Botany Research International 2 (3): 182-185, 2009
ISSN 2221-3635

Micropropagation of *Costus speciosus* (Koem.ex.retz) Sm., an Antidiabetic Plant by Using Explants of Pseudostems

[1]J. Philip Robinson, [2]S. John Britto and [1]V. Balakrishnsn

[1]Department of Biotechnology, K.S. Rangasamy College of Technology, Tiruchengode-637 215, Tamil Nadu, India
[2]The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous), Tiruchirappalli-620002, Tamilnadu, India

Abstract: Tissue culture techniques are useful for *ex situ* conservation of rare, endemic of threatened plant species. This report describes a protocol for micropropagation of *Costus speciosus*, an valuable medicinal plant species, using segments of psedostem as explants. Only 15% of the explants were contaminated by using this material to start the *in vitro* culture. The highest shoot proliferation was obtained on Murashige and skoog medium supplemented with 0.05 mg l^{-1} 6- benzylaminopurine. Shoot length gradually decreased by addition of increasing concentrations of 6- benzylaminopurine. Maximum number of roots was obtained in Murashige and skoog medium supplemented with 0.1 mg l^{-1} indole 3- butyric acid. High survival percentage, over 75%, was obtained when the plantlets were transferred to greenhouse conditions. *Costus speciosus* can be successfully micropropagated with a pseudostem with out any significant damage to the mother plant.

Key words: Micropropagation · Multiple shoots · Plantlets and plant growth regulators

INTRODUCTION

Genetic diversity is widely recognized as the key component for -long term survival of the species. It is the foundation of sustainability because it provides raw material for adoption, evolution and survival of species and individuals, especially under changed environmental and disease conditions [1]. Increasing conversion of natural forests for agriculture and urban development has resulted in the forest fragmentation and declining forest habitat. For forest fragmentation and increased rates of population extinction results in smaller population sizes and increased isolation of populations, particularly among the less common or rare species [2].

Costus speciosus belonging to the family Costaceae is an important medicinal and ornamental plant, cultivated in India. *Costus speciosus* is propagated vegetative methods using rhizome pieces. However, the planting material shows a very low multiplication rate. The lack of seed setting spoiled the breeding of this plant. Four different alkaloids responsible for pharmacological aspects and the presence of acetylcholine responses for anticholinesterase activity are also reported [3]. Conventional propagation is beset with problem of poor seed viability, low percentage of germination and scanty delayed rooting of vegetative cuttings. Therefore there is a need for alternative propagation methods [4]. Tissue culture techniques might be applied to generate large number for clonal propagules. Regeneration and reestablishment of the plant through *in vitro* culture is one of the most effective biological techniques to maintain its diversity. Since the harvest of medicinal plants on a mass scale form their natural habitats is leading to a depletion of plant resources the conservation of these valuable genotypes is imperative. In recent years there has been an increased interest in *in vitro* culture techniques which offer a viable tool for mass multiplication and germplasm conservation of rare, endangered and threatened and economically important plants [5- 7].

MATERIALS AND METHODS

Nodal segments of *Costus speciosus* were collected from the tender parts of mature plants grown in botanical garden of K.S. Rangasamy College of technology. These

Corresponding Author: J. Philip Robinson, Department of Biotechnology, K.S. Rangasamy College of Technology, Tiruchengode-637 215, Tamil Nadu, India

segments were washed under running tap water followed by a detergent, Tween-20 for min. after repeated washes in double distilled water, surface sterilization was done with mercuric chloride (0.1%) for 4-8 min. the sterilized segments were then washed thoroughly with sterile double distilled water and cut into appropriate sizes and cultured on Murashige and skoog [8] medium. MS medium was used as basal medium and was supplemented with 3% (W/V) sucrose and gelled with 0.8% (w/v) agar (Himedia). Different growth regulators (BAP, KIN, NAA, IAA and IBA) at different concentrations either alone or in combinations were added to the medium. The p^H of the medium was adjusted 5.8 before autoclaving at pressure of 1.06 kgcm-2. All the cultures were incubated at 25±2°C with 16/8 h photoperiod under white fluorescent tubes (25µ molm-2s-5). Then cultures were raised for each treatment and all experiments were repeated thrice. Means were compared using Duncan's multiple range test [9]. Rooted shoots were directly transferred to small rooting pots containing coir pith (moisture preserving material) and sand (1:1) and subsequently to the fields.

Single disinfected stem segments were cultured on MS medium for culture initiation and served as explant source for subsequent experiments, the p^H of the medium (supplemented with respective growth regulators) was adjusted to 5.8 with 1N NaoH or 1N Hcl before gelling with 0.8% (w/v) agar (Himedia). In all the experiments the chemicals used were of analytical grade (Himedia, Qualigens, Merkard and Sigma). The medium was dispensed into culture vessels (Borosil, Mumbai, India) and autoclaved at 105 kPa and 121°C for 15 min. the surface disinfected explants were implanted horizontally on the culture medium test tubes (150-X25) containing 50 ml medium and plugged tightly with non-absorbent cotton. All the cultures were incubated at 25+ 2°C under 16 h photoperiod of 45- 50 mol m^{-2} s^{-2} irradiance provided by the cool white fluorescent tubes (Philips, India) and with 55- 60 % relative humidity.

Statistical Analysis: Experiments were set up in a randomized block design and each experiment usually 10 replications and were repeated at least three times. Observations were recorded on the frequency of embryos from the explants and the number of germinating embryos. Means and standard errors were used through out the study and the values were compared using Duncan's multiple range tests according to Gomez and Gomez., 1976 [10].

RESULTS AND DISCUSSION

Pseudo stem segments of *Costus speciosus* were selected as explants for in vitro establishment. Pseudostems have been shown to be the best material for the *in vitro* establishment of the medicinal plant, such as *Centaurea spachii* [11] and *Gentiana lutea* L. spp. *Aurantiaca* [12]. Explants exhibited only 15% of contamination, lower than that of buds and leaves. During the shoot multiplication phase, the percentage of reactive explants (83- 100%) was consistently high in all media

Table 1: Effects of BAP, KIN and IAA concentrations on the percentage of reactive explants, the number of shoots and the maximum shoot length per explant after five weeks of culture on MS medium. The means and standard errors (±SE) are presented for each column. Mean sharing at least one letter are no significantly different at the $p \leq 0.05$ level (Duncan's multiple range test)

Hormone	Concentration mgl^{-1}	Percentage of responsive explants	No of shoots per explant	Maximum shoot length(mm)
Control	-	70	1.6±0.3 lm	0.9±1.0 hi
BAP	0.05	93.3	2.8±0.4 jk	38.3±2.9b
	1.00	97	3.4±0.6h	28.7±3.4cd
	0.50	100	11.7±0.8a	40.9±5.4a
	1.00	90	6.7±1.1e	26.1±2.8de
	1.50	87	4.9±1.1f g	25.1±3.3e
KIN	0.05	97	9.4±1.0c	26.9±3.8d
	1.00	90	7.0±1.2de	21.9±3.2f
	0.50	86	3.3±1.1hi	19.5±2.8fg
	1.00	83	3.0±1.0ij	9.2± 1.8h
	1.50	79	1.8±0.3l	8.0± 1.8i
IAA	0.05	93	7.3±1.3d	36.4±4.6bc
	1.00	97	11.3±1.3ab	3.4±5.2ij
	0.50	93	5.1±0.7f	29.3±5.7c
	1.00	83	2.9±0.7ijk	12.1±2.4gh
	1.50	80	3.3±0.8hi	4.0±2.0gl

Tabel 2: Effects of auxin on *in vitro* rooting of shoots cultured on MS medium. The means and standard errors (±SE) are presented for each column. Mean sharing at least one letter are no significantly different at the $p \leq 0.05$ level (Duncan's multiple range test)

Auxin	Concentration mg l^{-1}	Percentage of rooting	No of roots explant	Root length per explant(mm)
IAA	0.05	70	3.4±0.6f	3.7±0.5f
	0.10	80	4.9±0.7e	6.0±0.9de
	0.50	80	6.1±0.9c	8.5±1.0c
	1.00	90	5.3±1.2de	5.2±0.8e
IBA	0.05	90	11.1±0.6ab	10.0±0.7b
	0.10	100	12±1.1a	12.1±0.7a
	0.50	90	7.8±1.0b	8.2±0.8cd
	1.00	80	5.5±1.0cd	6.5±0.6d

Fig. 1: *In vitro* regeneration of *Costus specious* (koen.) sm.,

Fig. 2: Response of pseudostem explants on shoot initiation on BAP ($0.50 mgl^{-1}$)

studied. In the MS medium with out any hormone, an average of 1.6±1.1 shoots per explants was recorded after five weeks of culture (Table 1). It is significant to note that multiple shoots were induced from pseudostems explants with out any intervening callus phase on MS medium containing different concentrations of BAP and KIN.

Fig. 3: Hardened plantlet

Multiple shoots were induced from pseudostem explants after four weeks of culture on MS medium supplemented with different concentrations of BAP at 0.50 $mg1^{-1}$ (11.7±2.6) (Fig. 1) and KIN at 0.05 mg l^{-1} (9.4± 3.4) (Table 1) the induction of shoots by using BAP has been well documented in Piper spp.[13], *Ocimum* spp. [14], *Withania somnifera* [15], *Phyllanthus caroliniersis* [16]. Shoot elongation, decreasing slightly with increasing BAP concentration, has also been observed in other micropropagation protocols [17, 18]. With further increase in cutokinin concentration there is an enhancement in callusing. However, in the medium containing higher concentration of IAA, shoots showed stunted growth with a lesser number of shoots (2.9±2.3) produced.

Rooting and Acclimatization: The *in vitro* multiple shoots were sub cultured to develop whole plants for root induction in media supplemented with different concentrations of IAA and IBA. When the rooting media were supplemented with IBA concentration 0.1mg l^{-1} the number and length of roots greatly increased (Fig. 1) and then decreased at 2.0 mgl^{-1} (Tabel 2) IBA was more resistant than IAA to degradation in the tissue culture media, both during autoclaving and at room temperature

[19], as with *Wedelia chinensis* [20], this concentration of IBA seems to play a stimulatory role in the process of root formation in *C. speciosus* shoots. In the IBA medium, the majority of roots developed three weeks earlier than in the IAA. IBA concentration was beneficial also foe both root system development and for shoot quality. The medium supplemented with IAA (0.05- 1.0 mg l^{-1}) had poor rooting, with an intervening callus. For acclimatization a mixture of soil, vermiculate and sand (2:1:1) and healthy roots appeared after two weeks. The survival rate of the clones was about 95%. The plants were hardened for 10-15 days before being transferred to the green house. Based on these observations we propose an efficient protocol to micropropagate the rare species *Costus speioosus* from pseudostemens.

REFERENCES

1. Erikson, G., G. Namkoong and J.H. Roberds, 1993. Dynamic gene conservation for certain futures. Forest Ecol. Manage, 62: 15-33.
2. Rojora, O. and P.A. Mosseler, 2001. Challenges and opportunities for conservation of forest genetic resources. Euphytica, 118: 197-212.
3. Mandal, A. and S.K. Chatterjee, 1985. Effect of some trace elements on diosgenin formation in Costus speciosus in relation to other metabolites. Indian J. plant Physiology, 28(3): 223-226.
4. Sarasan, V., E. Thomas, B. Lawrance and G.M. Nair, 1993. Plant regeneration in *Piper longum* L. (Piperaceae) through direct and indirect shoor development J. Spices Arom. Crop., 2: 39-40.
5. Ault, J.R., 1994. *In vitro* propagation of Eriostemon myoporoids and Eriostemon "stsrdust". Hort science, 29: 686-688.
6. Sahoo, Y. and P.K. Chand, 1997. Micropropagation of *Vitex negundo* L. a woody aromatic medicinal shrub, through high frequency auxiliary shoot proliferation. Plant Cell Rep.,18: 301-307.
7. Prakash, E., P.S. Shavalli Khan, P. Sairam Reddy and K.R. Rao., 1999. Regenerationof plants from seed derived callus of *Hybanthus enneauspermus* L. Muell. a rare ethnibotanical herb. Plant Cell Rep., 18: 873-878.
8. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15: 473-497.
9. Duncan, D.B., 1955. Multiple range and multiple F-tests- Biometrics11:1- J. Pharmacology, 4(3): 178-179.
10. Gomez, K. and K.A. Gomez, 1976. Stastical procedures for agriculture research with emphasis on rice. Los Banos. International Rice Research.
11. Cuenca, S., J.B. Amo-Marco, 2000. *In vitro* propagation of *Centaurea spachii* from inflorescence stems. Plant growth Regulation, 30: 99-103.
12. Feijoo, M.C. and I. Iglesias, 1998. Multiplication of an endangered plant: *Gentiana lutea* L. subsp, *aurantica* Lainz. using *in vitro* culture. Plant tissue culture and Biotechnology, 4: 87- 94.
13. Bhar, S.R., K.P.S. Chandel and S.K. Malik, 1995. Plant regeneration from various explants of cultivated Piper species. Plant cell Reports., 14: 395-402.
14. Pattnaik, S.K., P.K. Chand, 1996. *In vitro* propagation of the medicinal herb *Ocimum americanum* (L.) Syn. *Ocimum canum* L. (Holy basil) and *Ocimum sanctum* L. (Holy basil). Plant cell Reports, 15: 846-850.
15. Manickam, V.S., R. Elengo and R. Antonisamy, 2000. Regeneration of Indian ginseng plantlets from stem callus. Plant cell, tissue and organ Culture, 62: 181-185.
16. Catapan, E., M.F. Otuki and A.M. Viana, 2000. *In vitro* culture of *Phyllanthus cartoliniensis*(Euphorbiaceae). Plant cell, tissue and Organ Culture, 62: 195-202.
17. Ault, J.R., 1994. *In vitro* propagation of Eriostemon myoporoids and Eriostemon "stsrdust". Hort science, 29: 686-688.
18. Iriondo, J.M., M. De la Iglesis and C. Perez, 1995. Micropropagation of *Elaeagnus angustifolia* from mature trees. Tree Physiology, 15: 691-693.
19. Nissen, S.J. and E.G. Sutter, 1990. Stability of IAA amd IBA in nutrient medium to several tissue culture procedures. Hort. Sci., 25: 800-802.
20. Kameri, M., S. Shashidhara and P.E. Rajasekaran, 2005. *In vitro* multiplication of *Widelia chinensis*(Osbeck) Merr. Plant cell biotechnology and molecular Biology, 6: 147-150.