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In vitro propagation and Microrhizome induction in *Alpinia galanga* (L.) Willd.

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ABSTRACT
A more advanced method for inducing microrhizomes in vitro in Alpinia galanga has been established. As the initial explants, newly sprouted shoot buds from rhizomes were used and replicated by in vitro techniques. In vitro produced shoots developed in MS medium fortified with cytokinin (BA) and various auxins (NAA, IAA and IBA). A maximum of shoots per explant were obtained inMS medium containing 0.5 mg/l BA and 1 mg/l IBA. Medium containing 1.0 mg/l BA in combination with 1.0 mg/l IAA showed the best response for induction of microrhizomes. The study further found that intermediate light (8 hr light and 16 hr darkness) greatly boosted the induction of microrhizomes, with 4% sucrose content being the optimal amount.Acclimatization of this microrhizome showed 55% of survivability rate in field condition.

Keywords: Galangal, In vitro, Organogenesis, Microrhizome, Auxin

INTRODUCTION

Alpinia galanga (Zingiberaceae), commonly known as galangal, is one valuable herbal plant that is extensively cultivated in Asia. The uses of this plant are numerous, and they include seasoning food and giving cookery a unique aroma. This plant can also be used

to treat a number of illnesses [1].The most utilized and researched portion of the plant is the galangal rhizome.acetoxyeugenol acetate, p-coumaryl diacetate and acetoxychavicol acetate are the importantbioactive compounds found inthe galangal rhizome[2].

Despite being a widely used medicinal plant, biotechnology interventions on galangal have not kept up with the crop's significance or the rising demand for it. Therefore, using biotechnological tools or using unconventional methods are required for the production and propagation of this valuable herb. Galangal can be cultivated in vitro, which allows for the quick creation of a large number of disease-free clones.

In the current study, we used sprouted shoot buds to design a direct regeneration strategy for galangal. This is an important technique for creating genetically consistent galangal propagation. Microrhizomes are small, in vitro-developed rhizomes. The development of microrhizomes is an excellent technique for storing germplasm and using directly in in vivo settings [3]. The current work thoroughly examined the variables that cause microrhizomes production, including photoperiod, sucrose content, and growth regulators.

MATERIALS AND METHODS

Aseptic culture establishment

Mature Rhizomes of *A. galanga* was collected from Medicinal plant garden of Unani Medical College, Kozhikode, Kerala and germinated in Jamal Mohamed College garden. Elongated sprouting shoot buds were carefully excised from the mother rhizome and employed as the explant for in vitro culture. The explants were thoroughly washed with running tap water for ten minutes in order to remove any adhering soil, and then they were submerged in a Tween-20 solution for five minutes. Following a 2-minute treatment with 1% (w/v) mercuric chloride ($HgCl_2$), the explants were carefully rinsed three times with distilled water that had been sterilized. After the outer scale was removed, the explants were placed in a Murashige and Skoog (MS) [4] medium that had been solidified with 0.8% (w/v) agar and supplemented with 3.0% (w/v) sucrose. The culture medium underwent autoclaving at 121°C and 104 kPa for 20 minutes, with the pH adjusted to 5.7. Cultures were upheld at a constant temperature of 25±1°C under white fluorescent light with an intensity of 40 µmol m^{-2} s^{-1}.

Effect of PGRs on shoot multiplication

In order to stimulate shoot multiplication, 30 days old shoot buds that were grown in vitro were carefully removed and subsequently subcultured in culture vessels, each containing 30 ml of MS medium. The culture medium were treated with several combinations

of Benzyladenine (BA) alone or in conjunction with Naphthaleneacetic acid (NAA), Indole-3-acetic acid (IAA), and Indole-3-butyric acid (IBA) were incorporated into the culture media. Following an incubation period of 40 days, the length of shoots, the mean number of shoots, and the biomass of microrhizomes were systematically noted for comprehensive analysis and evaluation.

Induction of microrhizome

Shootlets that were grown in vitro and reached a shoot length of 4–5 cm in a MS medium supplemented with 1.0 mg/l IAA and 1.0 mg/l BA were used for the induction of microrhizomes. These in vitro cultures were exposed to a range of sucrose concentrations, from 1% to 5%. A comprehensive evaluation was conducted to determine the effects of varying photoperiods, such as 0 hours, 8 hours, and 16 hours of light exposure. Growth characteristics of the cultures were noted after 90 days of culture.

Acclimatization

Plantlets with strong root and leaf systems were selected for the following phase and placed into paper cups that were filled with a 1:2:1 balanced mixture of sand, soil, and vermiculite that had been autoclaved. To create an optimal microenvironment for acclimatization process, the cups were shielded with perforated polythene bags to maintain high humidity levels. Until new leaves appeared and the plants began to show signs of robust development, the bags functioned as protective covers. After three months of the hardening process, the survival rate of the plantlets was meticulously calculated, providing a quantitative measure of the plants' adaptability and resistance to the changeover from lab conditions to the external field conditions.

Statistical analysis

All experiments were conducted in triplicates, each containing a minimum of 10 explants to ensure comprehensive and reliable results. Using SPSS software (Version 20), a one-way analysis of variance (ANOVA) was applied to all the data. Duncan's multiple range test was used to compare means, and a significance level of $P \geq 0.05$ was applied.

RESULTS

Effect of PGRs on shoot induction

*A. galanga*sprouting bud explants were cultivated on MS media supplemented with BA either in alone or in various ratios with NAA, IBA, and IAA. A maximum of 12.33±0.33shoots per explant were obtained after treatment with lower concentration of 0.5 mg/l BA and 1 mg/l IBA. It was noted that the longest shoots measured 9.66±0.33 cm (Table 1).At this concentration, the microrhizome's biomass and size were 1.7±0.35 g and 0.76±0.14

cm, respectively.

Effect of PGRs on microrhizome induction

The swollen shoot base served as the starting point for rhizome development. Microrhizome biomass was maximum at a concentration of 1.0 mg/l BA in combination with 1.0 mg/l IAA. In this medium, 3.66±0.33 g of microrhizome biomass and 3.0±0.57 cm of microrhizome length were achieved. Further research is conducted using the same medium.

Effect of Sucrose concentration and photoperiods on microrhizome induction

In 1% sucrose concentrations under various photoperiods, no rhizomes were found. However, when the concentration of sucrose increased, correspondingly increased the number of microrhizomes. The ideal sucrose content for inducing microrhizomes was 4% (Table 2).Three photoperiods were used to assess the development of microrhizomes from in vitro cultivated shoots (0, 8 and 16 hr). It indicates the induction of microrhizomes was much enhanced by an intermediate light.

Acclimatization

70% of the plants in paper cups survived after 4–5 weeks of hardening. After that, they were shifted to earthen pots in a shade hut. It was observed that the survival percentage beneath the shade house had dropped to 55%. The regenerates displayed no morphological differences.

DISCUSSION

Reports on numerous Zingiberaceae species have documented BA's influence on shoot induction. Shoot regeneration was stimulated by BAP, either by itself or in conjunction with other growth regulators. A beneficial effect of BAP on in vitro regeneration was reported on *Zingiber officinale* [5-7] and*Curcuma longa* [8-9].

The size and fresh weight of the rhizomes varied according to the various treatments of BA, NAA, and IAA (Table 1; Figure 1). In our study, a combination of 1.0 mg/l BA with 1.0 mg/l IAA was maximum for microrhizome production. Positive effect of BA on microrhizome is reported in *Curcuma longa* [10], *C. aromatica* [11], *Zingiber officinale* [12-13] and *Curcuma zedoaria*[14]. The effects of auxins like NAA were examined for microrhizome production in independent studies [15-17].

For shoot and microrhizome induction, sucrose supplies carbon and energy. Therefore, the in vitro microrhizome induction was significantly influenced by the sucrose content. In our study, 4% sucrose concentration was optimum for microrhizome induction

Different Zingiberaceae species require distinct ranges of sucrose concentrations for the induction of microrhizomes,8% in *Zingiber officinale* [7], 5% in *Z. cassumunar*[18],6% in *C. aromatica* [11], *C. zedoria* [14], 12% in Potato [19], 6% or 8% in *Ipsea malabarica*[20], *C. longa* [21] and 6-9% in *Hedychium stenopetalum*[22].

The biomass of the microrhizome was greatly decreased in an in vitro culture with 16 hours of light and total darkness. It suggests that in our investigation, the induction of microrhizomes was significantly boosted by an intermediate light. This is in accordance with several other studies in Zingiberaceae family [7,11,13].

CONCLUSION

The study investigates the impact of PGRs on shoot induction in *A. galanga* sprouting bud explants. The study found that a lower concentration of BA and IBA led to a maximum shoots per explant. The maximum microrhizome biomass was achieved at 1.0 mg/l BA in combination with 1.0 mg/l IAA. The ideal sucrose content for inducing microrhizomes was 4%. The study also found that intermediate light significantly enhanced the induction of microrhizomes.

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Table 1: Effect of plant growth regulators on shoot and microrhizome induction

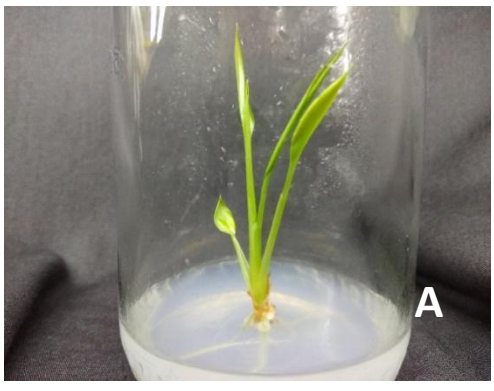
Plant growth regulators (mg/l)				Mean number of shoots per explant	Shoot length (cm)	Microrhizome size (cm)	Microrhizome Biomass (g)
BA	NAA	IAA	IBA				
0.5	0.5			8.0±0.57^{cd}	8.66±0.33^{ab}	0.66±0.16^{cde}	1.5±0.5^{def}
	1.0			6.66±0.33$_{ef}$	8.66±0.66^{ab}	0.83±0.16^{cde}	1.36±0.31^{def}
	1.5			6.66±0.33^{ef}	5.33±0.33^{f}	1.66±0.33^{bc}	3.2±0.61^{ab}
		0.5		7.0±0.57^{de}	7.0±0.0^{de}	2.0±0.0^{b}	3.13±0.59^{ab}
		1.0		5.66±0.33^{ef}	6.33±0.33^{def}	2.0±0.57^{b}	1.76±0.39^{cde}
		1.5		8.33±0.33^{c}	8.66±0.33^{ab}	0.83±0.16^{cde}	1.1±0.1^{ef}
			0.5	9.66±0.33^{b}	8.33±0.33^{bc}	0.83±0.16^{cde}	1.1±0.37^{ef}
			1.0	12.33±0.33^{a}	9.66±0.33^{a}	0.76±0.14^{cde}	1.7±0.35^{def}
			1.5	11.33±0.33^{a}	8.33±0.33^{bc}	0.73±0.17^{cde}	1.0±0.0^{ef}
1.0	0.5			2.0±0.57^{j}	3.33±0.33^{g}	0.0±0.0^{e}	1.3±0.33^{def}
	1.0			2.33±0.33^{j}	3.33±0.33^{g}	0.56±0.23^{de}	0.53±0.24^{f}
	1.5			4.33±0.33^{gh}	2.66±0.33^{g}	0.56±0.21^{de}	0.86±0.18^{ef}
		0.5		3.0±0.57^{ij}	3.33±0.33^{g}	0.83±0.16^{cde}	0.83±0.2^{ef}
		1.0		4.0±0.57^{hi}	5.33±0.33^{f}	3.0±0.57^{a}	3.66±0.33^{a}
		1.5		6.33±0.33^{ef}	6.0±0.0^{ef}	1.0±0.0^{cde}	2.33±0.33^{bcd}
			0.5	6.33±0.33^{ef}	2.33±0.66^{g}	0.83±0.58^{cde}	2.83±0.44^{abc}
			1.0	3.66±0.33^{hi}	3.66±0.88^{g}	1.5±0.5^{bcd}	1.1±0.1^{ef}
			1.5	5.33±0.33^{fg}	3.66±0.33^{g}	0.36±0.08^{e}	1.06±0.06^{ef}

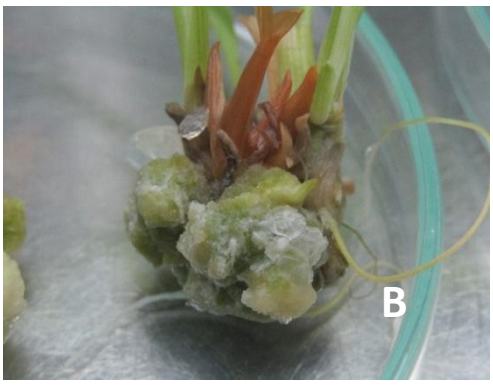
Values are expressed as the mean ± SE, taking ten explants in each experiment with three replications. Within each group, values with different letters indicate significant difference at P ≥ 0.05 using Duncan's multiple range test (DMRT)

Table 2 :Determination of sucrose concentration on microrhizome formation

Sucrose (%)	Mean number of shoots per explant			Microrhizome biomass (g)		
	0 h dark	8 h light	16 h light	0 h dark	8 h light	16 h light
1.0	0.0±0.0c	1.33±0.33c	1.66±0.66c	0.0±0.0d	0.0±0.0d	0.0±0.0c
2.0	2.33±0.33b	4.66±0.33b	4.66±1.45b	3.83±0.08bc	3.13±0.08c	1.06±0.23bc
3.0	2.0±0.57b	7.66±0.66a	9.0±0.57a	5.12±0.07a	5.53±0.18b	2.36±0.68a
4.0	4.33±0.33a	8.33±0.33a	6.0±0.57b	4.33±0.24ab	6.56±0.31a	1.83±0.32ab
5.0	2.33±0.33b	4.33±0.33b	4.0±0.57bc	3.03±0.51c	3.36±0.31c	0.86±0.18bc

Values are expressed as the mean ± SE, taking ten explants in each experiment with three replications. Within each group, values with different letters indicate significant difference at P ≥ 0.05 using Duncan's multiple range test (DMRT)





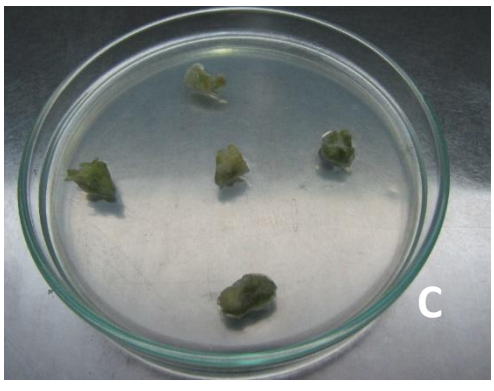


Figure 1: (A) Shoot induction on MS medium (B) Microrhizome formation on MS medium containing BA and IAA (C) Isolated microrhizomes.