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Analysis of growth, yield potential and horticultural performance of conventional vs. micropropagated plants of *Curcuma longa* var. Lakadong

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Complete randomized block design was applied to evaluate and compare the growth, yield and field performance of *in vitro* derived turmeric plants with conventional rhizome under field condition. *In vitro* propagated plants manifest consistently superior horticultural performance over the conventional rhizome. Among the different lines of *in vitro* propagated plants, plantlets treated with silver nitrate (AgNO₃) were studied and compared with the conventional rhizome which showed superior growth in almost all the different traits compared. Phenotypic variation was higher in *in vitro* (3.3%) than conventional plant (1.2%) with no statistically significance. Tissue culture plants grew vigorously and taller than conventional type after six months of propagation. The highest yield potential were observed in *in vitro* plants (13.96 ton/ha) as compared with the conventional rhizome (6.97 ton/ha). However, the agronomic traits observed during the present study in tissue culture plants are stable, and has to be ascertained in subsequent years. If provided stable, this clone can be incorporated into the crop improvement programs of turmeric var. Lakadong.

Key words: Turmeric, micropropagation, field performance, tissue culture.

INTRODUCTION

Turmeric (*Curcuma longa*) locally known as "Yaingang" has been used in preparing local cuisines and traditional medicine in Manipur, India for centuries and has gained reinvigorated interest due to its significant medicinal values like the plant extracts reported (Yin et al., 2008a, b; Li et al., 2011; Yang et al., 2012; Kong et al., 2009, 2011). Turmeric is exclusively propagated vegetatively using rhizomes in different parts of Manipur (Imphal, Bishnupur, Urkhul, Churachandpur, Senapati, Tamenglong and Thoubal). The North Eastern region of India offers great potential for large scale cultivation of turmeric plant. Its productivity in the region is only 1.5 tonnes against 3.9 ton/ha in the country. Since, rhizome multiplication is slow, recently, conventional propagation

through seed rhizome was replaced with the technologies of plant tissue culture over the past four decades. The demands for the rhizome have been increased rapidly, due to its medicinal application of turmeric and curcumin, a major constituent of rhizome (Chattopadhyay et al., 2004). Since conventional methods of propagation are unable to supply large quantities of genetically superior individuals required by cultivar development programs, new methods are required to meet the demands. The use of tissue culture in conjuction with classical breeding methods, can accelerate cultivar development programs of this potent species. Moreover, tissue culture technology enables the rapid production of a large quantity of disease free true-to-type plants from a single parent plant showing good genetic potential. Due to high susceptibility of turmeric crops to soft rot (*Pythium* sp.) and bacterial wilt (*Pseudomonas solanacearum*) which is a major constraint in the production, replicated field testing of plants from tissue culture is necessary to

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Table 1. Field evaluation and morphological traits of tissue culture and conventional grown plants of *C. longa* var. Lakadong.

Trait	Conventional rhizome propagated plants	*In vitro* plant	Difference
Off type (%)	1.2^z	3.3^z	2.2**
Number of tiller/plant	$5.0\pm.17^a$	$6.45\pm.15^a$	1.40**
Plant height (cm)	$73\pm.30^b$	$94.75\pm.27^c$	21.71*
Number of leaves	$8.0\pm.10^d$	$12.30\pm.12^e$	4.30*
Leaves length (cm)	$38.65\pm.18^f$	$49.30\pm.21^f$	10.65**
Leaves width (cm)	$13.60\pm.25^g$	$12.70\pm.14^g$	-0.90**
No. of finger/plant	$20.65\pm.33^h$	$53.75\pm.43^i$	33.10*
Average finger diameter (inch)	$4.05\pm.22^n$	$5.25\pm.16^n$	1.20*
Total rhizome weight/plant (g)	627.45 ± 4.01^j	1256.80 ± 4.9^k	629.35*
Weight of mother rhizome (g)	$81.45\pm.24^l$	$183.40\pm.58^m$	101.95*
Maturity*** (days)	250**	271**	21**

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

determine whether the potential benefits of micropropagated plants are being realized. There is limited report on field trial performance test in turmeric.

The purpose of this study was to evaluate the field performance of the *in vitro* derived plantlets and to observe whether the incorporation of silver nitrate interacts with propagation methods and has an effect on morphology and development of turmeric plants. In the current study, a number of morphological, rhizome yield and quality traits were studied simultaneously with the view to identify phenotypic abnormality or somaclonal variation if any, in micropropagated plants and to compare the over-all performance of tissue-cultured plants over conventional rhizome seed-grown plants.

MATERIALS AND METHODS

Explants were taken from plants grown in IBSD (Institute of Bioresources and Sustainable Development), Imphal (India) under shade house condition. *In vitro* plants were obtained through minirhizome induced plantlets acclimatized at shade house IBSD IBSD (Institute of Bioresources and Sustainable Development), following the procedure describe in the previous protocol (Dikash et al., 2012). Among the *in vitro* derived lines in the previous paper, plantlets treated with silver nitrate ($AgNO_3$) were used for the experiment. Conventional rhizome was obtained from true-to-type plants maintained in shade house. The later were selected based on highest curcumin content as per described in previous paper (Dikash et al., 2012). Plants of both factors were planted during year 2011 April, using a completely randomized block design with equal number of replication. Three plots were planted for each of the two factors, each plot consisting of 36 plants arranged in 6 x 6 per replication. Plants were spaced at 0.3 x 0.3 m, providing a density of 11111.11 plants/ha presumably. Each of the two factor plants were assessed individually for growth and yield characteristic; only the 25 plants in the middle of the plot eliminated border effect as per the methods of Dirk et al. (1996).

Data were collected on the important morphological characters such as, number of tiller per plant, plant height, 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 after one month interval, till the time of harvest. Statistical analysis was conducted for each experiment and pooled analyses over the experiments were 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. Data on phenotypic variation were analyzed using X^2 test.

RESULTS AND DISCUSSION

Phenotypic variant or off type plant are commonly observed in *in vitro* raised population of *C. longa* var. Lakadong. Variants were observed in micropropagated plants in all the three different replicated sites exhibiting the changes in leaf morphology and colour. The X^2 test for independence indicated that phenotypic variation and propagation methods were independent criteria. ($X^2 = 3.70$, p = 0.32) (that is, 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). The higher frequency of variations in rhizome derived plants and the higher dominant characteristics shown by micropropagated plant (Table 1) partly explain the lack of significant differences. Almost double the variation frequency in the micropropagated plants was not significantly different from that in the conventional rhizome derived plants. However, we did not expect both frequency distributions to be similar, as *in vitro* plant (Lakadong) showed much higher rates of variation than conventional rhizome derived plants. Similar result was observed in banana (Drew and Smith, 1990; Smith, 1988; Dirk et al., 1996). Among the variation observed in both the populations (*in vitro* and conventional plant), tillers of approximately 3.3% of *in vitro* and approximately 1.2% of conventional rhizome plants had leaves with one half of the lamina green and

the other half albino (Table 1). In some case, variegation was observed on the edge of the lamina. Similar results were also documented by Neeta et al. (2002) in turmeric cv 'elite'. However, we infer that results of phenotypic variation rate were not always consistent over trial, which however, may be greatly influenced by environmental factor. In general, however, off type frequencies are higher in *in vitro* plants than conventional rhizome derived plants, even if the former are produced by micropropagation involving direct plant regeneration from shoot cultures (Dikash et al., 2012) and without intervening callus phase (Dirk et al. ,1996; Smith, 1988; Smith and Drew, 1990; Vuylsteke et al., 1988). Plants regenerated from tissue culture exhibited various morphological and biochemical changes which those of Larkin and Scowcroff (1981) termed as somaclonal variation. However, the off type plants produced may be due to mutation. Small changes observed in the variants suggested that several genes or minor genes may have been altered (Stephens et al., 1991). If chromosomal aberrations were present or genes responsible for qualitative traits had been altered, then abnormal plants and greater variation would be expected. The results presented show that turmeric cultivation through plant tissue culture would not necessarily be detrimental mutations rather showing increase in the yield then conventional rhizome, which was in contrary with the results of Bhagyalakshmi and Singh (1988).

Only true-to-type plants were included in the analysis of variance of the horticultural performance of conventional propagated vs. micropropagated plants of turmeric var. Lakadong. There were significant difference between tissue culture and conventional derived plants in almost all the different traits studied except number of tillers, leaf width and average finger diameter (Table 1). *In vitro* turmeric was significantly taller than the conventional type, throughout the vegetative growth cycle, as compared with plant height every after 1 month of initial propagation. In fact, 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). During the initial stages upto 2 months after planting, the height of plants of both type did not show any significant differences. 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 (94.75±.27 cm) than that reached by conventional plant (73±.30 cm) of the same age (Table 1) after 6 months. Similar result was also documented by Beruto et al. (1996) in *Ranunculus asiaticus*.

Fast growing *in vitro* plants emit new leaves at a faster rate, resulting in larger leaf area during vegetative growth than that of conventional type. *In vitro* plant gives approximately 4.30 more number of leaves and produced higher number of leaves (12.30±.12) as compared to plants from conventional rhizome (8.0±.10) throughout the growing period (Table 1). The maximum differences in the leaf production were observed at 6 months after planting. This is in agreement with the finding of Neeta et al. (2001), Israeli et al. (1988) and Hwang et al. (1984), noted that micropropagated plant retained more healthy leaves than conventional plant due to fast rate of leaf emission. However, the induction of shoot development was independent on the propagation type. 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. This is contrary with the finding of Samir et al. (2007), which shows the dependent of shoot development on the propagation type in lowbush blueberry plant. Presumably, the increase in vegetative growth has contributed to the increase in shoot yield and number of leaves of tissue culture plants of turmeric. Even though comparison of *in vitro* plants with the conventional type showed significant variation ($p<0.05$) for plant height, number of leaves and leaves length but there was non for number of tiller and leaves width. Although the differences were significant ($p<0.05$), they were not large since the range for the *in vitro* lines was reasonably close to the means for the conventional plants (Table 1).

Tissue culture plants recorded significantly higher number of finger/plant (53.75±.43) than that of conventional plant (20.65±.33) (Table 1). An increased of 33.10 number of finger/plant 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 number of finger/plant ultimately increase in the yield of turmeric plant. This is in support with the finding of Neeta et al. (2001). Considering the number of fingers 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). However, the present finding is in contrary with those of Smith and Hamil (1996) showing significantly reduced rhizome yield *in vitro* ginger plant. Bhagyalakshmi and Singh (1988) also found significantly lower yield with micro ginger harvested at 8 months compared with seed derived ginger, and also attributed their difference to micropropagated plants lacking a rhizome (seed reserve) when planted.

Number of finger, weight of total rhizome and ultimately yield of the rhizome shows significance difference between the conventional and tissue culture plant but there were no significant difference between different type of propagation methods. The main yield component (plant height, number of leaves, and leaves length) also shows significant difference between conventional and *in vitro* plants (Table 1). With the significant increase in the growth of tissue culture plant, gradual increase in the yield was observed. The observed mean rhizome weight

Table 2. Comparative data of turmeric yield in tonnes/ hectare.

Parameter	Productivity (tonnes/ hectare) of turmeric in India (Spices board India)				Tissue cultured plant	Conventional rhizome propagated plant
Year	2007-2008	2008-2009	2009-2010	2010-2011		
Productivity (tonnes/hectare)	4.967	4.585	4.947	5.09	13.9644	6.9716

of *in vitro* plants was approximately double (1256.80±4.9 g) of the conventional rhizome weight (627.45±4.01 g). 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 fingers and the total weight of rhizome/plant had the greatest correlations with the yield. There was production of more leaves, greater plant height and the production of more number of fingers which ultimately increase the weight of rhizome per plant.

According to the presumption, if one plot consist of 36 plants in 6 x 6 arrangement and space at 0.3 x 0.3 m providing a density of 11111.11 plants/ha, then the approximately weight of rhizome produced by the tissue culture plant in one hectare will be 13.9644 ton. This amount of production will be approximately double the weight of rhizome produced by conventional rhizome seed (6.9716 ton/ha). The comparative production of turmeric from the last five years provided by Spices Board India with the present study (Table 2) indicated that the present investigation can be beneficial as they showed suitable agronomic performance than conventional plants and resulted in the increased production of finger numbers and subsequently marketable rhizome yields. However, the agronomic traits observed during the present study in tissue culture plants are stable; has to be ascertained in subsequent years. If provided stable, this clone can be incorporated into the crop improvement programs of turmeric var. Lakadong. Field evaluation of tissue culture and conventional plants revealed that *in vitro* plants were superior in performance over conventional plant exhibiting vigorous vegetative growth, increased and uniformity of rhizome production.

In vitro derived plants treated with $AgNO_3$ produced significantly more number of finger as compared with the conventional type and consequently provided a larger framework for rhizome production during the experiment than did those produced by conventional rhizome. This may be a direct result of residual $AgNO_3$ with the minirhizome left over from the *in vitro* treatment used to increase microrhizome production as reported earlier in the previous paper (Dikash et al. 2012). Although effects of $AgNO_3$ on number of finger/plant have not been studied previously, Dikash et al. (2012) and Chithra et al. (2005), found similar results with increased microrhizome production under *in vitro* condition. The mode of action of $AgNO_3$ in plant tissue culture is assumed to be associated with the physiological effects of ethylene; silver ions act as a competitive inhibitor of ethylene action rather than inhibiting ethylene synthesis per se. $AgNO_3$ which is an inhibitor of ethylene action was reported to act as a stimulant and inhibitor in several plant tissue culture (Al-Khayri and Al-Bahrany, 2001). However, we infer that addition of $AgNO_3$ in tissue culture plant improved rhizome production and ultimately yield of the turmeric plant.

Conclusion

We have shown that *in vitro* produced plants of turmeric var. Lakadong manifest consistently superior horticultural performance compared to conventional plants. Nevertheless, *in vitro* micropropagation remains an important technique for the establishment of large number of true-to-type newly bred or selected genotypes, and thus, it is worthwhile emphasizing that there were no detrimental effects of *in vitro* propagation on horticultural performance. Based on the improved yield and other superior characters of *in vitro* plants against conventional plants, a conclusion could be drawn that tissue culture methods can be used to induce an inheritable shift and to create superior varieties for agricultural use. In addition, this study shows the economic benefits of tissue cultured plants compared with conventional plant, in fact, although the estimated cost of *in vitro* tissue cultured plants production could be quite high in comparison with the cost of conventional rhizome, the better rhizome yields from the tissue cultured material provide a reduction of initial cost. This drastic increased in the yield of rhizome can be used to create superior lines for conventional propagation of newly selected superior genotype of *Curcuma longa* var. Lakadong.

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