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ORIGINAL RESEARCH

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, antiapoptotic, 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 Research from trials (unpublished) performed at the Scientific Research Council (SRC), Jamaica, indicated that the yellow variety of the Jamaican ginger have a lower multiplication rate than the Jamaican blue variety. Micropropagation of the Jamaica yellow explants was done on three growth media types. Explants were cut to approximately 1cm in height and transferred to media (S, F, M). 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. 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 and 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

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 (Abbas et al., 2011; Abdulrazaq et al., 2012). Ahmad et al., 2015 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, antiapoptotic, cytotoxic, antidiabetic, anti-proliferative and anti-platelet activities (Oyagbeni et al.,

2010; Bellik 2014; Pawar et al., 2015). Ginger is a warm-season crop adapted for growth in tropical and subtropical regions. The most favourable conditions for growth occurs in moist conditions with temperatures of 25-28 °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 (Shahrajabian et al., 2019). 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 (Ministry of Industry, Commerce, Agriculture and Fisheries, 2018). 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 (Dake & Edison, 1989; Shanmugam et al., 2013). It severely affects seed rhizomes and possible complete crop failure may occur under the right conditions (Stirling et al., 2009). 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 (Trujillo, 1964; Shanmugam et al., 2013). The disease has been found in all the major ginger production areas in Jamaica, with yield losses ranging from approximately 50-90 percent. This led to high import of ginger to supply local demands (Lancashire, 2014). 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 (Ministry of Agriculture and Fisheries, 2014).



A. Blue or flint ginger



B. Yellow or white ginger

Figure 1. Jamaican blue and yellow varieties of ginger (Ministry of Industry, Commerce, Agriculture and Fisheries, 2018).

Traditionally, ginger can be propagated by using the fingers of the ginger rhizomes. Guar et al. (2016); 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 germplasm conservation and the resuscitation of the ginger industry in Jamaica in response to the ginger rhizome rot disease (Chung, 1998). 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 (Suman, 2017). 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 METHODS

Tissue culture plant materials:

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

Growth Media:

Micropropagation of the Jamaican yellow plantlets was done on three growth media types. Explants were cut to approximately 1cm in height and transferred to media – S, F, M (Abbas et al., 2011). 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.

Abbreviations, Manufacturer

BA- 6-Benzylaminopurine, Phyto Technology Laboratories

NAA- 1- Naphthaleneacetic acid, Phyto Technology Laboratories

PPM- Plant Preservative Mixture, Plant Cell Technology

Media S

The multiplication medium was made up of Murashige and Skoog (1962) medium, BA (2.8 mg/l), NAA (4.0 mg/l), Sucrose (30g/l), PPM (1 ml/l) and Phytigel TM (2.5 g/l).

Media M

The multiplication medium was made up of Murashige and Skoog (1962) medium, BA (4.5 mg/l), Sucrose (30g/l), PPM (1 ml/l) and Phytigel TM (2.5 g/l).

Media F

The multiplication medium was made up of Murashige and Skoog (1962) medium, BA (3.0 mg/l), NAA (0.5mg/l), Sucrose (30g/l), PPM (1 ml/l) and Phytigel TM (2.5 g/l).

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.

Growth room conditions:

The culture vessels, covered with plastic caps and sealed with parafilm were maintained at $22-27 \pm 2^\circ \text{C}$ under daily photoperiods of 12 h of fluorescent and 12 hr of darkness.

The cultures and 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 \pm 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 (Murashige, 1974).

Researcher Kavyashree (2009) reported that shoot buds can proliferate to as many as 19 shoots per bud on Linsmaier and Skoog's basal medium (LSBM) fortified with $17.76 \mu\text{M}$ of 6-benzylaminopurine (BAP). Additionally, Sharma and Singh (1997) stated that 7 shoots per shoot bud were observed on MS basal medium with BA, 2 mg/l. None of the growth media used in this experiment induced productive growth among buds when compared to that observed by Kavyashree (2009) and Sharma and Singh (1997). This may be due to genetic diversity among varieties used in the different experiments and the concentration of cytokinin used in the growth media which reveals that the basal media and growth regulators are

important factors that affects proliferation of shoot buds (Ishmail et al., 2016).

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 ± 0.04 1a	1 ± 0.06 3b	2 ± 0.073 b	1 ± 0.05 1a	1 ± 0.057 ab	2 ± 0.064 a
S	1 ± 0.03 5a	1 ± 0.03 8a	1 ± 0.057 a	1 ± 0.05 7a	1 ± 0.066 a	2 ± 0.084 ab
M	1 ± 0.03 6a	1 ± 0.04 3a	1 ± 0.063 ab	2 ± 0.06 9b	2 ± 0.072 b	2 ± 0.092 b

Superscripts with different letters denote significant differences (p ≤ 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 ± 0.029b	0.90 ± 0.035a	1.18 ± 0.051a	0.97 ± 0.026b	0.96 ± 0.025a	1.11 ± 0.027a
S	0.64 ± 0.037a	0.80 ± 0.039a	1.07 ± 0.056a	0.88 ± 0.034a	0.98 ± 0.03a	1.08 ± 0.035a
M	0.68 ± 0.028a	0.81 ± 0.031a	1.07 ± 0.046a	0.92 ± 0.023ab	0.99 ± 0.033a	1.14 ± 0.031a

Superscripts with different letters denote significant differences (p ≤ 0.05). Values are mean ± SE n=90

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 (2009). This may be due to phytoplasma present in the plant tissue (Caglayan et al., 2019) which can inhibit the growth of plantlets.

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 ± 0.05 8a	1 ± 0.06 4a	1 ± 0.07 6a	2 ± 0.09 0a	2 ± 0.11 7a	2 ± 0.15 0a
S	1 ± 0.06 9a	1 ± 0.07 2a	1 ± 0.10 5a	2 ± 0.10 7a	2 ± 0.12 0a	2 ± 0.15 3a
M	1 ± 0.07 7b	1 ± 0.08 6b	2 ± 0.11 2b	2 ± 0.10 7b	2 ± 0.11 4b	3 ± 0.15 1b

Superscripts with different letters denote significant differences (p ≤ 0.05). Values are mean ± SE n=90

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 (1991) 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. 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 S was 2, 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 (2009) and Malamug's (1991) findings which indicated that an increase in the concentration of the cytokinin BAP may induce vegetative growth of in vitro plantlets. 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.

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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