

Novel Explants and Decontamination Protocol for In vitro Micropropagation of Red Ginger Lily (*Alpinia purpurata* Vieill., K. Schum) in Jamaica

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ABSTRACT

The range of different explants found in the red ginger lily (*Alpinia purpurata*) inflorescence included miniature plantlets, corm-like propagules with leaf scars or nodal rings at the base of the mini plants, pseudo-stem regions with nodal rings, sections of the bract stalk, some with emerged axillary buds, leaf whorl tissue and leaf explants. In vitro explant tissue cultures of red ginger lily were established from four explant types on MS solid media with BAP, Kinetin and carbendazim (Methyl 2-benzimidazolecarbamate) incorporated in the media at initiation. There was no significant difference in establishment rates between different explants types nor the biocides options. Establishment ranged from 50 to 100% in two separate replications. In rooted plants after two months, despite numerically more roots, leaves and greater height in plants on carbendazim media, ANOVA showed that there was no statistically significant difference in establishment between explants with carbendazim present or absent from the media.

Keywords

Ginger lily, in vitro culture, Inflorescence, Miniature plantlets, Alternative explants propagules, Biocides.

Introduction

Red ginger lily (*Alpinia purpurata*) is a relatively common ornamental in Jamaica. It is also known as jungle king, ostrich plume or pink cone ginger, and bokkepoot and billy-goat's foot in Suriname (per. comm. G. Rowe). Other synonyms *Languas purpurata* (Vieill.) Kaneh, *Alpinia grandis* K. Schum, *Guillainia novo-ebudical* F. Muell and *Guillania purpurata* Vieill also refer to *Alpinia purpurata* [1]. An herbaceous plant, it produces large colourful flowers, varying from white to intense red and aromatic rhizomes which grow in thick clusters [2,3]. The plants grow upright up to two meters tall with leafy cane-like stems [2].

The local significance of these exotic flowers to Jamaica is their potential in the cut flowers industry as the plants flower year-round

and produce durable blooms [3,4]. Several Caribbean, Central and South American countries cultivate *A. purpurata* as an ornamental crop. Dated figures of production in Hawaii showed red ginger annual production revenues in excess of the equivalent of Ja \$ 150 million with export revenues exceeding Ja \$100 million [5]. *Alpinia* plants are currently imported to Jamaica both as rooted and unrooted cuttings and seedlings (<https://www.moa.gov.jm/content/plants-import-permits-requirements>).

Red ginger lily also has medicinal value. Not dissimilar to the common ginger *Zingiber officinale* Rosc used in cooking and medicine, red ginger extracts exhibit antibacterial, antiviral, antifungal and anti-parasitic activities, and the plant is used to treat headache and cough [2,6]. Floral oils have potent larvicidal activity against the mosquito which is the primary vector for dengue fever, zika and chikungunya [7].

Typical of the Zingiberaceae family, gingers whether *Hedychium*

spicatum Smith [8], *Z. officinale* Rose [9], *Alpinia* sp., *Cucurma* sp., *Canna* sp. [10], they are all conventionally propagated from sprouted subterranean rhizomes. Cutting the shoots or decapitation removes apical dominance and promotes axillary shoots. They are also propagated by plant tissue culture [9]. Wild ginger (*Siphonochilus aethiopicus* (Schweif.) B.L. Burt.) is propagated from corm, leaf and root explants [11]. The Biotechnology Department at the Jamaica Scientific Research Council has been propagating local ginger cultivars *Jamaica Blue*, *Jamaica Yellow*, *China Blue* and *Hawaiian*, turmeric and other gingers exclusively from sprouted rhizomes. The similar approach described herein has been previously documented [12].

An archived document describing the inflorescence of *Alpinia purpurata* shed light on its unique potential to provide a wider range of explants for propagation. Miniature plants are produced on the old inflorescence after flowering with numerous plantlets developing among the bracts. These can be removed at 4 - 6" and set as individual plants [13]. The purpose of this communication is a revisit of a natural feature of red ginger described over 60 years ago by Sheehan [13] to discuss novel explants in a family where normally sprouted rhizomes are used for *in vitro* initiation. Whereas a complex of heavy bacteria and fungal contamination is observed from subterranean rhizomes the purpose was to use aerial explants with the use of a biocide included in the *in vitro* media to reduce contamination and enhance establishment.

Methodology

In the Biotechnology labs at the Jamaica Scientific Research Council (SRC), Kingston (Google map coordinates 18.0189,-76.7497), red ginger inflorescence was examined to determine how many potential explants were available for *in vitro* initiation. The red bracts were removed from the inflorescence stalk exposing axillary or preformed buds. Miniature plants were harvested from the inflorescence. Explant surface sterilization followed a protocol modified from Ahmad et al. [14]. Under a laminar flow cabinet, excised and surface sanitized explants were placed on MS media with and without carbendazim (Methyl 2-benzimidazolecarbamate). The initiation media comprised Murashige and Skoog (MS) basal medium with vitamins (M519 Phytotechnology Laboratories) supplemented with BAP (3mg L⁻¹), Kinetin (2 mg L⁻¹), sucrose (30 g L⁻¹). Media supplemented with carbendazim (0.25 g L⁻¹; Sigma- Aldrich 378674) which was dissolved in 1 M KOH then added to the media with pH 5.7 before autoclaving.

Data was collected to compare the contamination (establishment) percentage between four types of explants on both media with carbendazim and control media without carbendazim. Two-Way ANOVA with single observation and arcsine transformed data was used to test the null hypotheses of no difference between explant types and no difference between media in establishment percentage. After two month *in vitro*, data was collected on the number of leaves, roots and height from four plants randomly selected from each treatment group. One-way ANOVA with log

transformed data was computed to determine differences in mean number of leaves, roots and explant height between carbendazim treatment and controls.

Results and Observations

Miniature plantlets 3 - 4" with unopened leaves (analogues of sword suckers in *Musa*) were harvested from the inflorescence (Figure 1a). Leaves emerged and expanded with plantlets 6-8" still intact on the inflorescence (Figure 1b). Four explant types were used for *in vitro* initiation. Pseudo stem or stolon like sections with visible nodal rings were found at base of plantlets (Figure 2a). Numerous preformed shoots were found on the central axis bearing the red bracts, which were only visible once the red bracts were stripped away. Each ring had potential for *in vitro* initiation. Explants were taken from the top or apical end as well as the bottom or distal end (Figure 2b). The fourth explant used was the basal or distal region of the miniature plantlet (Figure 2c). The illustrations of the miniature red ginger plantlets showed that at initiation once the shoots were trimmed and the basal corm reduced to 2- 5mm or one nodal ring below the apical meristem, that basal tissue was also available for *in vitro* initiation with visible nodal rings or leaf scales (Figure 2d). The leaf whorls were also possible explants similar to an old method of tissue culture for sugar cane from transverse sections of the immature stems [15].



Figure 1a: Inflorescence with plantlets. **Figure 1b:** Plantlets with expanded leaves weeks later.

Contaminated cultures were removed from the growth room. The common contaminants were *Fusarium spp*, *Penicillin* sp. fungi and bacteria observed oozing from the explants. Cultures from four explant types were established *in vitro*. The percentage of explants successfully initiated for two replications of initiation are summarized (Table1). The ANOVA for both replications showed that the F values were not significant. In replication 1, s² within, 866.58 was larger than s² for Carbendazim treatment, 43.93 and s² for explant type s² 112.14. As such with F < 1, the differences between explant type and fungicide treatment were not significant (Table 2). In replication 2, F_{1,3} treatment 5.89, and F_{3,3} explants

4.18 were not significant (Table 3).

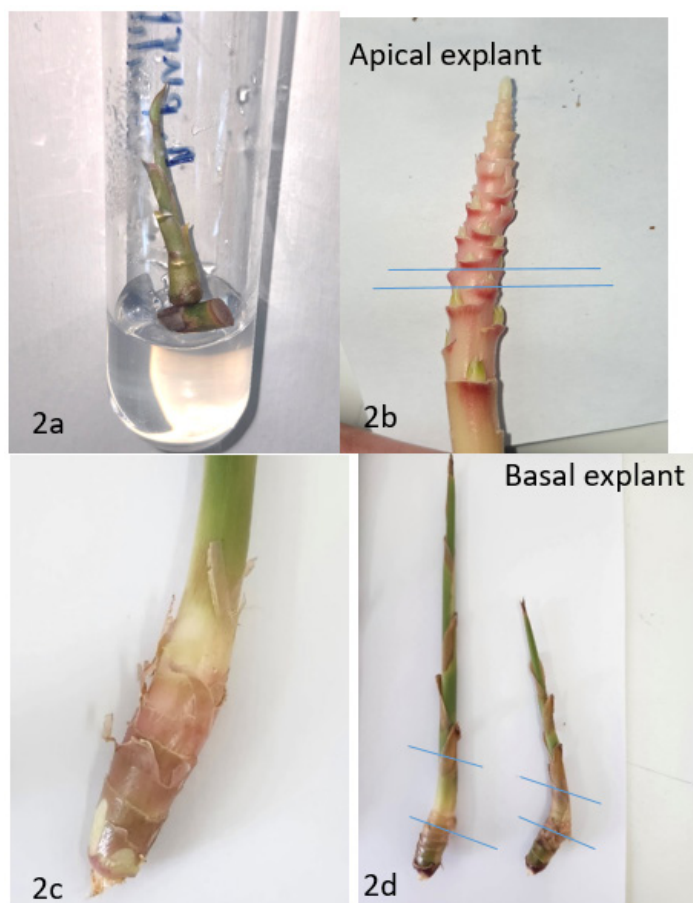


Figure 2a: Pseudo stem with emerging shoot; **Figure 2b:** Inflorescence with distal explant and apical explant; **Figure 2c:** Root/corm or basal section of miniature plant; **Figure 2d:** Guide lines for excision of miniature explants for *in vitro* initiation.

Table 1: Percentage (%) Establishment of Explants on Treatment Media.

Replication		Pseudo-stem	Distal bract stalk	Apical bract stalk	Basal plant corm
1	Carbendazim	80	94	100	50
	Control	67	86	57	100
2	Carbendazim	86	93	100	100
	Control	63	75	75	100

Table 2: ANOVA Summary Table for Replication 1.

Source of Variation	Sum of Squares	df	s ²	F
Variable A (treatment)	43.926	1	43.927	0.051
Variable B (explant)	336.423	3	112.141	0.129
Within	2599.745	3	866.582	
Total	2980.095	7	425.728	

Table 3: ANOVA Summary Table for Replication 2.

Source of Variation	Sum of Squares	df	s ²	F
Variable A (treatment)	463.297	1	463.297	5.894
Variable B (explant)	985.959	3	328.653	4.181
Within	235.816	3	78.605	
Total	1685.073	7	240.725	

Multiplication of Initiated explants was prolific from the bract stalk and pseudo stem (Figure 3) and explants held for two months exhibited spontaneous rooting on the initial explant (Figure 4). The data for rooted plants was summarised in Table 5. The number of leaves, roots and heights were all numerically higher in the explants grown on carbendazim supplemented media. However in the ANOVA because the within sample variance $s^2 0.076$ was higher than the between samples $s^2 0.028$, there was no need to compute F. With $F < 1$ there was no significant differences between the means for the two treatments. The wide within samples variance is illustrated with carbendazim only one unit higher than controls for number of roots and leaves (Figures 5). The variation in number of roots appeared greater. Plant height was almost identical with more variation in the heights on media without biocide (Figure 6).



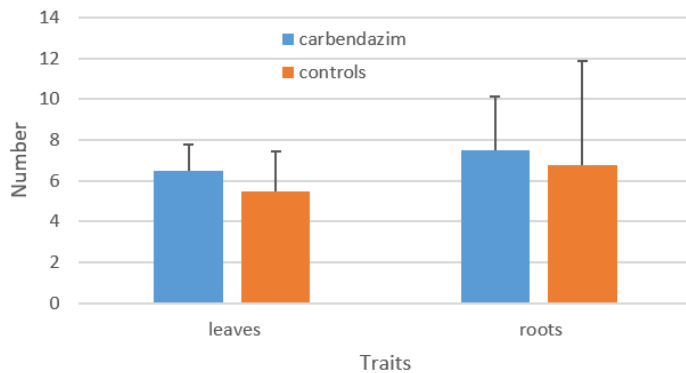
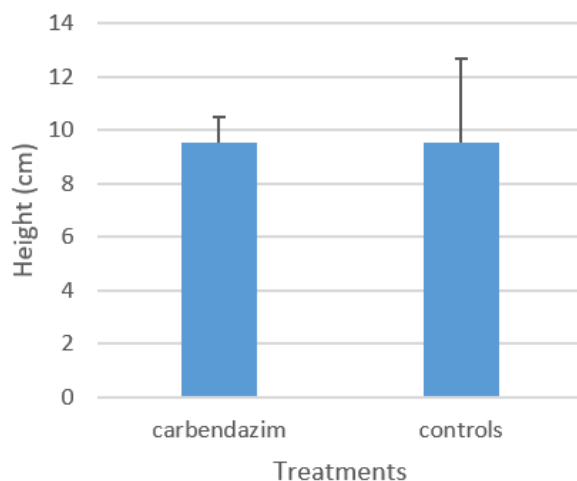
Figure 3: Vigorous *In Vitro* Shoot Formation in Red Ginger Inflorescence Explants.



Figure 4: Rooted Red Ginger Tissue Cultured Plants.

Table 5: Summary of Trait Means between Treatments.

Trait	Cardendazim	Control	F _{1,6}
Leaves	6.5±1.3	5.5±1.9	0.849
Roots	7.5±2.7	6.8±5.1	0.375
height (cm)	9.5±1.0	9.5± 3.2	0.040

**Figure 5:** Wide Within-Sample Variances in Numbers of Leaves and Roots.**Figure 6:** Wide Within-Sample Variance in Heights.

Discussion

The aim of the investigation was to highlight an age-old well-known morphological feature of red gingers [13]. It is unclear if miniature or mini plants have been well exploited in tissue culture initiation as described in this research. The research cited that was described as similar referred to inflorescence buds [12]. In Ill & Faria [12] perhaps only the axillary buds emerging on the bract stalk were used. Some other notable differences were the use of a different MS media formulation, more recently discovered and more transparent gelling agent, along with a recent practice of incorporating biocide in the media. The investigation did not address multiplication rates which appeared prolific. The greater focus was on investigating the improvement in establishment rates arising from aerial explants over subterranean rhizomes, as well as the addition of biocide in the media. Establishment rates where 50 – 100 % which is reasonably acceptable compared to the norm of

heavy bacterial and fungal contamination working with rhizomes. Currently sporadic endophytic bacteria remains one of the biggest constraints to maintaining *in vitro* cultures. It presents relatively greater challenges than fungus. Perhaps first noticed much earlier than the 1990s it was not in literature until much later [16]. The inclusion of biocides with dual anti-bacteria and anti-fungus activities as well as their systemic modes, incorporated in the *in vitro* growth media has been an option in addition to pre-sterilisation procedures. Opinions are split on their merits and risks of selecting for resistant strains of microbes but they represent a normal part of contemporary *in vitro* tissue culture [17-19]. Observations were that plants appeared to grow better with numerally more leaves, roots and taller. Albeit, the difference was not statistically significant. Much more research is required to make a determination but so far the benefits from inclusion of biocide appear positive.

The demonstration highlighted that almost every vegetative structure available on plants is exploitable for tissue culture initiation. Novel explants were used relative to the conventional practice of using sprouted rhizomes only. The observations included among other novelties, potential use of the leaf whorls for indirect organogenesis via a callus phase, but it was overlooked as well as leaf explants to produce plants via a similar callus route and somatic embryogenesis. These are methods that have been in routine use and long documented [11,20] with somatic embryogenesis for the leaf bases [21].

Equal chances of establishment among these aerial explants is a positive outcome. In comparison to rhizomes these explants from the inflorescence become available once blooms age. They are expected to be more convenient and easier to work with. Less contamination is expected and harvesting mini plants constitutes a less invasive technique and less damage to the mother plant. Relative to rhizomes they have potential to provide times more explants for initiation and potentially larger numbers from *in vitro* propagation.

Multiplication rates for red ginger inflorescence buds were reported to be as high as 15 - 20 every four weeks [12]. This may be adjusted by manipulating the BAP concentrations. The findings showed potential for high rates so this may warrant investigation. In addition, the ease of rooting is also an area for further research. Overall, with greater establishment rates, these are benefits of the inflorescence explants that make them more amenable for initiation than rhizomes.

Conclusion

Whereas edible ginger is propagated *in vitro* at SRC's lab from sprouted rhizomes, studies of these ornamental counterparts are useful in improving *in vitro* propagation of ginger generally. There is a plan to collect and conserve all the ginger diversity in Jamaica. Unfortunately, flowering is not a common feature in the edible ginger. There is therefore potential for research to induce flowering to study the potential exploiting the ginger inflorescence.

It is well appreciated why the aerial or higher explants show less contamination than the subterranean rhizomes. While true leaf and pseudo-stem explants along the ginger plant are a gateway to plants by indirect organogenesis, the ultimate and perfect explant for *in vitro* initiation remains the miniature plantlets. The prospects for *in vitro* conservation and multiplication of ginger at SRC are better with enhanced competence in the use of fungicides and bactericides incorporated in the *in vitro* growing media.

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