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In Vitro Propagation of *Zingiber officinale rosc*

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Abstract

Ginger *(Zingiber officinale rosc.)* has become more popular in recent years because of its low toxicity and its broad spectrum of biological and pharmacological applications. These applications include antitumor, antioxidant, anti-inflammatory, antiapototic, cytotoxic, antidiabetic, anti-proliferative and anti-platelet activities. Traditionally, ginger can be propagated by using the fingers of the ginger rhizomes however early in the crop, disease can cause severe losses. Ginger propagation is usually performed with rhizome which depends on the availability of good quality seed rhizome. Alternatively, tissue culture technology can be used for the propagation of ginger. Tissue culture ginger has been considered vital for germplasm conservation and the resuscitation of the ginger industry in Jamaica in response to the ginger rhizome rot disease. Research from trials (unpublished) performed at the Scientific Research Council (SRC), Jamaica, indicated that the yellow variety of the Jamaican ginger has a lower multiplication rate than the Jamaican blue variety. Micro propagation of the Jamaica yellow explants was done on three growth media types. Explants were cut to approximately 1 cm in height and transferred to media (S, F, M). The shoots developed *in vitro* were separated and sub-cultured on same medium for multiplication and rooting over a 6-week period. Growth parameters were evaluated during the course of the study. These included number of shoots, height of shoot and number of leaves. This study aims to investigate the performance of Jamaica's yellow ginger variety on different nutrient media. Media *M* appears to be the most effective for the growth and multiplication of Jamaican yellow ginger variety when compared to Media (S, F). However, there remains a need for further research in improving media M to obtain optimal growth of ginger explants *in vitro*.

Keywords: *Zingiber officinale*; Plant tissue culture

Abbreviations: BA: 6-Benzylaminopurine, Phyto Technology Laboratories; NAA-1: Naphthaleneacetic acid, Phyto Technology Laboratories; PPM: Plant Preservative Mixture, Plant Cell Technology

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Introduction

Ginger (*Zingiber officinale rosc*.) is a member of the tropical and subtropical, *Zingiberaceae.* It has been consumed for thousands of years in food for its nutritional and medicinal benefits [1-3] states that there are more than 60 active constituents present in ginger, which have been broadly divided into volatile and nonvolatile compounds. Ginger is becoming more and more popular in recent years because of its low toxicity and its broad spectrum of biological and pharmacological applications. These applications include antitumor, antioxidant, anti-inflammatory, antiapototic, cytotoxic, antidiabetic, anti-proliferative and anti-platelet activities [4-6]. Ginger is a warm-season crop adapted for growth in tropical and subtropical regions. The most favourable conditions for growth occur in moist conditions with temperatures of 25°C-28°C. Growth efficiency declines with temperatures above 30°C and below 24°C. Ginger grows well in full sun. Vegetative growth is promoted with long day lengths, and rhizome enlargement is promoted under shorter day lengths. Ideal pH is (5.5-6.5) and it requires a deep (25-40) cm, rock-free, sandy loam soil, high in organic matter with adequate drainage that allows for proper hilling of the crop [7]. It is reported that ginger was first introduced to Jamaica around 1525 and by 1547, ginger exports had been reported to be over 1.2 million kg. Between the 1930s and 1960s, Jamaica was considered to be one of the largest producers of ginger in the world, along with India and Sierra Leone [8]. However, ginger production has decreased immensely from close to 2 million kilograms of ginger in 1953 to around 0.4 million kilograms in 1995. In the early 1990s, the production of Jamaican ginger reached an all-time low, this was due to the impact of the rhizome rot and bacterial wilt diseases. Currently, production of this crop is still seriously hampered by significant losses caused by ginger rhizome rot. Ginger rhizome rot is incited by a complex of pathogens (*Phythium aphanidermatum*, *Fusarium spp*., *Rhizoctonia spp.* and *Ralstonia solanacearum*), adversely affects ginger production worldwide [9-10]. It severely affects seed rhizomes and possible complete crop failure may occur under the right conditions [11]. The affected rhizomes

become soft and pulpy, on pressing it collapses very easily, this is due to rotting of internal tissues. Leaves of affected plants become yellow and have water-soaked areas that rot at the basal portion and shoots finally wilt and collapse [10, 12]. The disease has been found in all the major ginger production areas in Jamaica, with yield losses ranging from approximately (50-90) %. This led to high import of ginger to supply local demands [13]. There are two main varieties of ginger grown in Jamaica (**Figure 1**), the blue or flint ginger and the yellow or white ginger. When the rhizomes of the blue variety are cut they establish a bluish tinge, while yellow flesh is observed on the yellow variety. It is reported that the blue variety is harder, more fibrous, poorer yielding and requires a longer time for drying compared to the yellow variety [14].

Traditionally, ginger can be propagated by using the fingers of the ginger rhizomes [15] reported that ginger is affected by leaf spots thereafter the leaves may reduce in size. Whitish spots with yellow edges appear on leaves, which eventually spreads and prevent photosynthesis, this leads to plant mortality. Early in the crop, the disease can cause severe losses. Ginger propagation is usually performed with rhizome which depends on the availability of good quality seed rhizome Alternatively, tissue culture technology can be used for the propagation of ginger. Tissue culture ginger has been considered vital for germ plasm conservation and the resuscitation of the ginger industry in Jamaica in response to the ginger rhizome rot disease [16]. Tissue culture technology is the process by which small pieces of plant tissue called explants are treated and placed on a culture medium, where they grow into complete plants identical to the mother plant [17]. Tissue culture technology has been more widely used in recent times due to the high occurrence of rhizome rot disease. Research from trials (unpublished) performed at the Scientific Research Council (SRC), Jamaica, indicated that the yellow variety of the Jamaican ginger have a lower multiplication rate than the Jamaican blue variety when using the MS media supplemented with 2.8 mgL^{-1} BA (6-Benzylaminopurine) and 4.0 mgL^{-1} NAA. In addition, stunted growth of explants was observed after six weeks on culture media. Therefore, the objective of this study was to identify a medium for optimal growth of the Jamaican yellow ginger *in vitro*.

Materials and Methodology

Tissue culture plant materials

6 weeks old Jamaican yellow ginger plants were chosen randomly from the Biotechnology Department *in vitro* Gene bank at Scientific Research Council of Jamaica Gene bank. These plants are from surface sterilized buds that were previously sterilized and screened for economically important pathogens by an accredited testing laboratory.

Growth media

Micro propagation of the Jamaican yellow plantlets was done on three growth media types. Explants were cut to approximately 1 cm in height and transferred to media (S, F, M) [1]. The shoots developed *in vitro* were separated and subcultured on same medium for multiplication and rooting over a 6-week period. Growth parameters were evaluated during the course of the study. These included number of shoots, height of shoot and number of leaves.

Media preparation

Media S*:* The multiplication medium was made up of medium, BA (2.8) mg/l, NAA (4.0) mg/l, Sucrose (30) g/l, PPM (1) ml/l and Phytagel TM (2.5) g/l [18].

Media M: The multiplication medium was made up of medium, BA (4.5) mg/l, Sucrose (30) g/l, PPM (1) ml/l and Phytagel TM (2.5) g/l [18].

Media F: The multiplication medium was made up of medium, BA (3.0) mg/l, NAA (0.5) mg/l, Sucrose (30) g/l, PPM (1) ml/l and Phytagel TM (2.5) g/l [18].

Media S, F and M consisted of 90 jars each with 6 batches labelled A-F with 15 jars in each group. Glass jars were used with one plant per jar.

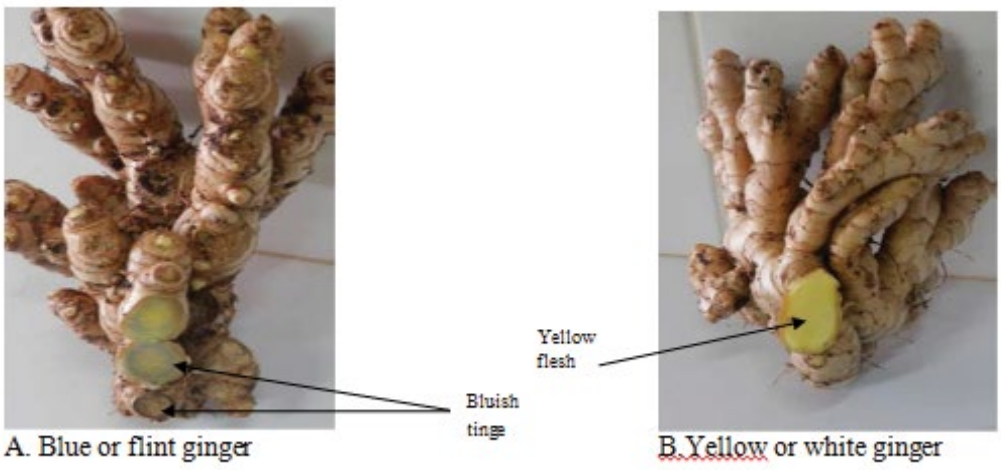

Figure 1 Jamaican blue and yellow varieties of ginger [8].

Table 1: Mean number of new shoot for *in vitro* Jamaican yellow ginger variety grown on media *S, M* and *F*.

Media	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
F	1 ± 0.041^{a}	1 ± 0.063^{b}	2 ± 0.073^{b}	1 ± 0.051^{a}	1 ± 0.057^{ab}	2 ± 0.064^{a}
S	1 ± 0.035^{a}	1 ± 0.038^{a}	1 ± 0.057^{a}	1 ± 0.057^{a}	1 ± 0.066^{a}	2 ± 0.084^{ab}
M	1 ± 0.036^{a}	1 ± 0.043^{a}	1 ± 0.063^{ab}	2 ± 0.069^{b}	2 ± 0.072^{b}	2 ± 0.092^{b}
Superscripts with different letters denote significant differences ($p \leq 0.05$). Values are mean ± SE n=90						

Table 2: Mean shoot height for *in vitro* Jamaican yellow ginger variety grown on media *S, M* and *F*.

Media	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
F	0.77 ± 0.029^{b}	0.90 ± 0.035^{a}	1.18 ± 0.051^{a}	0.97 ± 0.026^{b}	0.96 ± 0.025^{a}	1.11 ± 0.027^{a}
S	0.64 ± 0.037^{a}	0.80 ± 0.039^{a}	1.07 ± 0.056^{a}	0.88 ± 0.031^{a}	0.93 ± 0.037^{a}	1.08 ± 0.035^{a}
M	0.68 ± 0.028^{a}	0.81 ± 0.031^{a}	1.07 ± 0.046^{a}	0.92 ± 0.0238^{ab}	0.99 ± 0.033^{a}	1.14 ± 0.031^{a}
Superscripts with different letters denote significant differences ($p \leq 0.05$). Values are mean ± SE n=90						

Table 3: Mean number of leaves *in vitro* Jamaican yellow ginger variety grown on media *S, M* and *F*.

Media	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
F	1 ± 0.058^{a}	1 ± 0.064^{a}	1 ± 0.076^{a}	2 ± 0.090^{a}	2 ± 0.117^{a}	2 ± 0.150^{a}
S	1 ± 0.069^{a}	1 ± 0.072^{a}	1 ± 0.105^{a}	2 ± 0.107^{a}	2 ± 0.120^{a}	2 ± 0.153^{a}
M	1 ± 0.077^{b}	1 ± 0.086^{b}	2 ± 0.112^{b}	2 ± 0.107^{b}	2 ± 0.114^{b}	3 ± 0.151^{b}
Superscripts with different letters denote significant differences ($p \leq 0.05$). Values are mean ± SE n=90						

Growth room conditions

The culture vessels, covered with plastic caps and sealed with para film were maintained at (22 ± 2)°C-(27 ± 2)°C under daily photoperiods of 12 hr of fluorescent and 12 hr of darkness.

The culture sand their growth or contamination were observed throughout the processes of propagation and multiplication during the incubation period.

Statistical analysis

Statistical analysis was performed using statistical package SPSS version 19.0 (SPSS, Cary, NC, USA). Differences between treatments in the experiments were analyzed using ANOVA and Duncan's multiple range test, while values of $p \leq 0.05$ were considered significant. Data was reported as mean ± Standard Error (SE).

Results and Discussion

One of the most vital steps in tissue culture is selecting suitable growth medium for the *in vitro* cultivation. A growth medium or culture medium is a liquid or gel designed to support the growth of plantlets. Tissue culture media is generally comprised of inorganic compounds and organic compounds. A typical culture medium is composed of a complement of amino acids, vitamins, inorganic salts, a carbon source such as sucrose, plant growth regulators and growth hormones [19]. Researcher Kavyashree et al. reported that shoot buds can proliferate to as many as 19 shoots per bud on Linsmaier and Skoog's Basal Medium (LSBM) fortified with 17.76 µM of 6-Benzylaminopurine (BAP) [20]. Additionally Sharma et al. stated that 7 shoots per shoot bud were observed on MS basal medium with BA, 2 mg/l [21]. None of the growth media used in this experiment induced productive growth among buds when compared to that observed by Kavyashree et al. and Sharma et al. [20-21]. This may be due to genetic diversity among varieties used in the different experiments and the concentration of cytokine in used in the growth media which reveals that the basal media and growth regulators are important factors that affects proliferation of shoot buds [22]. The numbers of shoot buds produced by the three media were nearly similar. After 6 weeks, plantlets on media *S*, *M* and *F* produced approximately 2 new buds (**Table 1**) which is low compared to those documented by Kavyashree et al. [20]. This may be due to phytoplasma present in the plant tissue [23] which can inhibit the growth of plantlets. **Table 2** shows that the mean height of shoots produced by plants on all three media was 1 cm, this may be an indication of stunted growth. According to Malamug et al. the shoot forming capacity of the regenerated shoots was found to decrease on the third subculturing and the average height recorded thereafter is 7.4 cm [24]. The height of the shoot becomes important in the survival rate of plantlets after hardening, with greater survival rate (90%) reported with shoots at least 10 cm long, therefore 1 cm shoot height observed in this study may not be suitable for hardening. After 6 weeks, the mean number of leaves produced by media *F* and Swas2, which is less than the number of leaves produced by plantlets on the media *M* (**Table 3**). This may be due to the BAP concentrations in the media which can be supported by Kavyashree et al. and Malamug's et al. findings which indicated that an increase in the concentration of the cytokine in BAP may induce vegetative growth of *in vitro* plantlets [20, 24]. **Table 1** indicated that the mean number of new shoots for all the growth media was less than 2 buds, with M having the highest number of new shoots. There were no significant differences in shoot height among the growth media studied [25].

Conclusion

Media *M* appears to be the most effective for the growth and multiplication of Jamaican yellow ginger variety when compared to Media *S* and *F*. However, there remains a need for further research in improving media *M* to obtain optimal growth of ginger explants *in vitro*.

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