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Full Length Research Paper

One step method of plantlet regeneration in *Trichosanthes dioica* Roxb.: An approach towards cost effective and shorter protocol

Rashmi Komal

Tissue Culture Laboratory, Department of Botany, Patna University, Patna, India. E-mail: rashmi0911@gmail.com.

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In order to reduce the cost and time of *in vitro* raised plants of *Trichosanthes dioica* Roxb., a minimal medium has been formulated by substituting costly growth regulators from the medium with a cost effective constituent, the coconut milk. A semisolid Murashige and Skoogs's medium supplemented exclusively with 15% coconut milk showed the highest percentage of plantlet regeneration (99%) in the explants. When nodal, shoot-tip and immature leaf explants were cultured on this medium, rhizogenesis was observed in about 5 to 6 days of inoculation, followed by shoot formation in about 8 to 10 days. The fully developed plantlets, 10 to 12 cm in length with professed roots were obtained in about 20 days of inoculation in a single step without adding/changing growth regulators. After transplantation in the potted soil, these plantlets showed similar growth patterns as compared to the plants obtained from a conventional three-four step method of tissue culture experiments.

Key words: Coconut milk, tissue culture, *Trichosanthes dioica* Roxb.

INTRODUCTION

In vitro plantlet regeneration is a sequel to the exposure of explants to a series of media with hormone supplementation as a two or three step process. In a typical tissue culture experiment, the protocol consists of as a first step, the induction of callus formation, the second step morphogenetic/embryogenetic induction and the third step organogenetic differentiation. Each phase require different hormone and takes about eight weeks to show specific results. This tissue culture methods are mostly based on technologies developed over twenty-five years ago. The main factors which ultimately influence the commercial propagation of plants *in vitro* are: the selection of plant species, the physical environment and the chemical media for the production of large number of plants within limited time with minimum input expenses. There seems to be a good scope for substituting the expensive chemical nutrient media with low cost natural extracts. An experiment has been formulated which involves only a single step, and the culture medium is devoid of growth regulators, except coconut milk. It takes about three-four weeks from the day of inoculation for the explants to regenerate into a plantlet ready for acclimatization and potting thereafter.

This experiment has been tried on *Trichosanthes dioica* Roxb. (Pointed gourd), a plant belonging to the Cucurbitaceous family, having both nutritive as well as medicinal value. The stem and leaves are used as antipyretic, anthelmintic, aphrodisiac and in the treatment of blindness and bronchitis. The fruits are used as appetizer, cardiotonic, diuretic, laxative, stomachic and blood purifier (Kirtikar and Basu, 1975).

MATERIALS AND METHODS

Single nodes, shoot tips and tender leaves were taken as explants from mature plants grown at the Botany Department, Patna University, Patna, India. Explants were washed in running tap water in plastic pots for 10 to 15 min. The explants were surface sterilized with detergent and 1 to 2 drops of Tween-20 for 5 to 10 min in a beaker containing tap water. The explants were transferred to autoclaved plastic pot and treated with 0.1% (w/v) aqueous mercuric chloride solution and 2 to 3 drops of Tween-20 and shaken for 8 min under laminar air-flow cabinet. Then explants were shaken in ethyl alcohol (70%) for 3 min. Further, the explants were rinsed thrice with sterile double distilled water in an aseptic condition. Finally,

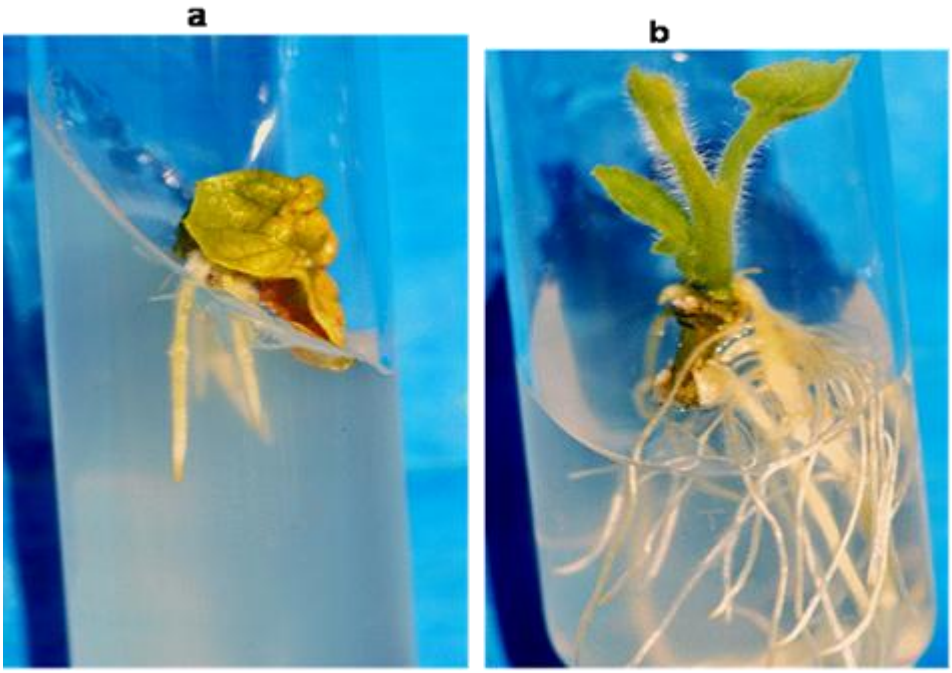

Figure 1. Whole plant regeneration of *T. dioica* Roxb. in MS medium supplemented exclusively with 15% coconut milk without growth regulators. a, Rhizogenesis in leaf explant after 6 days of inoculation; b, Shoot formation in rooted nodal explants after 12 days of inoculation; c, Plantlet regeneration after 20 days of inoculation; d, Multiple shoot formation after 25 days of inoculation.

using sterile forceps, explants were transferred to sterile Petri dishes and cut into small pieces (0.5 to 1.0 cm) with sterile scalpel.

Different concentrations of coconut milk (5, 10, 15, 20 and 25%) was tested for callus induction/shoot initiation, shoot proliferation and rooting. The pH of the medium was adjusted and maintained between 5.6 and 6.0 with the help of 0.1 N NaOH or 0.1 N HCl before adding 0.6% agar (Hi-media, Mumbai). The cultures were incubated in the culture room maintained at 26 ± 2°C with a relative humidity of about 55 to 65% and 16 h photo-period, with 2000 to 2500 lux light intensity from fluorescent tubular lamps. The media were sterilized by autoclaving at 121°C for 20 min at 15-psi pressure. After acclimatization, the rooted micropropagules were transferred in sterilized pots containing a mixture of sterilized soil, sand and farmyard mixture in the ratio of 1:1:1. About 75% plants survived and hardened properly, and were then transferred to natural soil and environment.

Some of the adventitious shoots were cut from the basal end of the regenerated plantlets leaving two-three nodes at the base and subcultured in fresh medium with same composition. The left over nodal portion regenerated into multiple shoots which served as the explants for further experiments/inoculations.

RESULTS AND DISCUSSION

A simple one-step method of plantlet regeneration from axenic node, shoot tip and leaf explants on MS semisolid medium supplemented with different concentrations of coconut milk only, has been experimented and reported by the author in this project. The regeneration occurred through direct organogenesis without callus formation. High frequencies of root formation was seen in the nodal and shoot tip explants cultured on semisolid MS media supplemented with 15% of coconut milk, and the quantity of agar used was 0.6%, but the tender leaves used as explants also showed rhizogenesis (Figure 1a). Among the various concentrations, MS supplemented with 15% coconut milk showed the highest percentage of shoot induction (99%).

Rooting was observed in about 5 to 6 days (Figure 1a) in all the types of explants inoculated followed by shooting in about 8 to 10 days of inoculation (Figure 1b). The fully developed plantlet, 10 to 12 cm in length with professed rooting was obtained in about 20 days of inoculation (Figure 1c). These plantlets were then acclimatized in an inorganic salt solution of MS media for one week before soil transfer. After transplantation in the potted soil (Figure 2), these plants obtained by one step method responded the same (without compromising the quality) way as the plants obtained from a conventional tissue culture experiment in which the explants were exposed to a series of media with hormone supplementation as a three-four step process.

Some of these regenerated plantlets before acclimatization (Figure 1c) were cut after 25 to 30 days from the basal end leaving two-three nodes at the base and subcultured again in a medium with similar composition and concentration for further plantlet regeneration. Multiple shoots originated within a week from the three nodes which were left in the culture tubes after cutting the upper

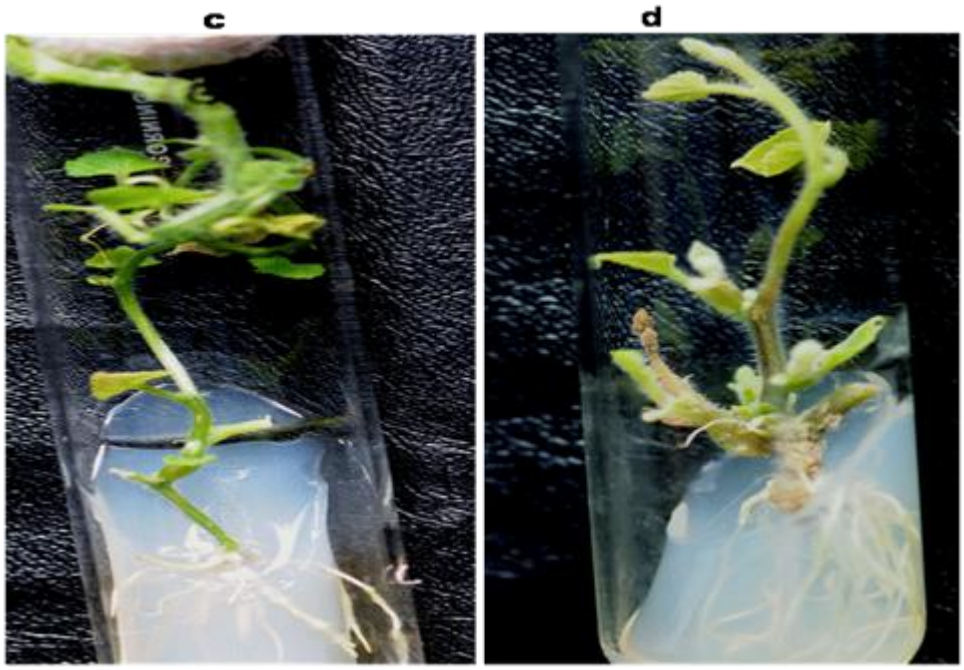

Figure 1. Continued.

Figure 2. A plant raised *in vitro* by one step method of plantlet regeneration after 30 days of potting.

shoots (Figure 1d). These regenerated shoots served as the explants for further inoculation, resulting in clone formation. The rooted micropropagules were acclimatized and transferred in sterilized pots containing a mixture of sterilized soil, sand and vermicompost in the ratio of 1:1:1 and successfully survived.

Coconut milk, the liquid endosperm of the fruit of *Cocus nucifera* L. is widely used as additives in plant tissue

culture experiments. The effects of using yeast and plant extracts in *in vitro* culture has been investigated by a number of workers. One of the earliest reports is that of Overbeek et al. (1941), who succeeded in growing immature *Datura* embryos in culture by including the liquid endosperm of *C. nucifera* L (coconut milk) in their culture medium. Other plant juices and liquid endosperms have been shown to possess stimulatory properties more or less similar to those of coconut milk. The inclusion of liquid endosperm from immature corn (Netien et al, 1951), tomato juice (Nitsch, 1951; Straus and La Rue, 1954), immature fruits and seeds (Steward and Caplin,1952; Steward and Shantz, 1959), orange juice, malt extract, yeast extract, leaf extracts from a number of plants and tumor extracts (Butenko,1968), have been reported.

The rapid cell division in the tissue of *T. dioica* Roxb. was induced by the addition of coconut milk in semisolid MS medium, which supports the presence of natural auxin as well as cytokinin in the medium and its activity gets increased by autoclaving due to its hydrolysis. There is no report regarding the use of coconut milk in *in vitro* culture medium of *T. dioica* Roxb. till date. The auxin-like and gibbereline-like activity was detected and reported earlier in both coconut milk and malt extract by several workers, but some of the recent reports have been mentioned. The auxin activity of coconut milk was stated by Edwin et al. (2007). The cytokinin activity in coconut milk was identified by Kobayashi et al. (2007). Due to the presence of natural cytokinin in coconut milk, its addition in the media gives similar effect as adding a recognized cytokinin. The presence of cytokinin and other phytohormones in coconut milk has also been identified by Jean et al. (2009). Indole-3-acetic acid was tentitatively identified as being present in malt extract (Dix and Van Staden, 1982).

This method of clonal propagation of *T. dioica* Roxb. in semisolid MS media supplemented with 15% coconut milk exclusively through *in vitro* culture of stem cuttings has been tested on large scale and is an example of successful industrial application of tissue culture in micropropagation of plants having commercial value. It can be applied on several other plants with economic and medicinal importance. The introduction of one step method is especially useful for preparing propagation stocks of required cultivar. It will result in economical plant production under green house conditions in a very short time with very less effort (less man power required). By applying this method, we can reduce the cost of expensive growth regulators, as we know that coconut milk is cheaper than the growth regulators, and save 2 to 3 times of the chemicals required for media preparation in a conventional three-four step method. Germplasm can be cloned and preserved on a large scale in short time, it has easy maintenance, less space, free from infection and above all, the production cost will be much reduced. Thus, due to the cost cutting measures applied in this experiment and successful single step, clonal plant production can be called a novel method of tissue culture, which gives successful result on other plants also.

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