Comparative studies on growth and Yield of Conventional and Tissue culture plants of Turmeric (*Curcuma longa*) var. CO2

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**ABSTRACT**

Turmeric (*Curcuma longa* L.) is an ancient spice, native of India and South East Asia used from antiquity as spice and a dye. It is commonly propagated through rhizomes. The availability of disease free quality planting material is scarce during the cropping season (June – September). An experiment was conducted to study the performance of *in vitro* derived turmeric plants with conventional rhizome under field condition. The results indicated that the tissue culture plants showed better performance over the conventional rhizome planting. Tissue culture plants grew vigorously and taller than conventional type. The highest yield potential was observed in tissue culture plants (40.83 tons/ha) as compared to the conventional rhizome planting (30.14 tons/ha). The rhizome rot incidence was lower (3.87%) in tissue culture plants than rhizome-derived plants (25.58%). However, the agronomic traits observed during the present study in tissue culture plants are stable and rhizome harvested from tissue culture plants can be used as disease free planting materials for further planting.

**Keywords:** Conventional propagation, Rhizome yield, Tissue culture plants and Turmeric.

Turmeric (*Curcuma longa* L.) is the third important spice crop, grown in tropical part of India. It is mainly used as condiment, dye and in drug and cosmetic industries. In India, it is grown in an area of 1,94,000 ha with a production of 9,90,000 tons. In the state of Tamil Nadu it is grown in an area of 35,700 ha with production of 1,90,000 tons and productivity of 5.3 tons of dried rhizome/ha. Turmeric is propagated through rhizomes and large quantity of rhizome is required as planting material for the commercial cultivation. Planting material requirement is about 2.5 t/ha and total requirement of the country is about 2 lakh tonnes per year. Storage loss of rhizomes due to rhizome rot disease is severe which causes tissue senescence and degeneration. Propagation of turmeric through seed is not economical because of poor flowering and seed set (Balachandran et al., 1990).

Curcumin, a major constituent of rhizome has been widely used in various medicinal purpose, which increased the demand of the rhizomes (Chattopadhyay et al., 2004). The conventional methods of propagation are incapable to produce large quantities of quality planting material, which necessitates the new method. Tissue culture is used to multiply large quantities of pest and disease free planting material and performance evaluation of tissue-cultured plants is necessary to determine the potential benefits. Main purpose of this study was to assess the field performance of the *in vitro* derived plantlets and its effect on morphology and development of turmeric plants.

The present study was carried out at HC & RI, Coimbatore, Tamil Nadu, India. *In vitro* plants were obtained by following the procedure described in the previous protocol (Babu et al., 2007). In this study, bud sprouts were used as explant and inoculated on to half strength MS media supplemented with 3 mg l⁻¹ BA and 1 mg l⁻¹ NAA. Contamination free one-week-old cultures were transferred into multiplication medium (MS medium containing 5 mg l⁻¹ BA and 1.0 mg l⁻¹ NAA). Sub-culturing was done using 7-8 weeks old culture for further multiplication. Well root *in vitro*
plantlets were transferred to polythene bags containing garden soil, sand and farmyard manure in equal proportions and kept in shade net for hardening and establishment. The rhizome was obtained from true-to-type plants maintained in shade house for conventional rhizome planting. Plants were planted during 2016-17, using a completely randomized block design with equal number of replication. Plants were spaced at 0.45 x 0.25 m with the plot size of the experiment was 3 x 5 m. A fertilizer dose of 25:60:106 NPK Kg ha$^{-1}$ was uniformly applied to all the plots. Data were collected on the important morphological characters such as plant height, number of tillers per plant, number of leaves, length and width of leaves, number of finger per plant, average finger diameter, total weight of rhizome per plant, weight of mother rhizome and number days for maturation of the plant. Each of the morphological characters except rhizome morphology was scored and recorded every two months interval, until the time of harvest. Statistical analysis was conducted for each experiment and pooled analysis over the experiments was conducted using a randomized complete block design method. Significance was determined at the 0.05 probability level. All statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to compare means.

Phenotypic variant or off type plants are commonly observed in in vitro raised population of C. longa var. CO 2. Variants were observed in micropropagated plants in all the three different replicated sites exhibiting the changes in leaf morphology and colour. The $\chi^2$ test for independence indicated that phenotypic variation and propagation methods were independent criteria. ($\chi^2 = 3.70$, $p = 0.32$) (i.e., the ratio of true-to-type to off type plants remained the same for both methods of propagation). The micropropagated plant showed two times higher variation frequency than rhizome-derived plants (Table 1). Similar result was observed in banana (Smith and Drew, 1990; Smith, 1988; Dirk et al., 1996). Among the variation observed in both the populations (in vitro and conventional plant), tillers of approximately 3.7% of in vitro raised plants and approximately 1.8% of conventional rhizome plants had leaves with one half of the lamina green and the other half albino and variegation on the edge of lamina (Table 1). Neeta et al. (2002) also documented similar results in turmeric var. ‘Elite’. However, conclude that results of phenotypic variation rate were not always

| Characters | Conventional rhizome derived plants | Tissue culture plants | Difference |
|------------|--------------------------------------|----------------------|------------|
| Off type (%) | 1.8$^a$ | 3.7$^b$ | 1.9** |
| Plant height (cm) | 65.61±.19$^b$ | 76.88±.27$^b$ | 11.27* |
| Number of leaves per plant | 10.93±.10$^b$ | 13.13±.12$^d$ | 2.20* |
| Leaf length (cm) | 39.38±.18$^e$ | 43.05±.21$^e$ | 3.67** |
| Leaf breadth (cm) | 12.69±.14$^f$ | 14.01±.25$^f$ | 1.32** |
| Number of tillers per plant | 2.73±.17$^g$ | 4.60±.15$^g$ | 1.87** |
| Weight of mother rhizome (g) | 105.83±.27$^h$ | 136.62±.22$^h$ | 30.79* |
| Weight of primary rhizome (g) | 148.30±.30 | 164.58±.58$^i$ | 16.28* |
| Weight of secondary rhizome (g) | 72.43±.35$^j$ | 85.58±.27$^m$ | 13.15* |
| Fresh rhizome yield/plot (15 m$^2$) (kg) | 45.62±.15$^n$ | 61.25±.10$^n$ | 15.63* |
| Estimated fresh rhizome yield/ha (t) | 30.14 | 40.83 | 10.69** |
| Maturity (days) | 276** | 255** | -21.00** |
| Rhizome rot incidence (%) | 25.58±.25$^o$ | 3.87±.40$^q$ | -21.71** |

Value are means ± SE, n=108.*Significant at $p$ d” 0.05; **non-significant; ***maturity date, when 95% of leaves become yellowish. Means followed by same letters are not significantly different at $p = 0.05$. 

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consistent over trial, which however, may be greatly influenced by environmental factor.

True-to-type plants were included in the analysis of variance of the horticultural performance of conventional propagated vs. micropropagated plants of turmeric var. CO 2. Growth and yield parameters were recorded on tissue culture and rhizome derived plants, which showed significant differences in all characters except number of tillers, leaf length and breadth (Table 1). In vitro turmeric was significantly taller than the conventional type, throughout the vegetative growth cycle. Plant height is a measure of plant vigour, indicating that in vitro plants established more quickly and grows vigorously than conventional plants (Dirk et al., 1996; Sheela et al., 2001). The tissue culture plants showed vigorous and fast increase in the length of shoots as well as new shoot emerged out from the base after one week. Development was more advanced (76.88±2.27 cm) than that reached by conventional plant (65.61±1.9 cm) of the same age after 6 months. Similar result was documented by Beruto et al. (1996) in Ranunculus asiaticus and Singh et al. (2013) in turmeric.

Fast growing in vitro plants produced new leaves at a faster rate, resulting in larger leaf area during vegetative growth than that of conventional type. In vitro raised plant gave approximately 2.20 more number of leaves and produced higher number of leaves (13.13±1.12) as compared to plants from conventional rhizome (10.93±1.10) throughout the growing period (Table 1). This is in agreement with the finding of Neeta et al. (2001), Israeli et al. (1988) and Hwang et al. (1984), noted that micro-propagated plant retained more healthy leaves than conventional plant due to fast rate of leaf emission. The tissue culture plant showed appreciable vegetative growth, produced longer shoots and more number of leaves, after 6 months of propagation in both the propagation type. Presumably, the increase in vegetative growth has contributed to the increase in shoot yield and number of leaves of tissue culture plants of turmeric. The comparison of in vitro plants showed no significant variation for number of tiller and leaves width.

Tissue culture plants recorded significantly higher weight of finger rhizome (164.58±.58 g) than that of conventional plant (148.30±.30 g) (Table 1). An increased of weight of finger rhizome (16.28 g) may be attributed to genetic uniformity of the plant, due to selection of superior types of micropropagated plants (Dikash et al., 2012). Increased in the weight of finger rhizome ultimately increase in the yield of turmeric plant. This is in support with the finding of Neeta et al. (2001). Considering the weight of mother and fingers rhizome produced per plant, tissue culture plants, gave significantly better rhizome yield per plant than conventional rhizome. This is consistent with the fact that tissue culture plant has more potential in growth, yield and rhizome production than conventional type (Neeta et al., 2002; Dirk et al., 1996; Sheela et al., 2001; Beruto et al., 1996).

The mean rhizome yield plot of in vitro raised plants was observed more (61.25±1.10 kg) than the conventional rhizome yield (45.62±1.15 kg). During the present investigation, there was no significant difference in the time taken for maturation for harvest between tissue culture and conventional plant. The enhanced growth rate exhibited by in vitro plants did not delay the plant maturation; however, it has shown less variability in the time taken for rhizome maturation under the same treatment. They were able to complete maturation in three weeks earlier as compared to plants from conventional rhizome. Among the yield attributes, the number of tillers and the total weight of rhizome plant had the greatest correlations with the yield. Estimated fresh rhizome yield plant was highest in tissue culture plants (40.83 t ha⁻¹) than rhizome derived plants (30.14 t ha⁻¹). Singh et al. (2013) also observed same trend in turmeric var. Lakadong. The tissue culture plant had the lowest rhizome rot incidence (3.87±40%) when compared to conventional plant (25.58±25%). Babu et al. (1997) reported that micro propagation technique could be used for production of disease-free planting material of elite plants. Apart from the production of pathogen free planting material, in vitro propagation of turmeric can also used for the conservation and exchange of germplasm (Cheethaparambil et al., 2014).

It should be concluded that the present investigation could be beneficial as the tissue culture plants showed suitable agronomic performance than conventional plants and resulted in the increased fingers weight and subsequently marketable rhizome yields. Field evaluation of tissue culture and conventional plants
revealed that in vitro plants were superior in performance over conventional plant exhibiting vigorous vegetative growth, disease free, increased and uniformity of rhizome production.

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