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Shoot Induction and Silver Nanoparticles Synthesis Using Bentong Ginger Extracts

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Abstract
The global demand for silver nanoparticles (AgNPs) is increasing due to their applications in medicine and agriculture. Utilizing Bentong ginger for eco-friendly, cost-efficient AgNP production through plant-based methods is advantageous. However, the scarcity of ginger rhizomes due to high demand hampers planting. To combat this, disease-free ginger plants are generated using tissue culture techniques. This research centers on optimizing the micropropagation of Bentong ginger by refining surface sterilization methods and adjusting plant growth regulator levels to promote shoot and root development. Sprouted bud explants were surface sterilized using varying bleach (Clorox) concentrations and 2 ml/L PPM. Diverse combinations of BAP and NAA concentrations were employed to induce shoots from sprouted buds over four weeks. The syntesis of AgNPs using extracts from field-grown rhizomes was analyzed using UV-Vis and FTIR. Successful sterilization encompassed treating explants with 70% (v/v) Clorox and 2 ml/L PPM, achieving an 80% sterilization rate. Specific NAA and BAP concentrations effectively stimulated shoot growth. Optimal conditions for ginger shoot growth encompassed an MS medium with 60 g/L sucrose, 15 µM BAP, and 7.5 µM NAA. In this study, the AgNPs synthesized by tissue-cultured Bentong ginger had better characteristics in UV-Vis and FTIR analysis compared to field-grown Bentong ginger.

Keywords: Ginger Bentong; Silver Nanoparticles; Plant Growth Regulators; Surface Sterilization

Introduction

The assessment of micropropagation techniques' effectiveness involves evaluating their capacity to successfully establish in vitro-grown plants when transferred to ex vitro conditions. Aseptic conditions, shoot regeneration, root development, and acclimatization are vital aspects for successful *in vitro* propagation (Zahid et al., 2021a). Rhizome buds from the Zingiberaceae family are commonly used as explants due to their meristematic tissues' heightened responsiveness to available nutrients, resulting in better sensitivity (David et al., 2018). This study concentrated on Bentong ginger, a member of the Zingiberaceae family. Nonetheless, early contamination-free cultivation is complicated by various soil-borne diseases (Manisha et al., 2018).

Silver nanoparticles (AgNPs) hold a crucial role as captivating nanomaterials extensively employed in biomedical applications and various nanoscience and nanotechnology fields. AgNPs possess unique attributes such as high electrical and thermal conductivity, chemical stability, and surface plasmonic behavior, rendering them significant in nanomedicine and beyond. Their distinctive

features, including enhanced antibacterial activity and catalytic properties due to a high surface area-to-volume ratio, differentiate AgNPs from their bulk counterparts. Their applications span *in vivo* imaging, biomarkers, cell signaling, and diverse medical uses. In agriculture, AgNPs find utility in food preservation, cosmetics, optoelectronics, and more.

Optimizing concentrations of Plant Growth Regulators (PGRs) in culture media is vital for favorable responses in ginger microrhizome induction. Yet, previous studies have employed differing measurement techniques, leading to experimental variations. Despite evaluating various PGR combinations, the optimal mix for desired shoot length, shoot count per explant, and root count per explant remains unidentified. The study aimed to identify the optimal surface sterilization method for sprouted buds of Bentong ginger, assessed the impact of various PGR concentrations on shoot and root induction in Bentong ginger, and analyzed the formation of silver nanoparticles (AgNPs) using field-grown Bentong ginger.

Materials and methods

Bentong ginger sourced from its growing region in Bentong, Pahang, Malaysia, was employed for the study. Sprouting buds of fresh mature ginger rhizomes, measuring 1 to 1.5 cm in length, served as the explants for culture initiation. The rhizome explants were cleansed with liquid detergent and Tween 20, then surface sterilized with 70% (v/v) ethanol for 1 minute followed by immersion in sodium hypochlorite (Clorox) solutions of varying concentrations (60%, 70%, and 80%) with Tween 20 for 30 minutes. Non-submerged explants were used as controls. Sterilized rhizome sprouting buds were aseptically trimmed to 0.5 cm and cultured in MS medium (Murashige & Skoog, 1962) supplemented with 60 g/L sucrose, solidified with Gelrite, and sterilized. Each test was replicated thrice. After a 4-week culture period at 25°C, percentage of the sterilized explants were calculated.

For shoot induction, rhizome explants were treated with 15 mg/L 6-Benzylaminopurine (BAP) and 7.5μM 1-Naphthaleneacetic acid (NAA) in the MS medium with 60 g/L-1 sucrose for 4 weeks. Different cytokinin and auxin concentrations were tested to identify the best conditions for shoot multiplication. Each combination was triply replicated.

The AgNP biosynthesis using ginger extract was prepared from dried rhizome pieces, blended to obtain a fine powder, and mixed with $AgNO_3$ solution. The color change of the solution was observed upon heating at 85°C. The resultant extract was filtered and centrifuged, and the AgNPs were characterized through UV–visible spectroscopy and Fourier transform infrared spectroscopy (FTIR) (Mehata et. al., 2021).

Data analysis was conducted using IBM SPSS Statistics version 27. Independent samples t-test assessed sucrose concentration effects on micropropagation, while ANOVA evaluated PGR combinations. Duncan's multiple range test (DMRT) differentiated significant differences with a threshold of $p \leq 0.05$.

Results and discussion

In this study, the effect of different bleach concentrations on the percentage of surface sterilization was assessed. The attempt to achieve surface sterilization using Clorox concentrations of 50%, 60%, and 70% (v/v) was found to be ineffective in preventing the growth of fungi and bacteria on the cultures. This resulted in a complete lack of sterilized explants, with a 0% success rate. However, a different approach utilizing 70% (v/v) Clorox, along with an additional 2 mg/L of Plant Preservative Mixture (PPM), yielded an 80% success rate in terms of sterilized explants. In contrast, the control group that did not involve the use of Clorox experienced contamination in all explants (as Table 1).

The contamination could have originated from pre-existing bacteria within the plant systems, particularly those that manifest during distinct growth stages, such as endophytic bacteria. This clarifies why the contamination control measures commonly applied in this study seemed to yield limited success, except for the surface sterilization method supplemented with 2mg/L PPM. PPM is a biocide or heat-resistant preservative employed in plant tissue culture, and it demonstrates efficacy in preventing or minimizing microbial contamination.

Table 1: Effect of bleach concentrations on percentage of sterilized explants.

Chlorox concentrations (v/v)	Percentage of sterilized explants, %
50%	0
60%	0
70%	0
70% + 2 mL PPM	80

Table 2 shows the growth of cultured explants in MS media, enriched with 60 g/L sucrose, 15 µM BAP, and 7.5 µM NAA—the most effective combination for ginger shoot induction. The sprouted buds, employed as sterilized explants, thrived following surface sterilization, exhibiting a green tint within a week, signifying their vitality. Subsequently, over four weeks culture, the explants demonstrated significant growth, particularly in shoot elongation, progressively increasing in size and length for approximately three months. Regrettably, only 1 out of 8 explants successfully triggered shoot growth, while 13 roots formed per explant—fewer than the typical counts in other studies (16-24). The presence of BAP, a stable growth medium component, was observed to impact shoot growth dynamics (Zahid et. al., 2021b). Notably, using smaller 1-1.5 cm explants helped mitigate contamination risk, although a sharp survival decline was noted as explant size decreased (Sathyagowri & Seran, 2011). Larger explants exhibited quicker sprouting, benefiting from existing nutrients, unlike smaller counterparts that relied more on the medium for nourishment.

Table 2: Effect of bleach concentrations on percentage of sterilized explants.

PGR treatment	Length of explant, cm	Length of shoot, cm	Percentage of Shoot Induction	Percentage of Root Induction	No. of Root Induced/Explant
15 µM BAP + 7.5 µM NA	2.5	4.3	12.5	12.5	13

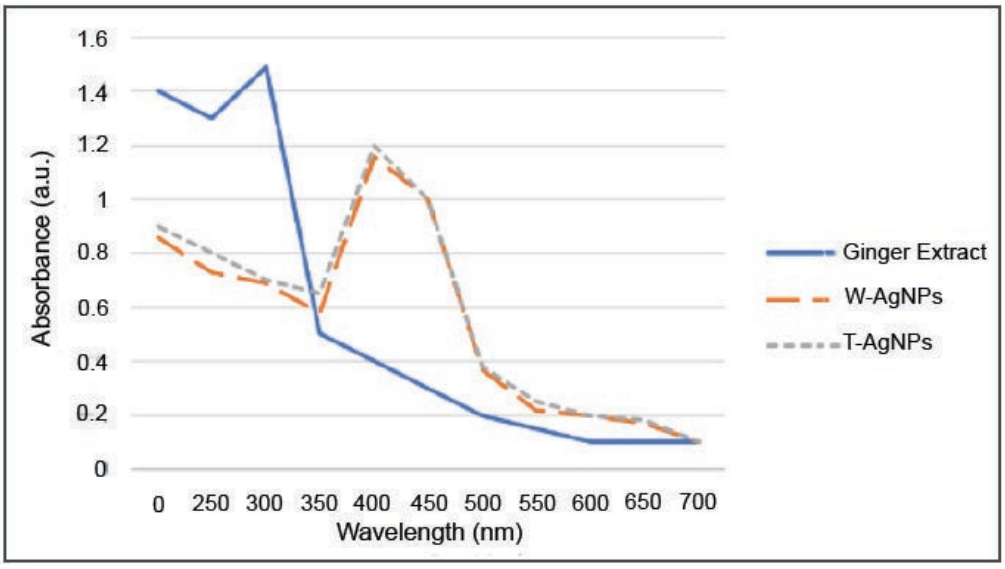

Figure 1 Absorption spectra of plant extracts: Field grown plant (W-AgNPs), Tissue culture plant (T-AgNPs) and ginger extracts.

The production of AgNPs was further explored using UV-vis spectroscopy. Figure 1 illustrates that the absorbance spectrum of AgNPs derived from tissue-cultured Bentong ginger (T-AgNPs) showed an absorbance of 1.2, while AgNPs from field-grown Bentong ginger (W-AgNPs) displayed a notable peak at 1.16. Both distinct peaks were observed within the 400 nm region, which corresponds to the typical λmax range of AgNPs. Previous research has indicated that AgNPs often exhibit significant peaks in the 400 to 500 nm range (Sadeghi & Gholamhoseinpoor, 2015). These outcomes suggest that the constituents of ginger extract acted as the necessary chemical reducing agents for AgNP synthesis. The absorption spectrum of the ginger extract displayed an absorption peak at 300 nm due to π-π transitions. Aside from a minor hump around 300 nm in the ginger extract, no other bands were evident within the 250-700 nm range.

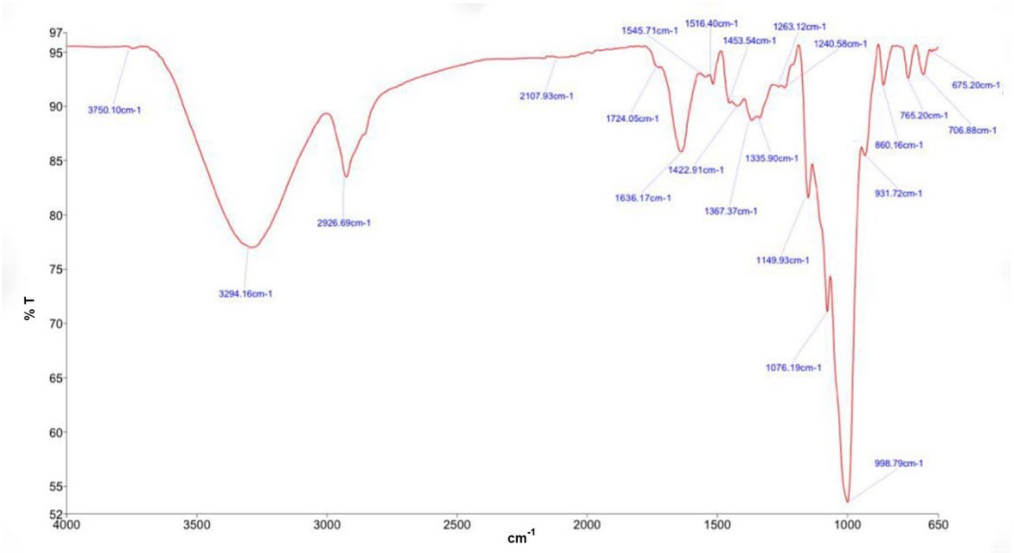

Figure 2 FTIR spectra of tissue cultured plant-AgNPs (T-AgNPs)

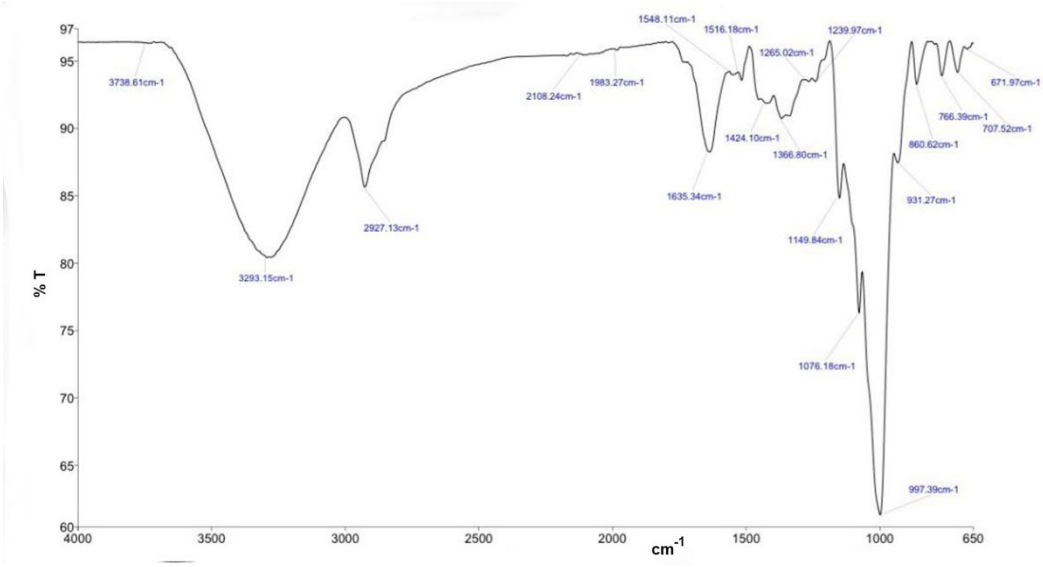

Figure 3 FTIR spectra of field grown plant-AgNPs (W-AgNPs)

This study also studied the presence proteins, amino groups, alkanes, and alkenes in the synthesized AgNPs. In Figure 2 and 3, the FTIR spectra of W-AgNPs and T-AgNPs in the 4000-650

cm^{-1} region were shown, respectively. The characteristic bands at approximately 3294.16, 1636.17 and 998.79 cm^{-1}, along with a faint band at 2107.93 cm^{-1}, were highly comparable to those observed for AgNPs synthesized from ginger extracts in previous studies (Ramzan et al., 2019; Mehata et al., 2021). The O-H stretching vibration was indicated by the band at 3294.16 cm^{-1}, while the C=C and C≡C vibrations were represented by the bands at 1636.17 and 2107.93 cm^{-1}, respectively. The C=O stretching vibration of the tertiary amine in amino acids was also associated with the peak at 1636.17 cm^{-1}. The C-Cl group of alkyl halides was represented by the strong peak at 998.79 cm^{-1}, which was found in both ginger extract and AgNPs. Additionally, minor peaks in the range of 1200-1000 cm^{-1} were observed in the ginger extract due to the presence of flavonoids and enzyme functional groups (alcohol, amine, ester, and carboxylic acid), which were absent in AgNPs. The functional groups presented in phenolic compounds, carbohydrates, starches, terpenoids, enzymes, etc., play a crucial role in the synthesis of AgNPs (Mehata et al., 2021). Table 3 presents the FTIR spectra comparison between T-AgNPs and W-AgNPs.

Table 3: FTIR spectra comparison of T-AgNPs and W-AgNPs.

FTIR Analysis	AgNPs	
	W-AgNPs (cm^{-1})	T-AgNPs (cm^{-1})
O-H stretching vibration	3293.15	3294.16
C=C stretching vibration tertiary amine of amino acid	1635.34	1636.17
C=C stretching vibration	2108.24	2107.93
C-Cl group of alkyl halides	997.39	998.79

Conclusion

In this study, the most effective approach for surface sterilization during the *in vitro* cultivation of Bentong ginger was determined to be a solution of 70% (v/v) Clorox combined with 2 ml/L PPM. It was observed that using a lower concentration of Clorox might not be sufficient to eliminate contaminants, while a higher concentration could hinder explant development. Furthermore, the optimal combination of plant growth regulators (PGRs) for successful shoot induction (12.5%) and root formation (12.5%) of Bentong ginger in this study was found to be MS medium supplemented with 10 µM BAP and 7.5 µM NAA. Notably, the AgNPs produced through tissue-cultured Bentong ginger (T-AgNPs) exhibited more pronounced absorbance spectra in UV-Vis analysis and featured more distinct characteristic bands in FTIR analysis compared to AgNPs derived from field-grown Bentong ginger (W-AgNPs).

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