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Full Length Research Paper

Optimized Rhizome Bud Culture for Accelerated Plantlet Regeneration in *Alpinia purpurata*

K.P. Kochuthressia[1], S.John Britto[1*], L. Joelri Michael Raj[1], M.O.Jaseentha[1] and S.R.Senthilkumar[2].

[1]The Rapinat Herbarium and Centre for Molecular Systematics,
St. Joseph's College (*Autonomous*), Tiruchirappalli – 620 002, South India.

[2]Department of Plant Biology and Plant Biotechnology,
St. Joseph's College (*Autonomous*), Tiruchirappalli – 620 002, South India.

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A reproducible and efficient protocol is described for multiple shoot induction of *Alpinia purpurata* using rhizome bud explants. Murashige and Skoog medium supplemented with BA (3.0 mg/l) and Kn (2.0 mg/l) exhibited regeneration rate up to 6.4±0.32 shoots/explants. Spontaneous rooting of shoots occurred in the same concentration of cytokinins. The number of leaves (4.8±0.67) and profuse rooting (8.4±0.70) facilitated 100% of plant recovery on acclimatization. This protocol proves its utility for rapid propagation of *A. purpurata* to be exploited for pharmaceutical and commercial purposes.

Key words: *Alpinia purpurata*, micropropagation, rhizome bud culture.

INTRODUCTION

Alpinia purpurata (Vieill.) K.Schum. is very popular garden plant in India and widely used as cut flower around the world((Kress *et al.,*2002;Sabu,2006);The plant is known to have medicinal value Prajapathi *et al*. (2003). Rhizome has a sharp odour, improves appetite, taste and voice. It is also used for head-ache, rheumatism, sore throat and renal disorders. The flowers used as decoction are a relief against cough (Victorio *et al*., 2009). It is extensively used globally due to the exquisite inflorescences and therapeutic potential (Victorio *et al*., 2009). In addition, this plant is important sources of raw material for many useful products such as foods, spices, medicines, perfumes, dyes and fiber paper (Tomlinson, 1969). Leaves of *A. purpurata* possess flavonoids, rutin, kaempferol-3-*O*-rutinoside and kaempferol-3-*O*glucuronide validating their therapeutic value (Victorio *et al*., 2009)

A. purpurata (red inflorescence form) used in this investigation is fast disappearing and threatened due to its indiscriminate collection and over exploitation for commercial exploitation to meet the requirements of the pharmaceutical industry. Commercial exploitation for production and conventional propagation are hampered due to poor seed viability, low rate of germination and poor rooting ability of vegetative cuttings (llg and Faria, 1995). *In vitro* propagation methods would be beneficial in accelerating large scale multiplication, quality improvement and conservation. *A. purpurata* has been regenerated from inflorescence buds (Chang and Criley, 1993, Illg and Faria, 1995). However, the multiplication rate achieved was very low. Literature reveals different regeneration systems for mass propagation of many *Alpina plantlets* from rhizome [Agretious *et al.* (1996); Anand and Hariharan (1997); Borthakur *et al.* (1999); Chinnasamy *et al.* (2007); Jinu and Aravindan, (2008). The objective of the current study was to develop a system for the mass propagation of *A.*

*Corresponding author Email: sjbrittorht@yahoo.com

Abbreviations

BA : 6 benzyladenine,
Kn : Kinetin
MS - Murashige and Skoog.

Table 1. Effect of different concentration of BA (0.5 mg/l – 3.5 mg/l) and Kn (0.5 mg/l - 2.5 mg/l) on direct multiple shoot induction from rhizome bud explants (Response noted after 15 days of initial culture)

Cytokinins (mg/l)		Shoot regeneration response (%)	No. of shoot / explant
BA	Kn		(mean ± SD)
0.5	-	0.0[a]	0.0[a]
1.0	-	30 ±2.5[b]	1.4 ± 0.12[b]
2.0	-	41 ± 3.4[d]	1.8 ± 0.14[c]
2.5		54 ± 4.1[cd]	2.2 ± 0.21[d]
3.0	**-**	**61 ± 3.7[c]**	**2.6 ± 0.19[c]**
3.5	-	39 ± 4.8[de]	2.2 ± 0.24[g]
0.5	0.5	29 ±1.5[b]	1.8 ± 0.17[b]
1.0	1.0	45 ± 3.4[d]	2.8 ± 0.16[c]
2.0	1.5	68 ± 5.2[ef]	4.0 ± 0.34[e]
3.0	**2.0**	**80 ± 6.7[h]**	**6.4 ± 0.32[h]**
3.5	2.5	69 ± 4.2[ef]	6.4 ± 0.31[f]

Mean ± SD: for each experiment marked with same letter do not differ significantly ($p<0.05$)

purpurata. Rhizome bud cultures are well established in a wide range of plant species and can be used for the clonal propagation. In the present study, shoots were regenerated from excised rhizome buds on cytokinins supplemented medium. This is the first report on *in vitro* culture of *A. purpurata* using rhizome buds.

MATERIALS AND METHODS

Plant material

A. purpurata plants were collected from Carmel College, Botanical garden Mala, Trichur Dt., Kerala. They were identified at The Rapinat Herbarium, St. Joseph's College (*Autonomous*) Tiruchirappalli, India and were grown in green house of Rapinat Herbarium, Tiruchirappalli and a voucher specimen was also deposited. Healthy and young rhizomes (2-3 months old) of *A. purpurata* were selected as explants.

Surface Sterilization and Inoculation of Explants

Emerging rhizome buds of *A. purpurata* were trimmed to 1-1.5 cm length and washed with 2% Bavistin for 5 minutes, 5% Tween - 20 solution for 15 min, immersed in 70% ethyl alcohol for 30 seconds and then washed thoroughly with double distilled water. They were surface sterilised using 0.1% mercuric chloride solution for 5–7 min, followed by three rinses in sterile water. Outer scale leaves were removed aseptically and explants of 0.6 - 0.8 mm size were inoculated in MS medium (Murashige and Skoog, 1962). Cultures were maintained at 25 ± 2°C under 16/8 h photoperiod provided by cool white fluorescent tubes (60 µ mol m^{-2} s^{-1}) with 55–60% relative humidity. Rhizome buds of (0.6 - 0.8mm) of *A. purpurata* cultured on MS medium fortified with various concentrations of either BA (0.5 mg/l – 3.5 mg/l) or Kn (0.5 mg/l – 2.5 mg/l), Explants were subcultured after every 2 weeks. Data were scored after 15, 30 and 45 days for multiple shoot induction and rooting respectively.

Acclimatization

The rooted plantlets were removed from the culture tubes and washed in distilled water. The number of leaves and roots per shoot and the average length of the leaves per shoot were recorded and then dipped in 0.1 (w/v) Bavistin for 5 minutes. The plantlets were then transferred to plastic cups containing vermiculite and soil (1:1), nourished with half strength MS basal liquid medium. Each one was covered with polythene wraps and watered on every alternate day to maintain humidity.

Statistical analysis

Only data which showed some advantageous effect were included in the tables and are presented as mean ± SE of 20 explants per treatment and experiments were repeated thriceMean values with the same superscript were not significantly different (p=0.05%) according to Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1976).

RESULTS AND DISCUSSION

Multiple shoot proliferation in *A. purpurata* rhizome buds could not be observed in basal MS medium. MS medium augmented with BA (3.0 mg/l) and Kn (2.0 mg/l) induced shoots (6.4 ± 0.32) per explant within 15 days of culture (Table 1, figure 1a). The induced shoots were subcultured on the same concentrations of cytokinins on which the multiple shoots were induced. The individual shoot subcultured on medium containing BA (3.0 mg/l) and Kn (2.0 mg/l) grew to 7.9 ± 0.70cm with 4.8±0.67 leaves within 45 days (Table 2, figure 2b). The effect of BA on clonal propagation of members of family zingiberaceae has been reported earlier [Balachandran *et al.* (1990);Hosoki and Sagawa (1977); Agretious, *et al.* (1996)] The efficacy of BA in inducing shoot multiplication is also reported in certain rhizomatous plants such as Musa (Krikorian and Cronauer 1984) and *Acorus calamus* (Harikrishnan et al.,1997). No additional step was required for rooting of the shoot. Shoots and roots simultaneously originated in the same medium fortified with BA and Kn within 15 days after the first subculture (figure 1c). Highest percentage of growth response with roots (8.4 ± 0.70) at the base of the shoot was noticed at

Table 2. Effect of sub culturing on different concentrations of BA (0.5 mg/l - 3.5 mg/l) and Kn (0.5 mg/l - 2.5 mg/l) on shoot growth and rooting (Response noted after 45 days of initial culture)

Cytokinins (mg/l)		Shoot length/ explant (mean ± SD) cm	Number of leaves/shoot (mean ± SD)	Length of leaves /shoot (mean ± SD)cm	Number of roots/shoot (mean ± SD)	Number of plants recovered on acclimatization (mean ± SD)
BA	Kn					
0.5	-	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
1.0	-	5.3±0.53[b]	1.7±0.13[b]	2.8±0.16[c]	7.4±0.66[b]	1.0±0.16[b]
2.0	-	5.4±0.45[b]	2.5±0.34[c]	3.4±0.34[d]	7.6±0.64[c]	1.5±0.21[c]
2.5		6.6±0.64[c]	3.8±0.41[d]	4.0±0.34[e]	8.4±0.45[cd]	1.7±0.21[d]
3.0	**-**	**7.8±0.66[cd]**	**4.2±0.51[cd]**	**4.5±0.37[f]**	**7.8±0.60[d]**	**1.9±0.13[c]**
3.5	-	7.8±0.60[d]	2.6±0.19[c]	4.2±0.34[e]	7.8±0.68[c]	1.0±0.25[d]
0.5	0.5	5.7±0.67[e]	3.2±0.51[c]	3.9±0.34[e]	8.7±0.67[e]	3.0±0.34[d]
1.0	1.0	6.8±0.63[ef]	3.5±0.44[cd]	4.0±0.37[f]	9.4±0.63[ef]	3.7±0.38[e]
2.0	1.5	7.4±0.91[g]	3.8±0.51[d]	4.2±0.34[e]	7.4±0.91[g]	5.2±0.44[f]
3.0	**2.0**	**7.9±0.70[h]**	**4.8±0.67[cd]**	**4.6±0.48[f]**	**8.4 ±0.70[h]**	**6.3±0.52[h]**
3.5	2.5	7.7±0.20[f]	2.6±0.19[c]	4.1±0.38[e]	8.2±0.67[e]	6.3±0.41[f]

Mean ± SD: for each experiment marked with same letter do not differ significantly ($p<0.05$)

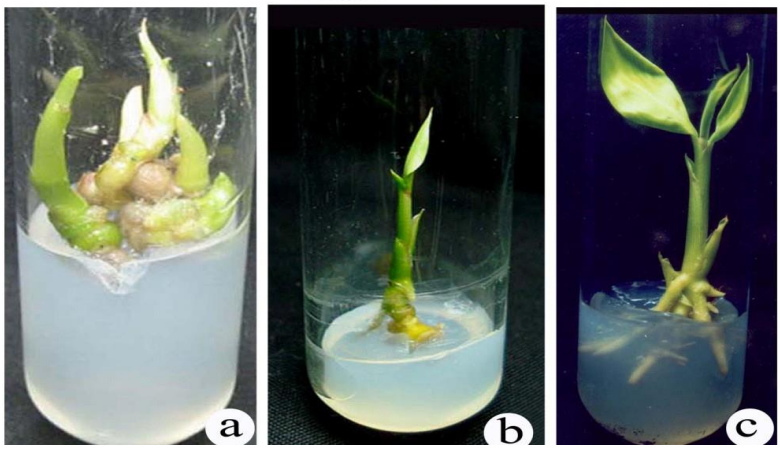

Figure 1. Multiple shoot induction from rhizome bud explant (a) shoot initiation after 15 days of inoculation in MS + BA (3.0 mg/l) and Kn (3.0 mg/l), (b) development of shoot and (c) rhizogenesis after 15 days of first subculture in same concentrations of cytokinins.

the combined effect of BA (3.0 mg/l) and Kn (2.0 mg/l) after 15 days of the second subculture (Table 2; figure 2a). This may be due to the rooting factors that are intrinstic in the rhizome as such a phenomenon has been reported in rhizomatous plants. Rhizome bud is one of the potent explants of Zingiberaceae where root induction from the shoots in shoot inducing medium itself was reported earlier by Balachandran *et al.* (1990) in *Curcuma longa* and *Zingiber officinale,* Agretious, *et al*. (1996) in *Alpinia calcarata,* Borthakur *et al*. (1999) in *Alpinia galanga* and *Prathanturarug et al*. (2003, 2005) in *Curcuma longa* L. The shooting and rooting are facilitated in the same concentration of cytokinins through it is a great advantage in preferring rhizome bud explants to other explants for propagation of *A. purpurata.*

When the leaves and roots had attained considerable growth in number and size plantlets from culture tubes were transferred to glass house for acclimatization. The number of leaves (4.8 ± 0.67) and size of the leaves up to 4.6 ± 0.48 cm provided enough photosynthetic activity for the plantlets (Table 2, figure 2b). This combined with profuse rooting (8.4 ± 0.70) facilitated 100% of plantlet recovery on acclimatization (Table 2, figure 2c). Similar observation has been reported earlier for *Curcuma zedoaria* where plantlets were acclimatized only on producing highest rate of 10.8 ± 1.6 roots/ shoot (Nguyen *et al.*, 2005). The essential

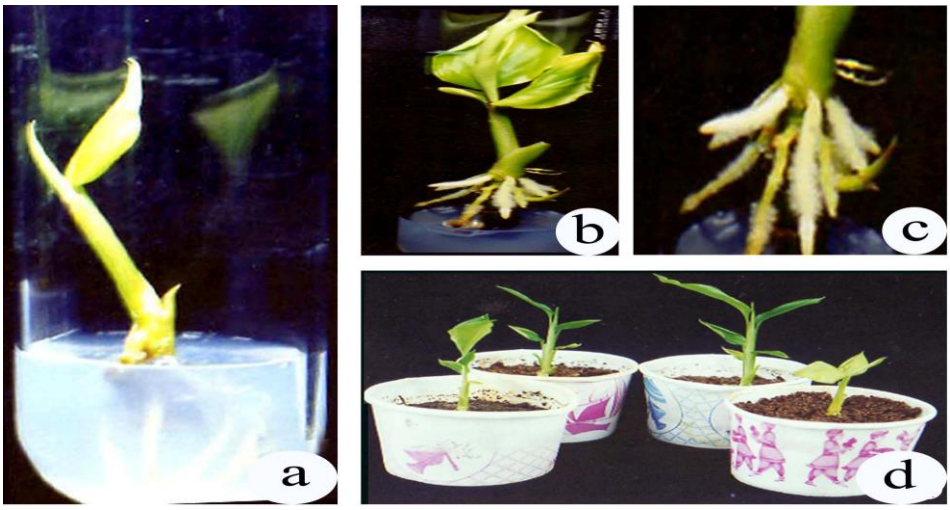

Figure 2. Rhizogenesis after 15 days of second subculture (a) & (b) in same concentration of cytokinins. (c) enlarged portion of profuse rooting (d) Hardened regenerated plantlets after five weeks from the day of inoculation.

requirement for sufficient watering and maintaining the plantlets under high humidity by wrapping them with polythene covers was noted.

Though the establishment of contamination free culture was difficult as explants were from underground rhizome, the protocol followed here provides tremendous potential for mass multiplication of *A. purpurata.*

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