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Research note

A protocol for micropropagation of *Alpinia galanga*

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Abstract

Emerging buds of rhizome of *Alpinia galanga* Willd produced shoots and roots simultaneously when cultured in MS medium supplemented with kinetin 3.0 mg l^{-1}. Each explanted shoot bud produced 8 shoots in average and roots simultaneously within 8 weeks. Shoot proliferation could be continued even after a year by transferring each divided shoot explant to the same medium. Regenerated plantlets could be sucessfully transferred to soil where they grew well within 10–12 weeks with 80% survivality.

Abbreviations: Kn – kinetin; BAP – 6-benzylaminopurine; NAA – napthalene acetic acid

Alpinia galanga WilId (Zingiberaceae) a perennial herb found mainly in the eastern Himalayas and South-west India, is used in traditional medicine in certain countries and also as condiment and spice. Phytochemical constituents of *A. galanga* rhizome include antitumour, antiulcer and anticalculi activity (Itokawa et al., 1987; Qureshi et al., 1992). Seeds of *A. galanga* also have cytotoxic and antifungal diterpenes (Morita and Itokawa, 1988). Several Zingiberaceae species having condiment and spice value have been investigated for *in vitro* multiplication (Hosoki and Sagawa, 1977; Inden and Asahira, 1988; Balachandran et al., 1990; Borthakur and Bordoloi, 1992). Information on such works on *A. galanga* are scanty. Induction of rooted multiple shoots had been reported with *A. purpurata* (Chang and Chiley, 1993). Realizing the importance of this taxon, the present study was undertaken to establish an efficient protocol for micropropagation of *A. galanga*. This technique would facilitate to obtain large number of uniform plants irrespective of season and will serve as an alternative source of seed materials. *In vitro* preservation of germ plasm is also a safe method to protect the species by reducing the risk of natural vagaries.

Emerging buds of stored rhizome of *A. galanga* were used as experimental material. Buds were trimmed and soaked with 5% tween 20 solution for 15 min and surface sterilized by immersing in 0.2% mercuric chloride solution for 5–7 min, followed by three rinses in sterile water. Outer leaves were removed aseptically and explants of 0.3 - 0.4 mm size were placed in culture medium. Cultures were maintained at 23 ± 2 °C under 16/8 h photoperiod of 2000 lux light intensity.

Murashige and Skoog's (MS) (1962) medium containing sucrose 3% (w/v), and agar 0.8% (w/v) was used as basal. Two cytokinins, Kn (1–4 mg l^{-1}) and BAP (2–3 mg l^{-1}) were added to the basal alone or in combination with NAA (0.5 mg l^{-1}). The pH of the medium was adjusted to 5.8 by using 0.1 N HCL or NaOH before autoclaving. After 8 weeks of culture, explants developing shoot clumps were divided and each clump containing 2–3 shoots was transplanted on the fresh medium of the same composition in 250 ml erlenmeyer flasks. Subsequently, regenerated plantlets at 6–8 weeks stage with well developed roots were sub divided and transferred to fresh medium after every 6 weeks. However, plants can be kept up to 5 months in the same nutrient medium in 250–500 ml flasks which remained green and alive without subculturing. Shoot and root length were shortened by slight trimming which helped in reducing contamination during

Figure 1 A–C. (*A*) The best shoot multiplication and growth was observed at 3.0 mg l^{-1}. (*B*) Proliferated shoot of 4–6 weeks old with well developed root systems. (*C*) Plantlets were hardened for 6–8 weeks and then transferred to potting soil. 80% of the transferred plantlets survived in the potted soil and did not show morphological abnormalities.

subculture. In one set of experiments thiamine at 10 mg l^{-1} was added to MS basal medium in place of MS standard three vitamins for assessing both shoot and root growth. Proliferated plantlets having 3–4 shoot clumps with well developed leaves and root systems were maintained in the original culture flask for about 30–45 days for hardening. Plantlets were removed when the medium was dried and kept for 4–6 weeks in half strength MS liquid medium. A filter paper bridge was provided to the shoot clumps for physical support. There after based on the hardening, plantlets were transferred into sterilized potting soil.

Establishment of aseptic cultures was difficult, but once a healthy culture was established, there was no further contamination. Similar findings were observed by earlier investigators with ginger (Hosoki et al., 1977; Inden et al., 1988; Balachandran et al., 1990). Under the conditions given and over a culture period of 30–45 days, explants from all the treatments produced multiple shoots and roots simultaneously. Such type of simultaneous production of shoot and roots were reported earlier for a few species of Zingiberaceae (Kuruvinashetty et al., 1982; Balachandran et al., 1990; Borthakur et al., 1992). In the present investigation, multiplication was found to occur by

Table 1. Effect of growth regulators on shoot multiplication and elongation in *A. galanga* after on 8 weeks incubation.

(mg) l^{-1}	Number of shoots	Number of leaves/ shoot	Length of leaf/ shoot (cm)	Number of root/ shoot	Root length (cm)	Plants recovered
Kn 1.0	2±0.63	2.5±0.92	2 -3.6	2.4±0.48	1.8-3.1	1.9±0.1
Kn 2.0	2.1±0.68	3.2±0.97	2.9-4.5	2.8±0.40	2.4-5.0	2.0±0.1
Kn 3.0	7.1±1.45	5.0±1.14	3.5-6.6	4.5±1.50	4.5-6.7	6.7±0.1
BAP2.0	3.3±0.94	3.1±1.29	2.6-4.8	3.1±0.68	2.3-4.5	2.6±0.4
Kn3.0+ NAA 0.5	5.7±1.16	5.0±1.36	2.7-6.2	4.5±1.39	4.0-5.4	4.8±0.5
BAP3.0+ NAA 0.5	3.4±0.90	3.4±1.17	2.5-4.8	4.5±0.90	3.4-5.2	3.1±0.5
Kn4.0+ NAA 0.5	5 ±1.63	4.8±1.51	2.4-5.0	3.0±0.81	2.5-4.0	4.6±0.2

development of axillary buds which is ideal for maintaining genetic stability. However, the rate of bud multiplication was significantly different according to the various concentrations of cytokinins supplemented. Cytokinin level produced a significant response upon the numbers of shoot formed per explant,and also showed influence on production of leaf number and rooting of the shoot (Table 1). Multiplication rate in the treatment with BAP and Kn at 1.0 mg l^{-1} level was intermediate between treatments with 2.0 – 3.0 mg l^{-1}, indicating that cytokinin alone can also induce shoot multiplication. Combinations of Kn and BAP at 2.0 mg l^{-1} with a lower level of NAA 0.5 mg l^{-1} also induced comparable numbers of shoots. However, profuse root branching and growth were observed in this case. The best shoot multiplication and growth was observed at 3.0 mg l^{-1} Kn, where a maximum of 8 shoots could be observed per flask (Figure 1A). Shoot cultures were thus maintained on this selected medium by regular subculturing at 6 weeks intervals. Within that period, development of 1–2 new axillary buds was also observed from almost all the cultured flasks. During subculture, all the plants were divided and cultured into same multiplication medium. They responded in the same manner as the mother culture, shoot showing similar shoot multiplication and roots. This multiplication rate did not decrease in the successive subcultures on the same medium even after more than one year. Thus, by repeating this procedure for 4–5 successive culture cycles, an average of 1000 plantlets can be produced per flask and complete plantlets could be produced within 45–50 days. No additional step was required for rooting of the shoots. However, root growth and elongation could be enhanced by supplementing thiamine alone at 10 mg l^{-1} in place of standard MS vitamins.

Proliferated shoot of 4–6 weeks old with well developed root systems were transferred to liquid nutrient solution containing half strength of MS medium (Figure 1B). Plantlets were hardened for 6-8 weeks and then transferred to potting soil composed of a 1:1 mix of autoclaved sand and soil, and were kept at 23 $\pm$ 2 °C with 16-h photoperiod. 80% of the transferred plantlets survived in the potted soil and did not show any morphological abnormalities (Figure 1C).

The present experiment demonstrates a very simple one step protocol for the rapid propagation of a medicinally important herb of *A. galanga* which can be applied as a part of *in vitro* conservation of germplasm.

References

Balachandran SM, Bhat SR & Chandel KPS (1990) *In vitro* clonal multiplication of turmeric (*Curcuma* spp). and ginger (*Zingiber officinale Rosc*). Plant Cell Rep 8: 521–524

Borthakur M & Bordoloi DN (1992) Micropagation of *Curcuma amada* Roxb. J. Spice and Aromatic Crops 1: 154–156

Chang BKW & Chiley RA (1993) Clonal propagation of pink ginger *in vitro*. Hort Science 28: 1203

Hosoki T & Sagawa Y (1977) Clonal propagation of ginger (*Zingiber officinale Rosc.*). Hort Sci 12: 451–452

Inden H & Asahira T (1988) Micro propagation of ginger. Acta Hort 230: 177–184

Itokawa H, Morita H, Sumitomo T, Totsuka N & Takeya K (1987) Antitumour principles from *A. galanga*. Planta Med 53: 32–33

Kuruvinashetty MS, Haridasan P & Iyer RD (1982) Tissue culture studies in turmeric (*Curcuma longa* L.) In: MK Nair et al., (eds) Proc. Nalt. Seminar on ginger and Turmeric CPCRI Kasaragad, Kerala (pp 39–41)

Morita H & Itokawa H (1988) Cytotoxic and antifungal deterpenes from seeds of *A. galanga*. Planta Med 54: 117–120

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

Nadgauda RS, Mascaenhas AF, Hendre RR & Jagannathan V (1978) Rapid multiplication of turmeric (*Curcuma longa* L.) plants by tissue culture. Indian J Exp Biol 16: 120–122

Qureshi S, Shah AH & Ageel AM (1992) Toxicity studies on *A. galanga* and *Curcuma longa*. Planta Med 58: 124–127