



## Commercial production of micropropagated *Coccinia indica* (Tondli) - A success story

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### ABSTRACT

*Coccinia indica*, a common vegetable from the cucurbit family. It is a perennial vine. Considering the poor survival of vegetatively propagated cuttings in the field, followed by low yields with misshapen fruits due to diseased plants, an alternative was sought to micropropagate selected mother plants of a locally cultivated variety.

A protocol was standardized for micropropagation using modified MS medium fortified with different concentrations of kinetin and Indole-3-acetic acid. After hardening, field planting was done. The first trials were taken in our own field in year 2009 when 100 plants were planted to see the flowering, uniformity of fruit size and yield. In subsequent year 50,000 plants were planted in farmers' fields. On an average, the farmer harvested every third day between 1000 to 1500 kg vegetable per acre as compared to 150 to 250 kg. A typical plantation of one acre gives a total yield of 48 to 50 tons per year. The plants produced by micropropagation outperformed the conventionally propagated plants by a factor of ten. This method of production of micropropagated plants has now become a common practice for tindora crop.

#### KEYWORD

micropropagation, *Coccinia indica*, ivy gourd, vegetable tissue culture, commercialization, medicinal properties

### INTRODUCTION

*Coccinia indica*, Wight & Arn., or ivy gourd, is commonly known as Tindora, Kundru, Tondli, Ghiloda, Gowai, Kovakka, Dondakaya in various states in India, and hong gua in Chinese and is synonymous with *Coccinia grandis* [HYPERLINK "http://www.plantnames.unimelb.edu.au/new/Coccinia.html"](http://www.plantnames.unimelb.edu.au/new/Coccinia.html) [\(L.\)J. Voigt](http://www.plantnames.unimelb.edu.au/new/Coccinia.html), or *Cephalandra indica*, W & A.

It is an underexploited cucurbit having an aggressive climbing behavior with fruits rich in carbohydrates, vitamin A and C. According to Ayurveda, there are two varieties, wild or bitter, and sweet or cultivated. The former is used in folklore medicine. The latter, although has no defined varieties, has been found to have two distinct type of fruit characters. First is having short and oblong type of fruit having white striations on the rind which is dark green in color, and produces more seeds. The second type has light green rind with no striations, fruits are slenderer and having less seeds. After the first scientific study by Chopra and Bose (1925), several workers have done pharmacological studies implicating the role of this plant in diabetes mellitus (Mukherjee et al., 1972; Hossain et al., 1992; Venkateswaran and Pari, 2003). It is also a subject of discussion on many websites (Yadav, 2011). Pharmacognosy in this plant is extensively reviewed recently by Tamilselvan et al., (2011). Dharmatti et al., (2008) have investigated 46 genotypes from Southern India in terms of yield and quality parameters, designating a particular DRC-1 genotype as most superior.

It is a perennial vine of the Cucurbitaceae family, producing small cucumber like fruit which turns scarlet red when ripe, hence the term scarlet gourd. The cultivated varieties produce bisexual flowers, and fruit is few inches long, up to one inch diameter and has a smooth rind which is light green or striped green in color. The fruits are used as a vegetable or salad in different countries. The young stems and leaves are eaten as food and also used as medicine, and reported to having hypoglycemic, hypolipidemic and antioxidant properties. The cultivated, or sweet *C. indica* is asexually propagated from cuttings of woody stems during monsoon, since the seeds if produced at all are not viable. Commercial cultivation is done by training plants on a bower system. Planting material is usually scarce, and the fruits are distorted in shape due to infection, presumably carried forward from previous source due to its susceptibility to powdery mildew caused by species of *Erisiphe*. As a result, farmers are reluctant to cultivate this easy to grow crop. Looking at the success of commercial production of other tissue culture raised crops such as banana, sugarcane etc., we attempted to apply similar technology for micropropagation of Tindora. There are few records of tissue culture and organogenesis from leaf callus of this plant. However, there is no mention of fully developed plants and their field performance in any of the published work.

### MATERIALS AND METHODS

Planting material was collected from a field of healthy growing, disease free plants showing good yields. The fruit was light green, having an average length of 2 inch and diameter of 1 inch. Apical portion of the stems were washed in running water for about half hour, and then sterilized using 0.1% mercuric chloride for 1 to 2 minutes. After rinsing twice in sterile distilled water, the young leaves were

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dissected off from the tips and the apical buds were inoculated onto sterile culture medium.

Culture medium having 3 % sucrose and gelled with 0.7 % agar. Different combinations of 6 BAP (0.1 to 5 mg/l) and indole-3 acetic acid (0.1 to 1.0 mg/l) were tried for culture incubation. Test tubes were incubated at  $25 \pm 2$  C at 16 h photoperiod.

Newly initiated shoots were sub-cultured on fresh media after three weeks. At every 4 week interval, single nodes were dissected and subcultured for multiplication. Well developed shoots were allowed to grow further on hormone free medium for root development. Rooted shoots were transferred to greenhouse and kept for primary hardening in mixture of cocopeat: soil (1:1) in portrays. After 4 weeks, the young plants were transferred to bigger trays in plugs containing a mixture of sand: farm yard manure and cocopeat (1:1: 2) and allowed to grow till field planting.

About 50 tissue culture derived plants were grown in field during the first season on a trial basis.

During subsequent years, more plants were produced using

the standardized technology and grown in farmers' fields. Data on economics was collected from them.

### Results and Discussion

During the early stages of growth, the medium contained modified MS salts (ammonium nitrate  $\frac{1}{2}$  times and calcium chloride  $1 \frac{1}{3}$  times that of MS). Instead of the low strength vitamins, higher levels were used (1 mg/l each of nicotinic acid, pyridoxine hydrochloride, thiamine hydrochloride).

Plant growth regulators used were 6BAP at 0.1 mg/l and 0.2 mg/l concentration, along with 0.1 mg/l IAA and 0.2 mg/l GA3. Among other organic constituents, coconut water (1 to 5 %) and casein hydrolysate (100 mg/l) were incorporated into the culture media.

Results indicated that when 6BAP was added to the medium, it showed better shoot growth giving a greater number of nodes in unit time as compared to having Kinetin in the medium, however there was more callus at the base of the explants and subsequently leaf yellowing was seen (Table 1).

**Table 1:** Tissue culture response of *C. indica* to various media components

Medium composition	No of cultures tested	No of cultures showing multiplication and %	Leaf development	No. of nodes/shoot	Degree of Callusing at base of shoot
6BAP 0.1 mg/l + 1% CW	14	3 (21%)	Small leaves, less greening	3-4	++
6BAP 0.1 mg/l +2.5 % CW	21	15 (71%)	Medium leaves, less greening	3-4	++
6BAP 0.1 mg/l + 5.0% CW	14	7 (50%)	Medium leaves, green	3-4	++
KIN 0.1 mg/l + 1% CW	14	8 (57%)	Medium leaves, green	2-3	+
KIN 0.1 mg/l +2.5 % CW	21	2 (10%)	Large leaves, green	2-4	+
KIN 0.1 mg/l+ 5.0% CW	14	5 (35%)	Medium leaves, green	3-4	+

It was observed that with incorporation of casein hydrolysate in the medium, maximum number of shoots developed from the axillary bud and the shoots and leaf growth were excellent. However, there was extensive callusing at the base of the explants in all cases. Similar results were obtained when

the 6BAP was doubled to 0.2 mg/l (Table 2).

A combination of 0.1mg/l KIN with 5 % coconut water was found to be giving best response and hence this was used for further micropropagation.

**Table 2:** Tissue culture response of *C. indica* to addition of casein hydrolysate in media components

Medium composition	Leaf development	Shoot development	Number of shoots	Degree of Callusing at base of shoot
6BAP 0.1 mg/l	Medium leaves, green	+++	3-5	++
6BAP 0.2 mg/l	Large leaves, green	++++	3-5	+++
6BAP 0.1 mg/l + 100 mg/l casein hydrolysate	Medium leaves, green	+++	3-5	++++
6BAP 0.2 mg/l+ 100 mg/l casein hydrolysate	Large leaves, green	++++	3-5	++++

After shoots developed having 5-6 nodes, they were transferred to medium devoid of any growth regulators in which root development was seen two weeks after transfer.

The objective of using the micropropagation was with the hypothesis that *C. indica* is asexually propagated ever since records are available and hence it must have accumulated many viruses or other disease-causing organisms causing poor performance.

Plants derived from meristematic tissue would be presumably free from such organisms and will give higher yield. However, we have not verified the hypothesis by conducting any pathological tests. But the

plants show a rapid growth rate and bear more fruit for a longer period of time thus increasing overall yield (see Table 3).

There are few reports on tissue culture of the medicinally important variety of *C. grandis*. For example, Sundari *et al.*, (2011) have reported rapid micropropagation in the *Coccinea grandis* (used for medicinal purpose) variety using MS + 0.5 mg/l Kn + 1 mg/l BA + 0.3 mg/l IBA. However, there is no report of hardening, and field studies of the tissue cultured plants. Similarly, Gulati, (1988), Sarker *et al.*, (2008) and Josekutty *et al.*, (1993) have also reported plant regeneration from various tissues but results on further growth of the plantlets during primary, secondary hardening are lacking. Ghantikumar *et al.*, (2013) as

**Table 3:** Comparison of plant performance in conventionally grown vs Tissue culture produced plants. Data from several farmers' field

	Conventionally grown	TC produced
Plant Habit	Plants grow up to support in 6-7 months	Plants grow up to support in 2-3 months
Yield/acre	100-250 kg	1000-2500 kg
Harvesting frequency	Once a week	Every third day
Duration of fruiting	Poor or no yield in cooler months (December to February)	Continuous fruiting even in cooler months
Fruit quality	'A" grade fruits 20 to 30 %	'A" grade fruits 70 %
Fruit characters	Fruits with more seeds, less pulp and less flavored	Fruits tender with more pulp, less seed and taste better

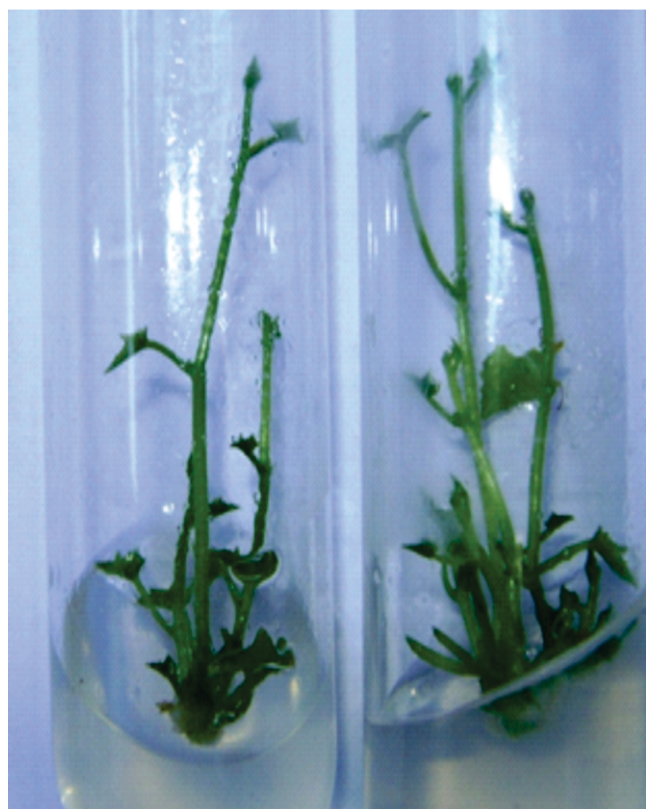


Fig. (a) Multiplying Cultures;



Fig. (b) Primary hardened plants;

well as Patel and Ishnava (2015) also described micropropagation of the medicinal plant with results only up to hardening stage.

We are reporting here for the first time a complete micropropagation protocol for the edible (sweet) variety of *C.*

*indica* up to field level. Moreover, we also give a comparative evaluation of the micropropagated plants in the field vis a vis the traditionally grown plants through cuttings. Since the last 8 years we have been producing and distributing about 1 lac plants per annum, and the demand is steadily increasing.



Fig. (c) secondary hardened, ready to plant plants;



Fig. (d) Tissue culture plants in growers shade house



Fig. (e) uneven and misshapen fruits from non-tissue cultured plants;



Fig. (f) Fruits from tissue cultured plants.

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