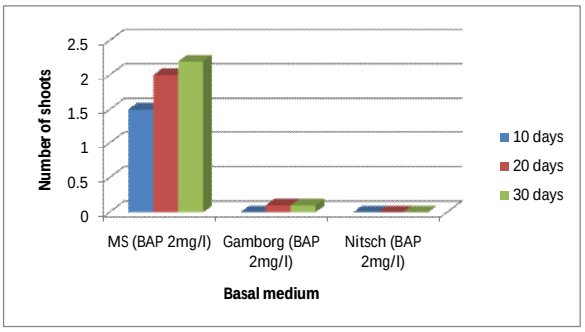

Fig. 1: Effect of BAP on shoot numbers of *A. paniculata* in MS, B_5 and Nitsch's media

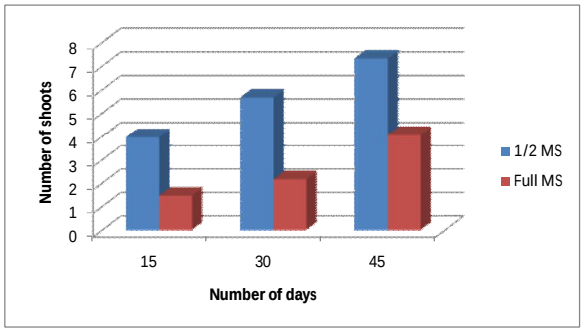

Fig. 2: Response of half and full MS media

This probably reflects difference between the physiological states of the buds on different regions of the stem (Vieitez *et al*., 1985). Hutchinson 1982 for instance, observed that shoot tips are not the best type of explants for maintenance of apple cultures, because most of them produce relatively fewer shoots (Borchetia and Handique, 2008). Different kinds of response shown by different explants may be attributed to the inherent genetic differences (Bhojwani *et al*., 1984). In *N. khasiana*, nodal explants were found to be the best explants for multiple shoot induction (Rathore *et al*., 1991). The differences in response of shoot tips and nodal explants could be the reflection of a probable difference of endogenous growth regulator level in the explants or different tissue sensitivity (Lisowska and Wysokinska, 2000; Chetia and Handique, 2003). Baruah and Das, 1979 observed restricted supply of required growth supplements to the growing primordial of the terminal bud in absence of vascular tissues below the growing shoot tip. It can be a cause which restricts the absorption and translocation of nutrients and growth adjuvants from the medium to the primordial cells of shoot tip explants (Borchetia and Handique, 2008). All the hormones did not respond to the same extent in *A. paniculata* nodal explants. Different factors, such as type of explant, concentration of phytohormone, inorganic concentration in the basal media affected the rate of shoot multiplication *in vitro*.

Table 1: Response of *A. paniculata* in the three basal media tested after 30 days of culture

	Hormone concentration (mg/l)			Mean number of shoots		
	MS	B5	Nitsch			
	-	-	-	2.0	0.00	0.00
BAP	2mg/l	-	-	2.2	-	-
	-	2mg/l	-	-	0.10	0.00
	-	-	2mg/l	-	-	0.00
		ANOVA				
Source of variation		F test			CD at 5%	
Time		34.975**			0.1	
Treatment		1.089 (NS)			0.1	

*Values represented in the table are the mean of 25 replications ** Significant at 5% probability level; NS: Not significant

Table 2: Response of nodal explants in ½ and full MS medium after 45 days of culture

	Hormone concentration (mg/l)	Mean number of shoots
	BAP	
½ MS	-	2.0
	2	7.36
Full MS	-	2.0
	2	4.1
	ANOVA	
Source of variation	F test	CD at 5%
Time	1.845 (NS)	0.2
Treatment	0.861 (NS)	0.2

*Values represented in the table are the mean of 25 replications ** Significant at 5% probability level; NS: Not significant

accordance to the present investigation. In *A. paniculata* shoots tips were found to produce lesser number of multiple shoots than the nodal explants. Nodal explants gives a maximum average of 18.36 shoots after 45 days of culture in ½ MS medium fortified with BAP 1mg/l and AdS 1.5mg/l. Whereas, shoot tips gives a maximum of 8.16 shoots after 45 dats of culture in ½ MS medium fortified with BAP 1mg/l and AdS 1mg/l. In *Morus nigra*, Yadav *et al*., 1990 reported that shoot tip explants show poorer proliferation and elongation than nodal explants. The control showed the development of first shoot after 17-18 days of culture. But no multiple shoot formation was seen indicating that multiple shoot formation depends on the optimum balance of growth regulators in the medium. The requirement of cytokinin for the initiation of primary cultures was reported by Huang *et al*., 1998 in *Cinnamonum camphora*. Chalupa, 1983 and Cao *et al*., 1993 also observed there is no bud multiplication on the basal medium without BAP and the optimum concentration of BAP was 1mg/l for the elongation and

multiplication of diploid black locust (Borchetia and Handique, 2008). In *A. paniculata* effect of a cytokinin along with an auxin gives basal callus and the explant fails to regenerate. IAA and IBA in combination with BAP give brown callus at the base of the shoot tip. Also, IAA or IBA in combination with NAA gives green callus which does not induce shoots but few roots can be seen which fails to elongate. Koroch *et al*., 1997 stated that cytokinin alone is sufficient in some cases to induce bud multiplication from shoot tip and nodal sections as in *Hedoema multiflorum*. However, the balance of auxin and cytokinin is a determining morphogenic factor. When shoot formation occurs on a medium containing a cytokinin alone, the explant must have contained sufficient endogeneous auxin to be capable of its *de novo* synthesis (Julliard *et al*., 1992). Lakshmanan *et al*., 1997 reported higher shoot proliferation in presence of BAP alone, however in presence of IAA shoot proliferation was significantly inhibited. The number of axillary shoots obtained reduced by about 70% upon culture in BAP and IAA supplemented medium and large amount of callus was observed at the base end. The formation of basal callus has been frequently observed in shoot culture of silver maple with strong apical dominance (Preece *et al*., 1991) and attributed to the action of accumulated auxins at the basal ends (Marks and Simpson, 1994). In *Cunila galioides* (Fracaro and Echeverrigaray, 2001) addition of auxin to BAP containing media reduced the number of shoots per explant. Combinations of cytokinin and auxin were found to be essential for optimum shoot proliferation of *Agave parrasana* Berger (Ruvalcaba *et al*., 1999). Patil and Jayanthi, 1997 reported that addition of an auxin with cytokinin did not help in shoot proliferation instead callusing at the base of the explants was seen which suppressed the growth of the *Rouvolfia* shoots (Chetia and Handique, 2003), which is in accordance with the present study. Of all the media tested, medium supplemented with 0.5mg/l IAA or IBA was found to be most effective for rooting of *Andrographis paniculata*. Similar results were also reported in *A. paniculata* by Prathanturarug *et al*., 1996 and Purkayastha *et al*., 2008. Fully grown plants were washed properly with sterilized distilled water in a sterile environment till the agar gets dislodged from the roots and transferred to pots containing sterile mixture of soil, sand and compost at the ratio of 2:1:3 and kept in the green house condition for hardening. Polythene covers ensures high humidity and irrigated with ½ strength sterile liquid MS salts for 10 days which enhances acclimatization. The plants were acclimatized at 25±2°C with 16 hours photoperiod cycle and eventually transferred to the field condition with 98% survivability rate (Plate 1). Thus, considering the need of the pharmaceutical industries, annual consumption, time and cost, delayed propagation via vegetative means and variation among seed derived progenies, the mass propagation of *A. paniculata* can be achieved by selecting the appropriate explant, plant growth regulators, and the culture medium.

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