ASIAN JOURNAL
ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 6, pp.119-122, October, 2010

RESEARCH ARTICLE

STANDARDIZATION OF STERILIZATION TECHNIQUES PRIOR TO *IN VITRO* PROPAGATION Of *Andrographis paniculata* (Burm.f) Nees

Ankita Kataky*[1] and Handique, PJ[1]

[1]Department of Biotechnology, Gauhati University, Guwahati-14, Assam

Received 7th July, 2010; Received in revised form; 25th August, 2010; Accepted 27th September, 2010; Published online 1st October, 2010

The success of micropropagation depends on a number of factors, which affect directly or indirectly on proper establishment of explants in the medium. In *Andrographis paniculata* fungal contamination occurs within 3-4 days of inoculation when the explants were collected from the field grown mother bushes. Spraying of 1000ppm carbendazim, a day prior to the collection controls the fungal contamination. While bacterial contamination can be overcome by surface sterilizing the explant with bleaching powder, broad spectrum antibiotics like amoxycillin, 70% alcohol and the chemical sterilent mercuric chloride. Addition of 200mg/l amoxycillin in the media can reduce the bacterial contamination by 90-95%.

Key Words: Micropropagation, *Andrographis paniculata*, Contamination, Mother bushes, Carbendazim, Amoxycillin. Mercuric chloride

INTRODUCTION

A. paniculata is in demand in terms of its medicinal properties (Kataky and Handique, 2010a). 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; Purkayastha *et al.,* 2008). Conventional propagation of this species is limited to vegetative means, which is difficult and slow in meeting the commercial quantities required. Variability among the seed-derived progenies and scanty and delayed rooting of seedlings curbs its propagation via seeds, Martin (2004). So alternatiFve techniques like micropropagation holds potential for producing large number of plantlets (Kataky and handique, 2010b). The success of micropropagation depends on a number of factors, which affect directly or indirectly on proper establishment of explants in the medium. Microbial contamination is a constant problem, associated with *in vitro* propagation of *Andrographis paniculata.* The nutrient media in which the plant is cultivated is a good source of nutrient for microbial growth. These microbes compete adversely with plant tissue culture for nutrient. The presence of these microbes in these plant cultures usually results in increased culture mortality, the presence of latent infections can also result in variable growth, tissue necrosis, reduced rooting (Kane, 2003; Odutayo *et al.,* 2007). In the present investigation, the seasonal variation and age of the explant also affects the survivability of *A. paniculata* in culture. Therefore, a systematic study was made considering all these factors to standardize a suitable sterilization protocol for rapid micropropagation of *Andrographis paniculata.*

***Corresponding author:** *ankita_lon03@rediffmail.com*

Establishment of field grown explants in culture medium

Explants prepared from field grown mother plant were treated with a number of antibiotics and fungicides to control the bacterial and fungal contamination respectively. A series of experiments were carried out to establish contamination free explants from field grown mother plants in the culture medium which are discussed below.

Effect of pretreated mother plant with fungicide:

In *A. paniculata* fungal contamination occurred within 3-4 days of inoculation and led to the death of the explant. An alternative to reduce the fungal contamination was however found when explants were pretreated with 1000 ppm of carbendazim on day prior to

collection of explants from field. The fungal contamination was totally controlled in carbendazim sprayed explants with proper surface sterilization. Moreover, it is less lethal to the mother plant. (Murali *et al.,* 2001) also reported their success in establishment of the contamination free explants in culture medium when tea bushes were sprayed with fungicides prior to collection of the materials. Efficacy of pre-chemical treatments to the mother plant was reported in many plants viz., apple (da Camara Machado *et al.,* 1991), coffee and cocoa (Duhem *et al.,* 1988) which is accordance to the present investigation. However, when the explants were collected from the greenhouse conditions 95% survivability rate was observed. Moreover, the bacterial and fungal contamination was totally controlled.

Explant sterilization

The shoot tips and nodal explants from field grown mother bushes maintained in the green house Department of Biotechnology, Gauhati University were washed under running water for 30 minutes. The explants were then soaked in 0.1% solution of bleaching powder containing 2-3 drops of tween 20 (v/v) for 8 minutes with constant stirring and then rinsed with distilled water for 4-5 times. The shoot tips and nodal explants were then disinfected with 0.1% solution of carbendazim for 8 minutes with constant stirring and washed several times with double distilled water. The explants were swirled in 0.2% amoxycillin solution for 10 minutes. The explants were then washed several times with sterile double distilled water in a laminar air flow cabinet and rest sterilization was done under it. The explants were thereafter surface sterilized with 0.1% mercuric chloride for 2 minutes followed by thorough rinsing with sterile double distilled water for 4-5 times. The explants are then treated with 70% alcohol for 30 seconds followed by a thorough rinse with sterile double distilled water. Damaged cut ends were trimmed on the either side using a sterile blade into 1.0-1.5cm pieces and explants were used for inoculation in culture medium (Kataky and Handique, 2010).

Monthly variation of explants survivability in cultures

It was observed that explants when collected during dry spells of the year showed lower bacterial contamination rates compared to rainy spells. Survivability of cultures gradually increased, due to lower contamination rates, from the month of August (75%) and September (75%) to the highest in the month of October, November, December and January of (95%). A slightly lower survivability rate was recorded in the month of February (80%) and March (75%). A low rate of survivability was recorded with the onset of rainfall during April (70%) which further reduced from May (60%) and a minimum of 50% in June and July (Figure 1). Bacterial contamination could be controlled upto 95% depending on the explants collection time. It was observed that the explants collected during the dry spells of the year showed only 5% bacterial contamination. In contrast to the observations in this study Amin and Jaiswal, 1993; Mohan *et al*.,1997 reported high percentage of plantlet formation during winter months. Evers *et al.,*1988 correlated bud sprouting in culture to the season in which nodal sectors are isolated from donor plants. When isolated in summer they show good response in culture, whereas resting buds on explants isolated in winter often fail to sprout, inspite of manipulating the growth regulator concentrations in the medium.

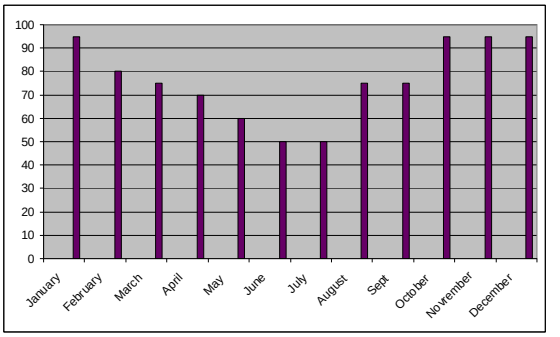

Fig. 1. Monthly variation in rate of survival of explants of *A. paniculata* in culture

In *Gmelina arborea* Roxb, explants isolated from seedlings showed sprouting of auxillary buds irrespective of season (Thakur and Bhargava, 1999). *In vitro* establishment and proliferation of explants from field grown trees of *Artocarpus heterophyllus* were also influenced by the season of the year during which the explants were collected. November to January were found to be the best months and yielded 100% bud break and only 5% explants death after 8 weeks of culture.

Effect of explant to surface sterilants

Both fungal and bacterial contamination was recorded higher in *Andrographis paniculata* when collected from the field conditions compared to the greenhouse condition ones. Due to heavy contamination by bacteria and fungi, after 3-4 days of inoculation, a pretreatment with the surface sterilants was done. To obtain contamination free explants for culture, the fungicide, carbendazim, bleaching powder, the broad spectrum antibiotic, amoxycillin, and chemical sterilant $HgCl_2$ was used as surface sterilant. Carbendazim and bleaching powder at 0.1% solution for 8 minutes showed better response. However, higher concentration leads to the death of the explants. Amoxycillin at 0.2% solution is

effective in controlling the bacterial contamination. The chemical sterilants, $HgCl_2$ at a concentration of 0.1% solution when treated for 2 minutes showed best response however higher concentration showed toxicity. Surface sterilization with carbendazim (0.1%) for 8 minutes to normal sterilization procedure, fungal contamination could be controlled upto 98%. Control of fungal contamination by using carbendazim has been reported earlier by Patel *et al.*,1997 in *Momordica dioica* and Reddy *et al.*,1998 in *Gymnema sylvestre*. 0.1% solution $HgCl_2$ was sufficient in establishing the explants in culture medium. The efficacy of $HgCl_2$ solution as surface sterilant in *in vitro* cultures of *A. paniculata* had been reported by Martin, 2004; Purkayastha *et al.*,2008; Kataky and Handique, 2010b).

Effect of antibiotics in controlling bacterial contamination

Role of different antibiotics in the control of bacterial contamination was studied since the endophytic bacteria carried by some of the explants and contaminated the subsequent subcultures. The basal media incorporated with different concentrations and combinations of broad spectrum antibiotics viz., amoxycillin, tetracycline, ampicillin were tested to prevent the cultures from contamination due to endophytic bacteria. Out of the different antibiotics tested amoxycillin at 200mg/l showed positive response. However, higher concentration of the antibiotic incorpotated media showed leaf necrosis, delayed in bud break and lower shoot proliferation rate. However, control of bacterial contamination required inclusion of antibiotics in the media. Although incorporation of antibiotics in the media have not been widely practiced for controlling contamination of plant tissue culture because of their high toxicity to the culture, certain antibiotics viz., tetracycline, ampicillin, carbendazim, cefataximine and chloramphenicol have been reported for their inhibitory effect on explant contamination without phytotoxic effects, Herman (1996). In the present investigation amoxycillin 200mg/l could reduce the bacterial contamination by 90-95% only. An increase in concentration 350mg/l could control bacterial growth but lead to 100% mortality of explants. Okkels and Pederson, 1983 demonstrated that a bacteriostatic effect could obtain with chloramphenicol at 128mg/l but toxicity of the antibiotic against plant tissue was observed at levels as low as 10mg/l. Therefore only those antibiotics should be applied which enable sufficient bacterial inhibition at a concentration level that does not harm the explant.

Effect of age of the explants

To find out the best suitable explants for micropropagation the response of the nodal explants taken from different positions of the branches of the field mother plant were assessed in the best medium found effective in producing the maximum number of multiple shoots. From the field grown mother plant, the shoot tips and axillary buds from 2^{nd} to 6^{th} nodal segments were considered for micropropagation and it was observed that the axillary buds of 2^{nd} to 4^{th} nodal explants produced the maximum number of shoots. Similar study was done in tea by Rajasekharan and Raman, 1993 and they observed lesser number of multiple shoots in the 4^{th} and 6^{th} nodal segment. Due to loss of juvenility, the 7^{th} and 8^{th} node was not considered for micropropagation in the present investigation. In general, the more juvenile the explant material, the greater the likehood of success (Channarayappa, 2006; Kataky and Handique, 2010). Thus, large scale propagation of *A. paniculata* can be achieved by collecting the appropriate explant in dry spells and by controlling the bacterial and fungal contamination in the culture medium.

REFERENCE

Amin, M. N. and Jaiswal, V.S. 1993. *In vitro* respose of apical bud explants from mature trees of jackfruit (*Artocarpus heterophyllus*). *Plant Cell Tiss. Org. Cult.*, 33: 59-65.

Banerjee, M. and Agarwal, B. 1990. *In vitro* rooting of tea, *Camellia sinensis* (L.) O. Kuntze. *Ind. J. Exp. Biol.*, 28: 936-939.

da Camara Machado, M.L., da Camara Machado, A., Hanzer, V., Kalthoff, B., Wei beta, H., Mattanovich D., Regner, F. and Katinger, H. 1991. A new efficient method using 8-hydroxy-quinolinol-sulfate for the initiation and establishment of tissue cultures of apple from adult material. *Plant Cell. Tiss. Org. Cult.*, 27: 155-160.

Duhem, K., Le Mercier, N. and Boxus, P.H. 1988. Difficulties in the establishment of axenic *in vitro* cultures of field collected coffee and cacao germplasm. *Acta Hort*., 225: 67-75.

Evers, P.W., Donkers, J., Prat, A. and Vermeer, E. 1988. In: Micropropagation of forest trees through tissue culture, Centre for Agricultural Publishing and Documentation (Pudoc), Wageningen. 221-242.

Gamborg, O.L., Miller, R.A. and Ojima, K. 1968. Nutrient requirements of suspention cultures of soyabean root cells. Exp. Cell Res., 50: 151-158.

Herman, E.B. 1996. Method of controlling contamination. In: Microbial contamination of plant tissue cultures. Agritech consultants, Inc. Shrub Oak, USA., 13-14.

Kane, M. 2003. Bacterial and fungal indexing of tissue cultures.http://www.hos.ufl.edu/mooreweb/TissueCulture/class1/Bacterial%20and%20fungal%20indexing%20of%20tissue%20culture.doc.

Kanjilal, P.B., Bordoloi, S., Kalita, R., Burman, P. and Singh, R.S. 2002. Cultivation practices for Kalmegh (*Andrographis paniculata*) and Spiderling. In: Recent progress in medicinal plants. Govil JN, Kumar PA, Singh VK (eds). Stadium Press, LLC, USA, 5: 175–180.

Kataky, A. and Handique, P.J. 2010a. Antimicrobial activity and phytochemical estimation of micropropagated *Andrographis paniculata* (Burm. f) Nees. *Asian Journal of Science and Technology*., 5: 91-94.

Kataky, A. and Handique, P.J. 2010b. Micropropagation and screening of antioxidant potential of *Andrographis paniculata* (Burm. f) Nees. *Journal of Hill Agriculture.*, 1(1): 15-20.

Mao, A., Wetten, A., Fay, M. and Caligari, P.D.S. 1995. *In vitro* propagation of *Clerodendron colebrookianium* Walp. A potential natural anti-hypertension medicinal plant. *Plant Cell Rep.,* 14: 493-496.

Martin, K.P. 2004. Plant regeneration protocol of medicinally important *Andrographis paniculata* (Burm.f) Wallich ex Nees via somatic embryogenesis. In Vitro Cell. Dev. *Biol-Plant.,* 40: 204-209.

Mohan, J.S.S., Bhadane, S.V. and Rao, P.S. 1997. A one step method for *in vitro* plantlet formation from shoots of *Dalbergia sisso* Roxb. *Plant Struct. Morph.*, 97: 104.

Murali, K.S., Baby, U.I. and Manivel, L. 2001. Combating microbial contamination and browning in tea culture. *Journal of Plantation Crops.,* 29(1): 55-58.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.,* 473-493.

Nitsch, C. and Nitsch, J.P. 1967. The induction of flowering *in vitro* in stem segments of *Plumbago indica* L. *Planta.,* 72: 355-370.

Odutayo, O.I., Amusa, N.A., Okutade, O.O. and Ogunsanwo, Y.R. 2007. Sources of microbial contamination in tissue culture laboratories in southwestern Nigeria. *Afr. J. Agric. Res.,* 2(3): 67-72.

Okkels, F.T. and Pederson, M.G. 1983. The toxicity of plant tissue and to *agrobacterium tumefaciens* of some antibiotics. *Acta Hort.,* 255: 199-207.

Pandidurai, V., Murali, K.S., Manivel, L. and Rajkumar, R. 1996. Factors affecting *in vitro* shoot multiplication and root regeneration in tea. *J. Plantation Crops.,* 24: 603-609.

Patel, D.R., Subash, N., Patel, A.M. and Patel, S.A. 1997. Mass *in vitro* multiplication of kankoda (*Momordica dioica* L.). *Plant Struct. Morph.,* 15: 105-109.

Phukan, M.K. and Mitra, G.C. 1984. Regeneration of tea shooyts from nodal explants in tissue culture. *Curr. Sci.,* 53: 874-876.

Prathanturarug, S., Schaffner, W., Berger Buter, K. and Pank, F. 1996. *In vitro* propagation of the Thai medicinal plant *Andrographis paniculata* Nees. Proc. Int. symp. Breeding Research on Medicinal and Aromatic plants, Quedlinburg, Germany., pp. 304-306.

Purkayastha, J., Sugla, T., Paul, A., Solleti, S. and Sahoo, L. 2008. Rapid *in vitro* multiplication and plant regeneration from nodal explants of *Andrographis paniculata*: A valuable medicinal plant. In Vitro Cell Dev. *Biol.-Plant.,* 44 (5): 442-447.

Reddy, P.S., Gopal, G.R. and Sita, G.L. 1998. *In vitro* multiplication of *Gymnema sylvestre* R Br. an important medicinal plant. *Curr. Sci.,* 75 (8): 843-845.

Ruffoni, B., Semeria, L., Doveri, S., Savona, M. and Allavena, A. 2001. Tissue culture and *A. rhizogenes* transformation in several ornamental *Limonium*. Abstract published in proceedings of the XLV Italian Society of Agricultural Genetics- SIGA Annual Congress, Salsomaggiore Terme, Italy-26/29 September. 80.

Sudha, C.G. and Seeni, S. 1994. *In vitro* multiplication and field establishment of *Adhatoda beddomei* C.B. Clarke a rare medicinal plant. *Plant Cell. Rep.,* 13: 203-207.

Thakur, J. and Bhargava, S. 1999. Seasonal variation in antioxidant enzymes and the sprouting response of *Gmelina arborea* Roxb. Nodal sectors cultured *in vitro*. *Plant Cell Tiss. Org. Cult.,* 59: 181-187.

Williams, R.R. 1999. *In vitro* ecology - see the light, raised the temperature. In: Johnson KA, McFarlane IJ. (Eds.) Plant tissue culture at the age of the new millennium. Proceedings of the International Association for Plant Tissue Culture and Biotechnology (Australian Branch) Sixth National Meeting, Sydney., pp. 40-52.

****