Acta Horticulturae Home
Login
Logout
Status
Help
ISHS Home
ISHS Contact
Consultation statistics index
Search

ISHS Acta Horticulturae 865: IV International Symposium on Acclimatization and Establishment of Micropropagated Plants

OPTIMIZATION OF *SPILANTHES ACMELLA* L. CULTIVATION BY IN VITRO NODAL SEGMENT CULTURE

Authors: M. Singh , R. Chaturvedi

Keywords: axillary shoot proliferation, clonal propagation, cytokinins, shoot multiplication

Abstract:
An efficient protocol for clonal propagation of *Spilanthes acmella* L., a medicinally valuable plant, has been developed. Nodal explants were collected at monthly intervals to initiate in vitro cultures. More than 60% aseptic cultures were established during the months from January to April. Long, multinodal shoots were developed from the nodes on full strength Murashige and Skoog (1962; MS) basal medium or basal medium supplemented with at least one cytokinin. Irrespective of the treatments, excessive adventitious root proliferation was observed from all over the surface of the in vitro developed shoots and on the explants as well. Of all the treatments, 6-benzylaminopurine (BAP) supported maximum percentage of cultures showing shoot proliferation. Interestingly, higher concentration of BAP resulted in slow growth of shoots while at lower concentration excessive adventitious roots were observed in the cultures, in addition to axillary shoot proliferation. However, intermediate concentration of BAP at 5 µM favored maximum rate of shoot multiplication with no adventitious roots. On MS + 5.0 µM BAP, 10 fold shoot multiplication was achieved every 5 weeks by cutting the solitary in vitro shoot into single node segments and culturing them onto the fresh medium of the same composition. The shoots were successfully rooted on half strength MS medium (major salts reduced to half strength) with 50 g/L sucrose; roots developed directly at the base of the shoot in 100% cultures. Plantlets were hardened and successfully established in the soil. Regeneration protocol developed in this study would provide a basis for germplasm conservation and mass cultivation of the plant for later biotechnological use.

- **Full Text** (PDF format, 350433 bytes)
- **Citation**
- Translate

Select Language

Powered by Google™ Translate

Download Adobe Acrobat Reader (free software to read PDF files)

Optimization of *Spilanthes acmella* L. Cultivation by In Vitro Nodal Segment Culture

M. Singh and R. Chaturvedi[a]
Department of Biotechnology
Indian Institute of Technology-Guwahati
Guwahati - 781039, Assam
India

Keywords: axillary shoot proliferation, clonal propagation, cytokinins, shoot multiplication

Abbreviations: BAP: 6-benzylaminopurine, Kinetin: N^6-furfuryladenine, 2-iP: 2-isopentenyl adenine, NAA: α-naphthalene acetic acid

Abstract

An efficient protocol for clonal propagation of *Spilanthes acmella* L., a medicinally valuable plant, has been developed. Nodal explants were collected at monthly intervals to initiate in vitro cultures. More than 60% aseptic cultures were established during the months from January to April. Long, multinodal shoots were developed from the nodes on full strength Murashige and Skoog (1962; MS) basal medium or basal medium supplemented with at least one cytokinin. Irrespective of the treatments, excessive adventitious root proliferation was observed from all over the surface of the in vitro developed shoots and on the explants as well. Of all the treatments, 6-benzylaminopurine (BAP) supported maximum percentage of cultures showing shoot proliferation. Interestingly, higher concentration of BAP resulted in slow growth of shoots while at lower concentration excessive adventitious roots were observed in the cultures, in addition to axillary shoot proliferation. However, intermediate concentration of BAP at 5 µM favored maximum rate of shoot multiplication with no adventitious roots. On MS + 5.0 µM BAP, 10 fold shoot multiplication was achieved every 5 weeks by cutting the solitary in vitro shoot into single node segments and culturing them onto the fresh medium of the same composition. The shoots were successfully rooted on half strength MS medium (major salts reduced to half strength) with 50 g/L sucrose; roots developed directly at the base of the shoot in 100% cultures. Plantlets were hardened and successfully established in the soil. Regeneration protocol developed in this study would provide a basis for germplasm conservation and mass cultivation of the plant for later biotechnological use.

INTRODUCTION

Spilanthes acmella L. a member of the family Asteraceae, is well known for its medicinal and insecticidal properties. It is a perennial herb and widely distributed in tropics and subtropics. The genus contains plethora of highly valuable bioactive compounds. It possesses anti-inflammatory, antibacterial, antimicrobial, antifungal properties and is used to cure toothache, flu, cough, rabies and tuberculosis (Burkill, 1966; Oliver-Bever, 1986; Ramsewak et al., 1999). These attributes are mainly due to an antiseptic alkaloid, spilanthol, present at a high concentration in the flowers (Khadir et al., 1989; Saritha et al., 2002). Spilanthol is effective against blood parasites at extremely low concentration and is a poison to most invertebrates whereas harmless to the majority of vertebrates (Watt and Brayer-Brandwijk, 1962). The hexane extract of dried flower buds of *S. acmella* is found to be effective against *Aedes aegypti* and *Helicoverpa zea* neonate larvae (Ramsewak et al., 1999).

S. acmella is conventionally propagated through seeds and grows generally in

[a] rakhi_chaturvedi@iitg.ernet.in, rakhi_chaturvedi@yahoo.co.in

moist and wet places, thus, is vulnerable to pest and diseases. In order to produce high value secondary metabolites, a constant source of diseased-free planting material is required. For the purpose, mass propagation of in vitro aseptic plantlets can be achieved which could be utilized as a ready stock to meet the demand of the pharmaceutical industries for the production of bioactive compounds from them. This may also help to steer clear of contaminated pharmaceutical raw materials. Additionally, a rapid and efficient propagation protocol would reduce the pressure on natural population, and thus, may prevent the plant from becoming endangered. However, tissue culture studies on *S. acmella* are in its infancy and so far only two reports are available on micropropagation of this species. Saritha et al. (2002) reported multiple shoot proliferation by adventitious mode from hypocotyl explant. However, chimera formation is very common during adventitious shoot proliferation, which may perpetuate in shoot-buds and cause the change in ploidy sometimes. Additionally, the adventitious bud formation method may require de novo differentiation of shoot-buds, which is not always possible, and wherever it is possible, it makes the rate of propagation slower. In comparison to this, clonal propagation by axillary shoot proliferation favors more uniformly diploid plants where the bud present at priori on the initial explant grows into a multinodal shoot. Thus, it promotes faster rate of multiplication with least genotypic changes. Consequently, Haw and Keng (2003) attempted clonal propagation by axillary shoot proliferation in *S. acmella* but the study lack crucial information on percent culture response, throughout the experiment. However, the present study gives a systematic report on highly reproducible and recurrent method of clonal propagation by investigating: 1) the effect of growth regulators, and 2) whether in vitro shoot formation dependent on season when the nodal segments were collected.

MATERIALS AND METHODS

Plant Material and Initiation of Aseptic Cultures

Plant materials from plants growing wild around the IIT-Guwahati campus were collected at monthly intervals to establish in vitro cultures. Single node segments bearing two opposite axillary buds were used as explants. After removing the leaves, nodal segments were thoroughly washed in 1% (v/v) savlon solution with two drops of tween 20 for 20 minutes followed by rinsing with sterile distilled water (SDW). All subsequent operations were carried out inside the laminar air-flow-cabinet (Saveer Biotech Ltd., India). The clean cuttings were given a quick rinse (30 s) in 90% ethanol before surface sterilizing with 0.1% (w/v) mercury chloride solution for 6 minutes. Each treatment was followed by repeated washing (three times) in SDW. The cuttings were slightly trimmed at both ends to expose fresh tissue before planting them on MS basal medium or basal medium supplemented with growth regulators.

Shoot Proliferation and Culture Conditions

Clean cuttings (2 cm) with two opposite axillary buds were cultured on Murashige and Skoog (MS; 1962) medium containing different concentrations (at 1–15 µM range) of a cytokinin (BAP/Kn/2-iP) alone or in combination with an auxins, NAA (5 µM) to evaluate their effects on in vitro shoot proliferation.

The pH of the media was adjusted to 5.8 before autoclaving at 1.06 kg cm^{-2} and 121°C for 15 minutes. Unless mentioned otherwise, all media contained 30 g/L sucrose and were solidified with 0.8% agar (Hi Media Laboratories, India). The cultures were maintained at 25±2°C temperature with 50–60% relative humidity and a 16/8 h (light/dark) photoperiod provided with diffuse light (1000–2000 lux). Twenty-four cultures were raised for each treatment and each experiment was repeated at least three times. Observations were taken at weekly intervals. Final observation on the number of shoots per explant, number of nodes per shoot, shoot length and percent axillary bud proliferation were recorded at the end of the fifth week, and standard error of the mean was calculated.

Rooting and Transplantation

For rooting, terminal 3 cm long portions from 5-week-old in vitro differentiated shoots were excised and transferred to half-strength MS basal medium containing 50 g/L sucrose. After 4 weeks, rooted shoots were washed with water to remove the agar and were transferred to the plastic pots (5 cm) containing a mixture of autoclaved vermiculite, perlite and garden soil (1:1:1) and maintained at standard culture room conditions. The plants were acclimatized by covering the pots with polythene bag to maintain high humidity for 6–7 days. After 7 days, 3–4 small holes were made in the bag and the potting mixture was drenched with half strength MS major inorganic salts through these holes, at frequent intervals. After 25 days, polythene bags were removed and the acclimatized plants were transferred to a shaded area under natural conditions.

RESULT AND DISCUSSION

Establishment of Aseptic Nodal Segment Cultures

Contamination of *S. acmella* nodal explants was a major problem during initiation of cultures under in vitro conditions. The extent of contamination was strongly influenced by the season during which the material was collected. The cultures initiated during January–April showed much higher (≥60%) survival rate than those raised in October–December. The reason for the difference in response may be due to high meristematic activity and fresh shoot growth in spring season form January till April. By October–December shoots became old, and thus, make it difficult to break down the dormant state of the buds. Therefore, routinely, the cultures were raised in January–April because of the higher number of aseptic culture establishment and shoot induction. Seasonal effects on establishment of cultures have earlier been reported for other species, viz, mulberry (Vijaya Chitra and Padmaja, 2002), neem (Chaturvedi et al., 2004), litchi (Kumar et al., 2006) and lotus (Shou et al., 2008).

Effect of Growth Regulators and Multiplication of Shoots

Since the aim of the present report is to achieve recurrent shoot multiplication, therefore, nodal segment cultures were raised on MS medium supplemented with a cytokinin (BAP/Kinetin/2-iP) alone or in combination with an auxin (NAA) or GA_3. Although fairly good percentage (64%) of bud-break was observed on MS basal medium, the presence of cytokinin, particularly BAP, in the culture medium helped in the year round multiplication of shoots. Inclusion of BAP at 1–15 μM in the culture medium promoted 100% shoot-bud induction from axillary buds as against only 20% response on Kinetin supplemented medium. Addition of 2-iP was found to have negative impact on shoot bud induction. Many reports have described the beneficial effect of BAP on shoot multiplication (Dantu and Bhojwani, 1987; Rao and Purohit, 2006). Incorporation of GA_3 at low concentration (1 μM) in the BAP supplemented medium promoted multiple shoot formation but the shoots were remained weak and stunted. On the other hand, single shoot with long internodes was observed when NAA (5 μM) was added to MS + BAP medium.

Irrespective of the treatments, excessive adventitious root proliferation was observed either at the base of the explant or all over the surface of the in vitro developed shoot. Of all the treatments, BAP supported maximum rate of shoot proliferation at optimal concentration of 5 μM. On MS + 5 μM BAP, 10 times shoot multiplication was achieved with no adventitious root formation (Table 1). On this medium, bud break started within a week (Fig. 1A). An average of 2.9 shoots/explant were developed with 3.5 nodes/shoot and an average height of 4.9 cm, after 5 weeks (Fig. 1B). There is a decline in the rate of shoot multiplication with the increasing concentration of BAP and BAP at 15 μM, resulted in stunted shoot with pale coloured leaves. The higher concentration of BAP (7–13 μM) supported slow growth of shoot where the rate of shoot multiplication comparable to that of MS + 5 μM BAP could be achieved if the cultures were incubated for longer duration in the medium up to 8 weeks. Surprisingly, lower concentration of BAP (1–3 μM) inhibited overall rate of shoot multiplication and

increased rate of adventitious root proliferation from all over the surface of the in vitro developed shoots. Thus, 10 fold shoot multiplication could be achieved in every 5 weeks on MS + 5 µM BAP by cutting the in vitro shoots into single node segments and culturing them onto the fresh medium of the same composition. Compared to this, Haw and Keng (2003) were able to achieve only 3.4 shoots/explant on MS + 2.2 µM BAP in nodal segments cultures of *S. acmella*. However, information on percent culture response and cycle of multiplication was not provided.

Rooting and Transplantation

Terminal 3–4 cm long portions of shoots, from 5-week-old cultures on MS + 5 µM BAP, were used for rooting. The remaining portions of the shoots were cut into single node segments and utilized for further multiplication. On ½ MS + 50 g/L sucrose, which proved to be the best rooting medium, 100% shoots formed roots directly from the basal cut end of the shoots (Fig. 1C). On this medium root primordia developed after two weeks, which later formed, branched nodulated roots after 4 weeks.

Following the protocol described under materials and methods, rooted shoots from MS + 50 g/L sucrose were transferred out of culture. The plantlets were acclimatized successfully.

CONCLUSION

The present study demonstrates the possibility for mass clonal propagation of *S. acmella* by nodal segment cultures. MS medium supplemented with 5 µM BAP was the best for recurrent shoot multiplication at a rate of 10 fold every 5 weeks and this rate of shoot multiplication was maintained for almost 1 year. The shoots could be readily rooted with a frequency as high as 100%. Rooted plantlets were acclimatized and transferred to the green house.

Literature Cited

Burkill, I.H. 1966. A dictionary of the economic products of the Malay Peninsula. Vol II. Governments of Malaysia and Singapore by the Ministry of Agriculture and Co-operatives, Kuala Lumpur.

Chaturvedi, R., Razdan, M.K. and Bhojwani, S.S. 2004. In vitro clonal propagation of an adult tree of neem (*Azadirachta indica* A. Juss.) by forced axillary branching. Plant Sci. 166:501–506.

Dantu, P.K. and Bhojwani, S.S. 1987. In vitro propagation and corm formation in gladiolus. Gartenbauwissenschaften 52:90–93.

Haw, A.B. and Keng, C.L. 2003. Micropropagation of *Spilanthes acmella* L., a bio-insecticide plant, through proliferation of multiple shoots. J. Appl. Hort. 5(2):65–68.

Khadir, H.A., Zakaria, M.B., Ketchil, A.A. and Azirum, M.S. 1989. Toxicity and electrophysiological effects of *Spilanthes acmella* Murr. extracts on *Periplaneta americana* L. Pesticide Sci. 25:329–335.

Kumar, M., Prakash, N.S., Prasad, U.S. and Sarin, N.B. 2006. A novel approach of regeneration from nodal explants of field-grown litchi (*Litchi chinensis* Sonn.) Fruit Trees. J. of Plant Sci. 1(3):240–246.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473–497.

Oliver-Bever, B. 1986. Medicinal Plants. In: Tropical West Africa. Cambridge Uni. Press, Cambridge.

Ramsewak, R.S., Erickson, A.J. and Nair, M.G. 1999. Bioactive N-isobutylamides from the flower buds of *Spilanthes acmella*. Phytochemistry 51:729–732.

Rao, M.S. and Purohit, S.D. 2006. In vitro shoot bud differentiation and plantlet regeneration in *Celastrus paniculatus* Willd. Biol. Plant. 50:501–506.

Saritha, K.V., Prakash, E., Ramamurthy, N. and Naidu, C.V. 2002. Micropropagation of *Spilanthes acmella* Murr. Biol. Plant. 45(4):581–584.

Shou, S.-Y., Miao, L.-X., Zai, W.-S., Huang, X.-Z. and Guo, D.-P. 2008. Factors

influencing shoot multiplication of lotus (*Nelumbo nucifera*). Biol. Plant. 52(3):529–532.
Vijaya Chitra, D.S. and Padmaja, G. 2002. Seasonal influence on axillary bud sprouting and micropropagation of elite cultivars of mulberry. Sci. Hort.92:55–68.
Watt, P.M. and Brayer-Brandwijk, M.C. 1962. The medicinal and poisonous plants of Southern and Eastern Africa, 2nd edn. Edinburgh: E&S Livingstone.

Tables

Table 1. Effect of BAP concentrations on shoot proliferation from nodal segments of *S. acmella*. Age: 5 weeks.

Concentration of BAP (µM)	Av. shoot length (cm)	No. of shoots per explant	No. of nodes per shoot	Rate of shoot multiplication
	3.5±0.1	1.6±0.0	2.0±0.7	3.2±1.1
1.0	4.0±0.1	1.7±0.3	2.8±0.6	4.8±0.1
3.0	3.8±0.1	2.0±0.6	3.0±0.7	6.0±0.2
5.0	4.9±0.4	2.9±0.3	3.5±0.7	10.2±0.4
7.0	3.3±0.5	2.8±0.6	3.2±0.6	9.0±0.4
9.0	2.7±0.1	2.8±0.4	1.8±0.4	5.0±0.9
11.0	1.8±0.2	2.8±0.6	1.3±0.6	3.6±1.4
13.0	1.8±0.2	2.8±0.6	1.2±0.4	3.4±0.9
15.0	1.0±0.6	1.8±0.7	1.0±0.3	1.8±0.3

Results are shown as mean ± SE.

Figures

Fig. 1. Shoot Proliferation: (A) A week old nodal segment at culture (×1); (B) A nodal segment on MS+5 μM BAP, showing long, multinodal shoots after 5 weeks of culture initiation (×1); (C) A shoot from B, rooted on 1/2 MS + 50 g L^{-1} sucrose. Branched roots have developed directly from the basal cut end (×1.1).