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Short Communication

In vitro propagation of spiral ginger [*Costus speciosus* (Koen.) Sm.]

Ravindra B. Malabadi

Division of Plant Tissue Culture, Department of Botany, Karnatak University, Dharwad 580 003

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Spiral ginger [*Costus speciosus* (Koen.) Sm.] (family Zingiberaceae) is a rhizomatous perennial herb with pinkish white flowers in reddish bracts. It is distributed below 1500 m altitude in tropical forests throughout India. The plant is ornamental and the rhizome is a source of an antihelmintic compound and an alternative source of diosgenin [1, 2]. It is also used locally for treating diabetes. The root extract acts as an astringent, aphrodisiac, depurative, purgative and useful in catarrhal fever, coughs, skin diseases and snake bites [3, 4]. The plant is conventionally propagated by vegetative techniques using rhizome and sucker segments and through seeds, which are very slow for large-scale plantation. Due to large indiscriminate collection of its medicinal rhizome from the wild and insufficient attempts either to allow its replenishment or its cultivation, *Costus* speciosus is rapidly disappearing from its natural habitat. The rate of regeneration of these plants are poor which make it imperative to go in for augmentative research on the development of suitable tissue culture and micropropagation methods. Therefore, there is an urgent need to develop methods for the mass propagation and conservation of this threatened species [5].

The rhizomes of Costus speciosus collected from the Western Ghat Hills near (Belgaum-Panaji highway road) Goa, India were thoroughly washed in Tween-20 for 15 min and then under running tap water for 2 hours Whole rhizomes were sterilized with 70% ethanol for 5 minutes followed by immersion in 0.1% $HgCl_2$ for 2 minutes, rinsed 4-5 times with sterile double distilled water. Rhizomes were cultured (Fig. 1) on modified Murashige and Skoog's basal medium (MS) [6] containing 50, 100, 150 and 200 mg/l^{-1} adenine sulphate (AS), 5, 10, 15 & 20% v/v coconut water (CW), 1.0 g/l^{-1} casein hydrosylate (CH), 1 g/l^{-1} L-glutamine, 0.5 g/l^{-1} meso-inositol, 30 g/l^{-1} sucrose (Analar), 7 g/l^{-1} agar (Difco-bacto) and supplemented with various permutation and combinations of 6-Benzyladenine (BA) 2.22, 4.44, 8.87 and 22.19 µM), Kinetin (KN) 2.32, 4.65, 9.29 and 23.23 µM) and ∝-Naphthalene acetic-acid (NAA) (2.69, 5.37, 10.74 and 26.85) respectively. The cultures were maintained at 25±2°C with 55-60% relative humidity on a 16 hour photoperiod under cool white fluorescent light (100 µmol m^{-2} s^{-1}). The stock solutions of L-glutamine, case in hydrosylate and coconut water were filter sterilized and added to the media after autoclaving.

Each replicate contained 10 cultures and for each set of experiment, 3 replicates were maintained. All the experiments were repeated three times. Observations

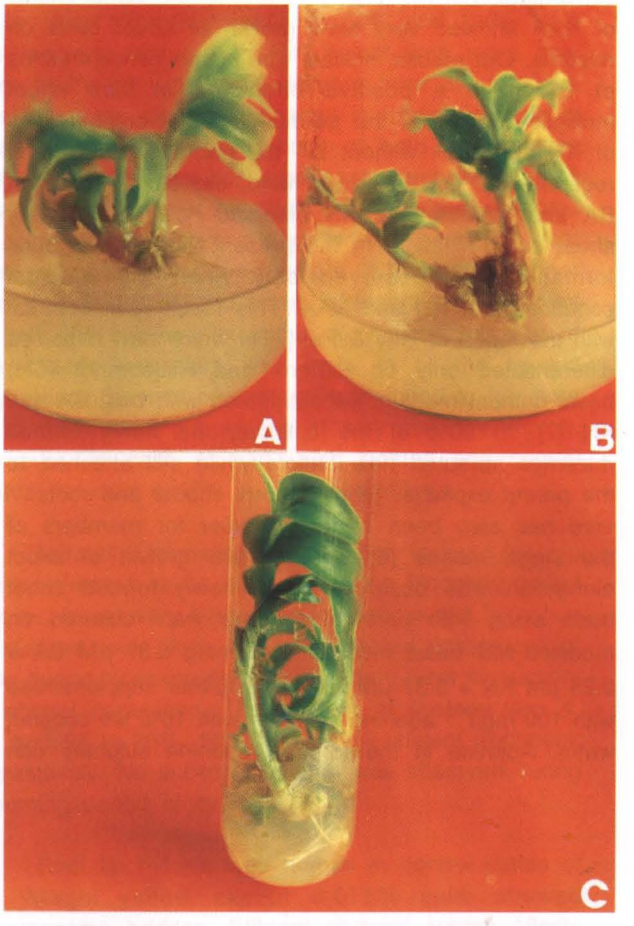

Fig. 1. Regeneration of plants from the in vitro cultured rhizomes of spiral ginger [Costus speciosus (Koen.) Sm.] A. In vitro cultured rhizome showing shoot regeneration with roots (bar = 1cm); B.&C. isolated shoots showing luxuriant growth with newly formed rhizomes (bar = 1cm)

on the number of shoots per rhizome and number of roots per shoot were recorded after 6 weeks of culture.

The present study demonstrates the successful shoot regeneration from the *in vitro* cultured rhizomes of spiral ginger. Only the rhizomes cultured on modified MS basal medium supplemented with 8.87 μM BA + 9.29 μM KN + 5.37 μM NAA (Shoot Initiation Medium) were able to form maximum number of shoot buds with 10 to 11 roots (Fig. 1). The combination of KN (2.32 and 4.65 μM), NAA (2.69 and 10.74 μM) with 8.87 μM BA resulted in the formation of 4 to 5 shoot buds. On the other hand the combination of KN (23.23 μM), NAA (26.85 μM) with 8.87 μM BA showed least number of shoot buds. Lower concentration of BA (2.22 and 4.44 μM), higher concentration of KN (23.23 μM) with different concentrations of NAA, rhizomes remained green but completely failed to produce shoot buds. Higher concentration of BA (22.19 μM) and lower concentration of KN (2.32 μM) with all the combinations of NAA showed very slow growth of shoot buds on rhizome. Our results indicate that higher concentrations of BA have a positive effect on *in vitro* shoot multiplication. In all the permutation and combinations of KN and NAA without BA did not show any shoot regeneration. On the other hand, the combinations of BA and NAA without KN also did not result in any shoot buds initiation. In the present study shoot buds formation was observed only when MS medium contained BA, KN and NAA. This result is in contrast with the report of Roy & Pal [7] in which new rhizomes differentiated only on Schenk and Hildebrandt (SH) basal nutrient medium supplemented with both BA and KN [7]. In 20% of the cultures, the newly formed rhizomes sprouted new shoots while still attached to the parent explants. Simultaneous shoots and roots *in vitro* has also been reported earlier for members of the Zingiberaceae [8, 9]. Luxuriant growth of shoot elongation was observed when newly formed shoot buds along with parental rhizome were cultured on modified MS basal medium containing 8.87 μM BA + 9.29 μM KN + 5.37 μM NAA and further supplemented with 100 mg/l^{-1} adenine sulphate with 10% v/v coconut water. Adenine in the form of adenine sulphate can stimulate cell growth and greatly enhance shoot formation. It provides a source of organic nitrogen to the cell and can generally be taken up more rapidly than inorganic nitrogen. However, on isolation and culture the new rhizomes produced plantlets. The plants with well developed roots were transferred to pots containing vermiculite and hardened for one week at 80% relative humidity at 25±2°C. After establishment, the plants were transferred to a green house. *In vitro* production of rhizomes, not only provided microrhizomes for storage and distribution but also for propagation through regeneration of multiple shoots. This simple and very efficient protocol for rapid propagation of spiral ginger (*Costus speciosus*) can be applied as a part of conservation of this species.

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