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RESEARCH ARTICLE

STANDARDIZATION OF MICROPROPAGATION FROM NODAL SEGMENTS OF *COSTUS PICTUS*- AN ANTI-DIABETIC PLANT

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ABSTRACT

Medicinal plants are widely used in traditional and modern system of medicine. Indiscriminate collection of these plants from the natural habitats is leading to their depletion. Hence there is a need to conserve and propagate important medicinal plants. The present study was taken up to standardize micropropagation protocol from nodal segments of *Costus pictus*. The explants were cultured on SH media supplemented with different concentrations and combination of BAP alone and in combination with IBA, IAA, NAA, KN and AdSo4 (Adenine sulphate) for inducing direct regeneration. Shoot buds appeared within 15-20 days and elongated to produce one to two shoots within one week. Among all combinations, highest shoot regeneration (86%) was observed in SH media with 1 mg/l BAP+ 1 mg/l IBA+25 mg/l AdSo4. To induce rooting, regenerated shoots were transferred to SH media with different concentrations of IAA and IBA. Roots appeared in both the combinations within 2-3 weeks. Among the two combinations, 1 mg/l IBA gave more rooting response (63%). Regenerated plantlets were transferred to field with 92% survival rate.

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INTRODUCTION

The demand for the medicinal plant resources has been on the rise due to their extensive utilization in herbal health care formulations, cosmetic products and nutritional supplements. About 95% of the medicinal plants used are obtained from wild habitats, especially from forests. The continued commercial exploitation of these plants resulted in depletion and extinction of many important species from their natural habitats. In the recent years, Plant cell and tissue culture has emerged as a powerful tool for large-scale multiplication of many plants. Plant tissue culture technology plays a significant role in establishing a reliable protocol for conservation and large scale propagation of many plant species. The *C. pictus* (Family: Costaceae) is an important medicinal plant widely used in traditional and commercial formulations. It is commonly known as spiral ginger or crepe ginger, used as tonic, stimulant, diuretic, digestive, antiseptic and anthelminthic. The rhizome is a major source of diosgenin (Chopra *et al*, 1956, which is antidiabetic in nature and is used in the treatment of diabetes-mellitus.

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The genus *Costus* consists of many species, of which *C. speciosus*, *C. pictus* and *C. igneus* are widely used for treating diabetes. Many nurseries are now promoting *C.pictus* as the 'insulin plant' that would dynamically bring down the blood sugar levels. The present study is taken up to standardize the direct regeneration of *C.pictus* using nodal explants.

MATERIALS AND METHODS

Collection and Sterilization of explants: The nodal segments were collected from the field grown plants of *C.pictus.* The explants are washed thoroughly under running tap water followed by Tween 20 (detergent) for 10 minutes. Then, the explants were surface sterilized with 0.1 % mercuric chloride ($HgCl_2$) for 3 minutes followed by 3-4 washes with sterile water**.**

Direct shoot regeneration: The nodal segments were cut into pieces of appropriate size and inoculated on onto SH media (Schenk and Hilderbrandt, 1972) containing different concentrations of cytokinins i.e BAP and KN and auxins i.e. IAA, IBA and NAA either singly or in combination with each other. The explants were incubated at 25 ± 2ºC temperature with 16 hours photoperiod. For each experiment 3-4 replicates were maintained.

Multiplication of shoots: The regenerated shoots from nodal explants were sub cultured on SH media with BAP and IBA along with different concentrations Adenine Sulphate ($AdSo_4$) for large scale multiplication of shoots. Also the effect of individual hormones i.e AdSo4 alone, BAP with $AdSo_4$ and IBA with $AdSo_4$ were tested.

Root induction: For initiation of roots, the regenerated shoots from nodal segments were separated and the individual shoots were carefully transferred on to SH media containing different concentrations of IAA, IBA and BAP either alone or in combinations with each other.

Hardening of Plants: Rooted shoots were carefully taken out of the medium and washed thoroughly under running tap water to remove all the traces of medium. The plantlets were transferred to small plastic pots containing a mixture of sand and soil (1:1) and covered with polythene bags to maintain humidity. After 10-15 days, the established plants were transferred to the field containing garden soil and farm yard manure.

RESULTS

To standardize the direct plant regeneration, the nodal explants of *C.pictus* were cultured on SH media supplemented with different concentrations of BAP alone and in combination with KN, IAA and IBA. To see the effect of cytokinin, various concentrations of BAP alone ranging from 0.25 to 3.0 mg/l was tested for shoot induction from the nodal explants (Table.1). The shoot buds appeared from the nodal region within 15-20 days of inoculation. The shoot buds elongated and produced one or two shoots within one week of culture (Fig.1). The percentage of shoot induction varied from 52% to 76% with different concentrations of BAP. The BAP at 1.0 mg/l had showed higher percentage of shoot regeneration (76.1%) with 1-2 shoots and shoot length of 2.8 cms. The increase in concentration of BAP above 1.0 mg/l resulted in decrease in frequency of shooting and also in number of shoots and shoot length. To study the effect of different hormones on shoot induction, BAP (1 mg/l) in combination with various other plant growth regulators like KN, IAA and IBA was tested (Table 2).

Table 1. Standardization of direct regeneration from nodal explants of *C.pictus* on SH media using BAP

S.No.	Hormone BAP mg/l	No of Explants inoculated	No of Explants Responded	Multiple shoot Regeneration(%) Mean±SE	No. of shoots /Explants	Shoot length(cm) Mean±SE
1	0.25	26	18	69.2± 1.2	0-1	2.3 ±0.6
2	0.50	21	15	71.4±0.27	0-1	2.4±0.11
3	1.0	21	16	76.1± 0.8	1-2	2.8± 0.4
4	2.0	20	12	60±1.8	1-2	2.6± 0.16
5	3.0	21	11	52.3±1.6	0-1	2.1± 0.21

*Values are mean ± SE of 3replicates

Table 2. Effect of different hormones in combination with BAP on shoot induction from nodal explants of *C. pictus*

S.No	Hormones (mg/l)	No of Nodal explants inoculated	No of Explants responded	Regeneration (%)	No of shoots/explants	Observation
1	1.0 BAP+0.25 KN	22	14	63.6±0.36	1-2	Moderate Regeneration
2	1.0 BAP+ 0.50 KN	15	10	66.6±0.48	1-2	
3	1.0BAP+1.0KN	21	13	61.1±0.52	1-2	
4	1BAP + 0.25 IBA	22	15	68.1±0.27	2-3	High Regeneration.
5	1BAP + 0.5 IBA	20	15	75.0±0.5	2-3	
6	1BAP + 1.0 IBA	23	18	78.2±0.61	2-3	
7	1BAP + 1.5IBA	23	17	73.9±0.13	1-2	
8	1BAP+0.25 IAA	24	12	50.0±0.81	0-1	Low Regeneration
9	1BAP+0.5 IAA	13	7	53.8±0.46	0-1	
10	1BAP+1.02 IAA	15	7	46.6±0.67	0-1	

* Values are mean ± SE of 3replicates
** Percentage of regeneration after 4 weeks of culture

Table 3. Effect of different concentrations of Adenine sulphate on multiplication of shoots regenerated from nodal explants of *C.pictus*

Treatment mgl^{-1}	Shoot multiplication (%)	Average No of shoots	Mean length of shoots (cms)
$AdSo_4$ 25.0	8.6±0.53	1.0±0.4	0.8±0.11
BAP1.0+ $AdSo_4$ 25.0	78.0±0.41	3.2±0.6	2.8±0.14
IBA1.0+ $AdSo_4$ 25.0	65.1±0.20	2.8±0.9	1.6±0.67
BAP1.0+IBA1.0+ $AdSo_4$ 10	79.2±0.32	3.6±0.2	3.0±0.23
BAP1.0+IBA1.0+ $AdSo_4$ 15	80.0±0.61	4.8±0.3	3.2±0.02
BAP1.0+IBA1.0+ $AdSo_4$ 20	83.4±0.60	5.2±0.2	3.4±0.06
BAP1.0+IBA1.0+ $AdSo_4$ 25	86.0±0.32	6.1±0.4	3.8±0.01

* Values are mean ± SE of 3replicates
** Percentage of shoot multiplication after 4 weeks of culture

Table 4. Effect of BAP in combination with IAA and IBA in rooting from regenerated shoots of C.pictus

S.No	Hormones (mg/l)	No of shoots inoculated	No of shoots with rooting responded	Rooting %	No of shoots / roots	Root length(cm) Mean±S.E
1	0.25BAP+ 0.25IAA	15	5	33.3	2.7±0.52	0.6±0.14
2	0.5 BAP+ 0.5 IAA	13	5	38.4	3.2± 0.20	1.0±0.40
3	1.0 BAP+1.0 IAA	15	6	40.0	3.25±0.46	1.2±0.01
4	0.25 BAP+0.25 IBA	17	8	47.0	3.9±0.35	1.7±0.20
5	0.5 BAP+0.5 IBA	15	9	60.0	4.41±0.21	2.1±0.17
6	1.0BAP+1.0 IBA	11	7	63.6	4.78±0.25	2.40±0.29

* Values are mean ± SE of 3replicates
** Percentage of rooting after 4 weeks of culture

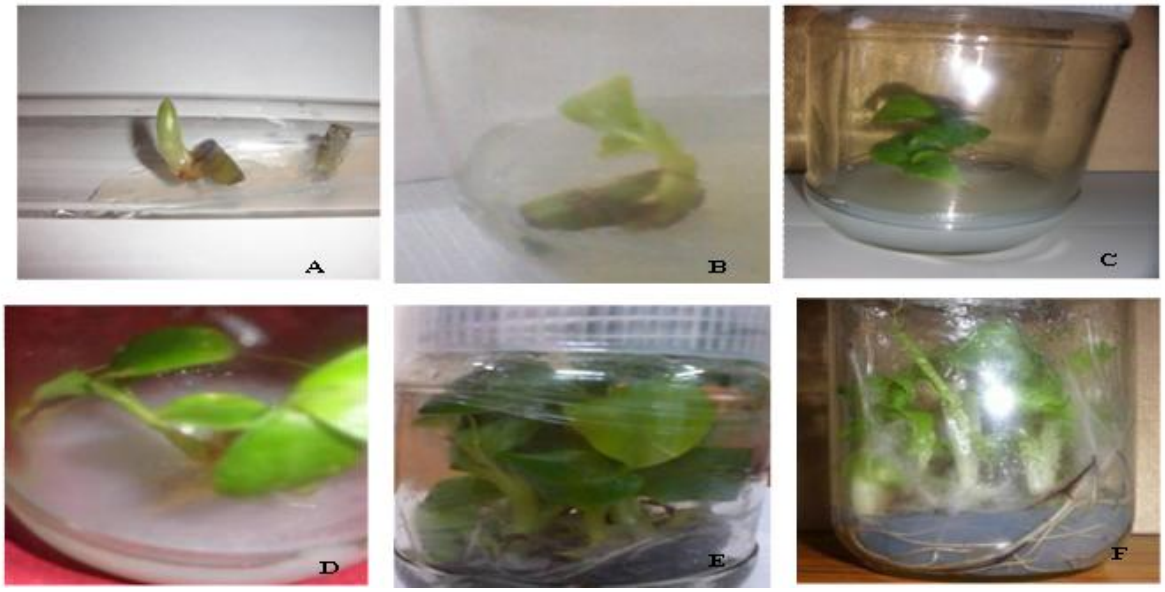

Fig.1. Direct plant regeneration from nodal segments
A to D- shoot induction from no dal explants of C. Pictus E-shoot multiplication
F-Rooting from regenerated shoots

BAP with different concentrations of KN exhibited moderate frequency of shoot regeneration (61 to 66%). The IBA combination at different concentrations (0.25 to 1 mg/l) showed rapid increase in percentage of plant regeneration from 68 to 78%. But, high concentration of IBA (1.5 mg/l) has lowered the regeneration potential. The frequency of shoot regeneration was low in BAP supplemented with IAA (46% - 53%) Of all the hormonal combinations tested, highest regeneration was recorded at 1 mg/l BAP along with 1 mg/l IBA (78.2%). The individual shoots developed using 1.0 mg/l BAP and IBA were subcultured onto SH media containing different concentrations of $AdSo_4$ for shoot multiplication. The supplementation of varying concentrations of adenine sulphate (10 to 25 mg/l) in combination with other hormones showed significant increase in shoot multiplication. The adenine sulphate alone without any growth regulators resulted in very low frequency of multiplication (8.6%). Adenine sulphate at 25 mg/l with individual hormone BAP alone (1.0 mg/l) exhibited 78% and IBA alone (1.0 mg/l) showed 65.1% of shoot proliferation. The BAP (1.0 mg/l) in combination with IBA (1.0 mg/l) along with varying concentrations of adenine sulphate (10 to 25 mg/l) showed increase in shoot multiplication ranging from 79 to 86% (Table.3). To induce rooting, regenerated shoots (2-3 cm) were transferred to SH media supplemented with BAP along with the two auxins i.e. IAA and IBA.

Roots appeared in all the combinations from basal ends of shoots within 2-3 weeks. In the medium supplemented with BAP and IAA, the frequency of root induction ranged from 33% to 40%. The combination of BAP with IBA, the percentage of root induction ranged from 47 to 63%. Among the two hormones tested, BAP in combination with IBA gave high frequency of rooting. Of all the combinations, 1.0 mg/l BAP with 1.0 mg/l IBA gave 63% of rooting (Table.4). Rooted plantlets were transferred to small plastic pots with mixture of sand and soil (1:1) and covered with polythene bags to maintain humidity**.** After 10-15 days, plantlets were transferred to soil and it showed 92% survival rate.

DISCUSSION

BAP is a commonly used cytokinin which is reported to stimulate shoot multiplication in many plants (Kachar *et al*, 1993; Ali *et al*, 2007; Haque *et al*, 1997). Therefore to see the effect of cytokinin, various concentrations of BAP alone (0.25 to 3.0 mg/l) was tested for shoot induction from the nodal explants. At low concentrations of BAP i.e 0.25 to 1.0 mg/l, the frequency of plant regeneration ranged from 69 and 76%. Similarly, the effective role of low concentration of BAP in shoot proliferation has been reported in other species belonging to the family Zingiberaceae (Ikeda and Tambe, 1989; Balachandran *et al*., 1990; Smith and Hamil, 1996; Rout

et al., 2001; Panda *et al*., 2007; Mohanty *et al*., 2011; Abdelmageed *et al*., 2011). High frequency of regeneration (76%) was observed at 1.0 mg/l BAP. The increase in concentration of BAP above 1.0 mg/l resulted in decrease in frequency of shooting and also in number of shoots and shoot length. These observations support the well-known inhibitory influence of higher concentrations of cytokinins on shoot elongation and shoot formation as observed in *Mellissa officinalis* (Tavares *et al*., 1996) and *Hedeoma multifolium* (Koroch *et al*., 1997). BAP at 3.0 mg/l reduced the frequency of plant regeneration to 52%. These findings resembles the report of Sasikumar *et al*. (2009) indicating an upper limit in concentration of BAP in induction of shoots.

In the present study, nodal segments of *C.pictus* when cultured on SH media supplemented with BAP alone showed better efficiency in shoot induction (76%). Therefore to further enhance the percentage of shooting, BAP (1 mg/l) in combination with various other plant growth regulators like KN, IAA and IBA was employed. Among all the hormonal combinations, BAP with IBA has shown good response towards the development of shoots from the nodal explants. Similarly, enhanced plant regeneration was reported with BAP and IBA combination in other Zingiberaceae members like *Curcuma zedoaria* (Miachir *et al*, 2004) and *Zingiber zumbret* (Stanley and Keng, 2007). The regenerated shoots using BAP (1mg/l) and IBA (1mg/l) were separated and subcultured onto the same media for further multiplication. The subculture on the same media resulted in growth in length of shoots but not in number. To increase the number of multiple shoots different concentrations of $Adso_4$ was employed. Adenine sulphate provides an available source of nitrogen to cell and can be taken up more rapidly than inorganic nitrogen (Thom *et al*, 1981). The benefits of adenine sulphate are often noticed only when it is associated with ammonium nitrate or cytokinins such as BAP or KN (Van *et al*, 2008). In the present study, varying concentrations of AdSo4 in combination with cytokinin (BAP) and Auxin (IBA) were employed. Individual hormones separately (BAP and IBA) and Adso4 alone without any growth regulators were also tested for promotion of shoot multiplication.

The supplementation of higher concentrations of adenine sulphate (10 to 25 mg/l) in combination with other hormones showed significant increase in shoot multiplication. Similarly high concentration of adenine sulphate was used for shoot multiplication and proliferation in many other plant species such as *Holarrhena antidysenterica* (Raha and Roy, 2001), *Curcuma angustifolia* (Shukla *et al*, 2007) and *Bacopa monnieri* (Ramesh *et al*, 2006). The frequency of shoot multiplication was high (86%) with an average number of 6 shoots/explant and with a shoot length of 3.8 cms, at an optimized concentration of 1.0 mg/l BAP and 1.0 mg/l IBA along with 25 mg/l AdSo4. A similarly observation was reported with a combination of cytokinin (BAP) and auxin (NAA) and Adso4 for shoot multiplication in *Cerapegia bulbosa* (John Britto *et al*., 2003). The combination effect of BAP, IAA and Adso4 has also been reported in number of plants using nodal explants (Dhar and Upreti, 1999; Hussain *et al*, 2008; Rout *et al*., 2008). The regenerated shoots were transferred on to rooting media.

The high frequency of rooting (75%) was observed with 1.0 mg/l IBA. Thus, IBA was found to be superior to IAA. There are many other previous studies, which reported IBA as more effective than IAA for root induction (Epstein and Ludwig-Muller, 1993).

Conclusion

In the present study, high frequency of direct shoot regeneration was standardized from the nodal explants of *C.pictus.* In multiplication media, addition of adenine sulphate has resulted in 3-6 multiple shoots for explant. Individual shoots were separated and subcultured into the same media. Through this procedure, a total of 160 plants were produced from each nodal explant through successive subculture in second and third cycle. Therefore, this protocol can be utilized for large scale multiplication and supply of uniform planting material. The commercial cultivation of *C.pictus* will reduce the collection form wild habitat and also help in supply of raw material for the industrial production of diosgenin.

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