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Effect of Planting Material on Growth and Seed Rhizome Yield of Ginger (*Zingiber officinale* Roscoe)

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ABSTRACT

Meristem derived ginger plants cultivar Khing Yai were micropropagated *in vitro*. Small rhizomes were produced from these plantlets under different photoperiods (8,10,12,14, and natural daylength (12.01-11.19 hrs.)). The performance of small rhizomes produced from micropropagated plantlets as compared with conventionally planting big rhizomes and direct transplanting of micropropagated plantlets was evaluated from growth, rhizome yield and rhizome branching. The conventionally propagated old rhizomes produced more fresh rhizome yield than rhizomes and plantlets from micropropagation but tillering and rhizome branching was more in rhizomes from micropropagation as compared with conventional method and direct transplanting. The result revealed that ginger plantlets produced through micropropagation should be planted for seed rhizome production under greenhouse conditions and after harvest these rhizomes should again be planted for two generations of multiplication which can then be used as mother plants for commercial ginger production.

Key words: ginger, micropropagation, conventional method, growth and rhizome formation

INTRODUCTION

Ginger (*Zingiber officinale* Roscoe) is a herbaceous perennial commercially grown as an annual which is one of the very ancient cultivated plants and has long been used as spice and medicine in Asia. Since flowering is rare and no viable seeds are produced, ginger is then vegetatively propagated exclusively through underground rhizomes with a very low multiplication rate. Conventional multiplication produces only 10-15 lateral buds in a season of 8-10 months (Bhagyalakshmi and Singh, 1988). In addition, this crop is heavily attacked by bacterial wilt (*Pseudomonas solanacearum*), rhizome rot (*Pythium spp.*), fusarium yellows (*Fusarium oxysporum* f. sp. *zingiberi*) and root knot nematode (*Meloidogyne incognita*). Dohroo (1989) reported that about 87% of the field infection with *Fusarium oxysporum* f. sp. *zingiberi* is transmitted through infected rhizomes. Hosoki and Sagawa (1977) estimated that a three fold increase in rhizome yield of ginger could be attained with sound disease control practices.

Bacteria, fungi and nematodes elimination are very commonly used in micropropagation (Sahavacharin, 1995). *In vitro* propagation of ginger has

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been reported by various workers (Hosoki and Sagawa, 1977; Bhagyalakshmi and Singh, 1988). However, the shoot tip culture method seems to be the most suitable technique in ginger leading to the production of pathogen free plants (Sakamura and Suga, 1989). As the rhizomes are used for conventional propagation of ginger, but it is completely lacking in micropropagated ginger plants at planting. This poses many questions related to survival, growth, yield and duration of cultivation in the open field condition. Therefore, micropropagated plantlets were transplanted for small seed rhizome production under different photoperiods (Pandey *et al.*, 1996) in the previous season and these rhizomes were again planted comparing with conventionally grown big rhizomes and direct transplanting of micropropagated plantlets.

MATERIALS AND METHODS

In vitro shoot multiplication

New emerging buds from rhizomes of ginger, cultivar Khing Yai or Khing Yuak were surface sterilized in 0.2% Tetracycline and 0.2% Metalaxyl for one and half hour before treated with 10% Clorox for 15 minutes followed by 5% Clorox for 10 minutes. Shoot tip explants excised from the buds were planted onto MS medium (Murashige and Skoog, 1962) supplemented with 3 mg/l BA. The uncontaminated shoot tips were then transferred to 25 ml of fresh MS medium supplemented with 5 mg/l BA. Subcultures were then repeated every 6 weeks the fifth subculture, an aseptic stock plant was established for further multiplication.

Shoot tips of *in vitro* plantlets were cultured on MS medium supplemented with 5 mg/l BA in combination with 0.5 mg/l NAA. The condition of the culture room was 25± 2°C temperature and 12 hours photoperiod. The multiplication rate was more than 5 times within 5 weeks of culture.

Planting materials

Two types of planting materials from tissue culture were used in the experiment as followed:

(i) Micropropagated plantlets: Shoots 7-8 cm tall with 4-5 good roots/shoot were selected for transplanting. These plantlets were transplanted in plastic baskets containing sand, burnt rice husk and coconut fiber in equal volume and were kept with mist spray under greenhouse condition for a month. After well establishment, these plantlets were directly transplanted in the experimental field.

(ii) Rhizomes from micropropagated plantlets: Small rhizomes were produced from micropropagated plantlets in the previous season. As described above, plantlets were produced from tissue culture and transplanted for establishment. Well established plantlets were again transplanted in plastic baskets containing equal volume of soil, sand, burnt rice husk and coconut fiber and kept outside greenhouse for acclimatization for 2 months. These plants were exposed to 8, 10, 12, 14 hours and natural (12.01-11.19 hours) photoperiods for 4 months under room temperature (Pandey *et al.* 1996). Small rhizomes were harvested and planted separately in this experiment except 14 hours photoperiod. Rhizomes produced from 14 hours photoperiod were shriveled and wrinkled which could not be used as seed material. Seed rhizomes produced under 10 hours and natural photoperiods were bigger in size and better than produced under other photoperiods (Pandey *et al.*, 1996).

Besides these small rhizomes and plantlets, rhizomes produced from conventional method were purchased from the local market and planted in the experiment. The experiment was included the following 6 treatments:

1. Seed rhizomes produced from micropropagated plantlets under 8 hours photoperiod

2. Seed rhizomes produced from micropropagated plantlets under 10 hours

photoperiod

3. Seed rhizomes produced from micropropagated plantlets under natural (12.01-11.19 h.) photoperiod

4. Seed rhizomes produced from micropropagated plantlets under 12 hours photoperiod

5. Seed rhizomes produced conventionally and purchased from the market

6. Plantlets produced from micropropagation and directly transplanted

Planting procedure and conditions

Experimental field was selected in Tropical Vegetable Research Center (TVRC) research field at Kasetsart University, Kamphaengsaen Campus, Nakhon Pathom. The field was planted with grain legumes for the past 5 years before this experiment. After field preparation, fertilizer was applied at 300:100:200 kg NPK/ha as split dose:

a) Basal application: 100:100:100 kg NPK/ha through Complete (15:15:15)

b) First top dressing (30 days after planting): 100:00:100 kg NPK/ha through Urea and Murate of Potash

c) Second top dressing (60 days after planting): 100:00:00 kg NPK/ha through Urea.

Three days after fertilizer application, soil was treated with Basamid (soil fumigant) at 50 g/m^2. After 12 days of Basamid application, soil was aerated for 3 days and ginger rhizomes were planted. The experiment was arranged in a randomized complete block design (RCBD) with 4 replications. Plot size was 2x1 sq.m., each plot consisting 4 rows of 25 cm and 7 hills/row maintaining 25x25 cm spacing. Rhizome seed pieces having only one sprouted bud from all the treatments were planted on June 18, 1996. The weight of individual seed piece was 1.55 g from 10 hours and natural, 1.10 g from 8 and 12 hours photoperiods and 25 g from conventionally produced rhizomes. Seed rhizomes were covered with soil and mulched with rice straw. Immediately after direct transplanting of tissue culture plantlets, light irrigation was given. However, irrigation was given to the entire field whenever it was necessary. Besides rainfall, irrigation was given 9 times, manual weeding 9 times and insecticides was applied 7 times during the whole growth period.

Harvesting and yield

The crop was harvested on January 15, 1997, after 7 months of planting. The clumps were gently dug, soil and other adhered materials were removed from the rhizomes along with roots and aerial stems. Randomly 10 clumps were selected and marked from each plot before harvesting. From these clumps number of tillers, plant height, fresh rhizome weight and number of seed pieces were calculated per clump with a standard error and the significance was found using the F-Test.

RESULTS AND DISCUSSION

Sprouting and establishment

Though the seed rhizomes were already sprouted before planting, sprouting in the field was observed after 12 days and completed after 20 days of planting. No effect of seed materials was observed on sprouting in the field. The sprouting of rhizomes in all the treatments was 100 percent. However, the survival of plantlets produced from tissue culture and directly transplanted in the field was 42.9 percent. The plants were directly transplanted after well establishment under greenhouse condition. In our experience, micropropagated ginger plants do not require stringent stepwise acclimatization if planted at the onset of monsoon but, after transplanting in the field there was a long drought with scorch followed by heavy rainfall and flooding. The plants got sudden shock of heat and flood which caused high mortality.

Babu *et al.* (1992) reported that *in vitro*

plantlets were transplanted in the soil (garden soil and sand in 1:1 ratio) and survival or establishment of the plants was more than 80% when high humidity was maintained for the first two weeks after transfer to soil by placing them in a humid chamber. Bhagyalakshmi and Singh (1988) reported that the survival of tissue culture plants under greenhouse condition was 90% when transplanted to pots containing an equal mixture of red soil, vegetable manure and sand.

Malamug *et al.* (1991) reported that ginger plantlets were potted into a vermiculite perlite (3:1) medium for acclimatization for 3-4 weeks and transplanted in the soil. Plantlets with shoot lengths of less than 5 cm had a lower survival rate (70% or less) than plantlets with at least 10 cm long shoots (greater than 90%).

In our present study, though the survival rate was low (42.9%) when transplanted directly in the field but Pandey *et al.* (1996) reported that survival rate was more than 90% when transplanted in the medium containing an equal volume of sand, burnt rice husk and coconut fiber and kept under greenhouse condition with mist spray.

Growth and yield

One month after planting, tillering was observed and it continued until November. The difference among the treatments on average number of tillers/clump was highly significant (Table 1). The highest average number of tillers was produced by small rhizomes which were produced from micropropagated plantlets under natural and 10 hours photoperiods at 18.11 and 16.86 tillers/clump respectively. The lowest number of tillers / clump (3.89) was produced by micropropagated plantlets. The difference among the treatments on plant height was also highly significant. The highest length of shoot was produced by market rhizomes (conventional method and larger seed pieces ~ 25 g) with an average of 57.27 cm and lowest by micropropagated plantlets with an average of 25.55 cm.

The highest rhizome yield was obtained from market rhizomes with an average of 212.31 g/clump. The average weight of rhizomes/clump was 152.4 g, 149.09 g, 101.81 g and 67.98 g from seed rhizomes produced from tissue culture under natural, 10, 8 and 12 hours photoperiods respectively. The average rhizome weight was the lowest (13.25 g/clump) from tissue culture plantlets. However, the ratio of seed rhizome to fresh rhizome yield was the highest from tissue culture seeds produced under natural photoperiod with a ratio of 1:98 *i.e.*, one gram seed rhizome produced 98 grams of fresh rhizome at harvest. The ratio of old rhizomes was 1:8 from seed rhizome to fresh rhizome yield. The ratio was higher in all the treatments of small seed rhizomes which were obtained from micropropagated plantlets produced under different photoperiods.

Though the fresh rhizome yield was produced more from market rhizomes (conventional method), the ratio of seed rhizome to fresh rhizome yield was more in seed rhizomes produced from micropropagated plants. Number of seed pieces or ratio is more important than the total weight of rhizomes for seed purpose. More seed pieces will cover more area, reduce the cost of seed and transportation. The result indicated that the rhizome yield in the next season planting would be more from these rhizomes produced from micropropagation as compared with conventional method.

Bhagyalakshmi and Singh (1988) reported that the yields of plants grown by tissue culture were compared with those of conventionally grown plants but no difference was obtained. Bhagyalakshmi *et al.* (1994) reported that the survival rate of ginger plantlets under greenhouse condition in pots was more than 90%, whereas with direct transplanting on rainy days the value was

Table 1 Average rhizome yield and other yield components of ginger planted with different planting materials under field condition at Kamphaengsean, Nakhon Pathom.

Seed rhizome[1]	Original planting material (g/piece)	Average No. of tillers/clump	Height (cm)	Fresh yield (g/clump)	Ratio of seed to rhizome yield
8 hours	1.10	14.66 b[2]	44.54 cd	101.81 c	1:93
10 hours	1.55	16.86 a	46.73 bc	149.09 b	1:96
Natural	1.55	18.11 a	47.08 b	152.40 b	1:98
12 hours	1.10	13.07 bc	43.84 d	67.98 d	1:62
Old Rhizomes	25.00	11.19 c	57.27 a	212.31 a	1:08
Tissue culture	-	3.39 d	25.55 e	13.25 e	-
CV %	-	10.9	3.3	9.2	-
F Test[3]	-	**	**	**	-

[1] 8 hours = seed rhizomes produced from tissue culture under 8 hours photoperiod
10 hours = seed rhizomes produced from tissue culture under 10 hours photoperiod
Natural = seed rhizomes produced from tissue culture under natural (12.01-11.19 h.) photoperiod
12 hours = seed rhizomes produced from tissue culture under 12 hours photoperiod
Market = rhizomes produced conventionally and purchased from market
Tissue culture = micropropagated plantlets transplanted directly in the field.

[2] Means followed by a common letter are not significantly different at the 5% level by DMRT.

[3] ** = Significant at 1% level of probability

about 73%. Yield of ginger rhizome and fiber content in micropropagated plants was significantly lower than conventionally propagated plants.

Though there is hardly any report on seed rhizome production of ginger *in vitro* there are many reports on underground organ formation in other crops. Mason and Osborn (1988) reported that the scheme for certified seed potato production involves the *in vitro* multiplication of plantlets and production of micro-tubers in laboratory, three generations of multiplication for foundation seed and two generations of multiplication for certified seed. The multiplication rate of certified seed was one ton per mini-tuber over 5 years.

The result of this experiment revealed that the rhizome yield of ginger is not directly influenced with the number of tillers. Average number of tillers/clump was higher in tissue culture seeds as compared with market rhizomes and micropropagated plantlets. Disease-free seed rhizome production through tissue culture should not be compared with the conventional method of propagation for fresh rhizome yield after one generation. In general, optimum size rhizome could be obtained after 2-3 generations through tissue culture. The principal objective is to produce disease-free seed rhizomes and multiplication should be continued until it produces optimum size rhizomes and good quality and quantity of seed material for commercial production.

The difference in the fresh weight yield of rhizomes may be attributed to the fact that in the conventional method the rhizome is the starting material which, after planting, not only produces shoots but also increases in size, producing shoots and the process continues adding to the rhizome yield. Total fresh rhizome yield was less from small rhizomes produced from tissue culture but tillering and branching of rhizome was more giving more yield for seed purpose. This might be due to

the fact that the size of seed rhizomes was too small to produce big rhizomes but tillering, branching and ratio of seed rhizome to fresh rhizome yield was more because of healthy and vigorous of the seed rhizomes produced from micropropagation. From the result it could be concluded that the fresh rhizome yield from these seed rhizomes would be more and better than conventional method in the next planting. The performance of directly transplanted plantlets was very poor in tillering and rhizome formation. This might be due to the absence of rhizome (reserve food) at planting which could not produce vigorous shoots for rhizome formation immediately after planting as in conventional method. Further, micropropagated plants undergo shock while acclimatizing from *in vitro* to greenhouse and then to field conditions.

Although the difference in the yield of rhizomes in tissue culture seeds is marginal compared with conventionally propagated seeds, there is a significant difference in growth, rhizome branching and ratio of seed rhizome to fresh rhizome yield. The difference would be considerable when these rhizomes from micropropagated plants are planted again in the next season. Therefore, the study implies that micropropagation has advantage over conventional method for seed rhizome production.

CONCLUSION

Ginger plantlets produced from tissue culture could be transplanted year round under greenhouse condition and produce rhizomes. These rhizomes as a source of disease-free seed material could be planted in the pathogen free soil or field conditions for seed rhizome production. Rhizomes produced from second planting could be used as disease-free mother seed material for commercial planting.

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