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Research Article
Gelrite and Aerated Condition Enhanced Rooting and Growth of Pine Apple

[1]Bui Van The Vinh, [2]Nguyen Ba Nam and [2]Hoang Thanh Tung

[1]Hutech University, Ho Chi Minh, Vietnam
[2]Dalat University, Lam Dong, Vietnam

Abstract

Background and Objective: The *in vitro* development of *Ananas* sp. can be significantly influenced by the choice of gelling agent and culture conditions. Integrating agar and gelrite with aerated culture systems improves nutrient uptake and reduces physiological stress, enhancing plantlet quality and uniformity. The objective of this study was to optimize micropropagation of *Ananas* by evaluating the effects of gelling agents and aeration on growth and physiological responses. **Materials and Methods:** Shoots of 2 cm length were cultured on MS medium supplemented with 30 g/L sucrose and 1 mg/L IBA. Two gelling agents (agar and gelrite) were tested under aerated conditions (with or without filter paper) to assess their effects on rooting, growth and physiological stress parameters. Growth parameters, medium utilization, antioxidant enzyme activities (SOD and CAT), leaf yellowing and hormonal balance (IAA, CK, GA3 and melatonin) were recorded. All treatments were replicated thrice (15 vessels/treatment) and data were analyzed using Microsoft Excel 2016 and SPSS 20.0 with Duncan's test at $p<0.05$. **Results:** Plantlets grown on gelrite under aerated conditions showed the highest growth, reaching 11.17 cm in height and 2170 mg fresh weight, with 90.16% medium utilization. Restricted aeration caused significant stress, including elevated SOD (189.88 U/g) and CAT (378.37 U/g) activities, 80% leaf yellowing in non-aerated agar treatments, reduced medium consumption (7.54% in non-aerated gelrite) and lower IAA/CK ratios. Aeration stabilized GA3, IAA and melatonin levels, reduced basal callus formation and produced uniform, healthy plantlets. **Conclusion:** Aerated culture with gelrite as a gelling agent enhances the efficiency of *Ananas* micropropagation. This method produces high-quality, uniform plantlets suitable for local cultivation and provides a valuable *in vitro* resource for future research.

Key words: Abnormal phenomena, callus at the root, gelrite, rooting, yellowing of leaf

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Corresponding Author: Hoang Thanh Tung, Dalat University, Lam Dong, Vietnam

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pineapple (*Ananas* sp.) is known as a fruit with high nutritional value with a high content of vitamins (A, B, C), calcium, iron and bromelain enzyme and accounts for nearly 20% of global tropical fruit production (27.81 million tons with an area of 1.07 million hectares) after mango and banana[1]. Pineapple contains a lot of sugar, have quite high calories, rich in minerals, especially potassium and has all the necessary vitamins, especially in pineapple trees and fruits. Besides, it contains bromelain, a protein-hydrolyzing enzyme that can cure digestive disorders, inhibit edema and blood stasis and help wounds heal quickly. In Vietnam, the An Xuan pineapple is a pineapple variety endemic to Phu Yen province (now Dak Lak); however, there are still some limitations because this pineapple variety only grows naturally, the regeneration process is slow, depends a lot on weather conditions and it is difficult to expand the raw material area. The supply of seedlings is unstable and insufficient to meet the demand.

Pineapple seeds are only used in breeding because the seeds are quite hard and take a long time to germinate. Pineapple seeds are very small, so in nature they are difficult to develop into seedlings, so the main method of propagation is usually by cutting and separating buds. Seed propagation by cutting can use apical buds or fruit buds, dormant eyes, axillary bud sand peduncle buds[2-4]. Axillary buds are often used for propagation more than other types of samples because they are larger in size and are usually broken 1 month after harvesting the fruit. After 2-3 months of harvesting the fruit, the peduncle buds can be broken. Fruit buds are rarely used as seeds because the growing time is much longer than using axillary buds and peduncle buds. Pineapple has a relatively low shoot multiplication coefficient, to provide 1 hectare of Pineapple, 50,000 to 60,000 shoots are needed. Therefore, traditional propagation methods are difficult to produce a large number of uniform shoots at once.

Plant tissue culture is a rapid technique for producing many high-quality, uniform, disease-free plant varieties in a short period of time[5]. *In vitro* propagation is widely deployed to provide large-scale *Ananas* plantlets[3]. *In vitro* propagation has been applied to many *Ananas* varieties worldwide[6]. The commonly used tissue types are axillary buds, peduncle buds, shoot tips, or dormant buds from the above types of buds[7]. The commonly applied method of *in vitro* propagation is direct or indirect regeneration. Direct propagation is done by stimulating shoot growth from dormant buds[8,9]. He *et al.*[10] has developed an industrial-scale *in vitro* propagation process from dormant buds of fruit shoots. Meanwhile, indirect propagation is often through the stage of callus formation and asexual embryogenesis[8,11]. Some studies have also applied propagation by liquid culture[11,12] or bioreactor system to improve plantlet production efficiency. Indirect propagation through callus can cause somatic variations but gives a higher multiplication coefficient than direct propagation from dormant buds or shoot tips and can form variations that are beneficial for new breeding[8].

Several studies have established a protocol for the propagation of *Ananas* sp. based on shoot elongation and proliferation from field-grown explants[2,13]. Different explants were cultured on MS medium[14] supplemented with plant growth regulators (BA, Kinetin, NAA and IBA) singly or in combination at different concentrations. During *in vitro* culture, factors such as high humidity, light and nutrients can cause oxidative stress. Antioxidant enzymes (SOD, CAT, APX) help minimize the negative effects of oxidative stress, protecting cells from damage and death. By eliminating free radicals and hydrogen peroxide, antioxidant enzymes help maintain the stability of cell structure and cell function, thereby promoting tissue growth and development. The activity of endogenous antioxidant enzymes can improve the efficiency of tissue culture, help increase the survival rate, increase the proliferation and maintain the quality of *in vitro* cultured plants. This study was conducted to improve the efficiency of micropropagation of An Xuan aromatic plants, create a source of uniform, quality plantlets for the locality and create a rich source of *in vitro* plants to use as a source of materials for future research.

MATERIALS AND METHODS

Study area and duration: The experiment study was carried out from June, 2025 to December, 2025 at the HUTECH University (Hochiminh, Vietnam) and the analysis of antioxidant enzyme activities, endogenous hormone extraction and data were carried out from December, 2025 to March, 2026 at Dalat University (Lamdong, Vietnam).

Plant material and culture conditions: Shoots were obtained from meristem cultures of An Xuan pineapple (Daklak, Vietnam) and were subcultured many times on MS medium. The shoots were about 2 cm in size, healthy and relatively uniform. The explants were inoculated into nylon bags (15 cm×20 cm) and placed in a light room with a temperature of about 25±2°C, relative humidity of 55-60% and placed under fluorescent light with a photoperiod of 12 hrs/day and a light intensity of about 40-45 µmol/m^2/sec.

Effect of gelling agent and aerated condition on rooting: *In vitro* shoots (2 cm) were cultured on MS medium containing 30 g/L sucrose, 1 mg/L IBA[2] and different gelling agents (agar or gelrite) under aerated conditions (with/without filter paper) to evaluate rooting. Millipore membrane with Milliseal™, pore size 0.5 µm (Nihon Millipore Ltd., Tokyo, Japan) and 2 cm diameter, used as ventilated membranes for culture systems. After 8 weeks of culture, the ratio of rooting (%), number of roots, root length (cm), number of leaves, plantlet height (cm), fresh weight (mg), dry weight (mg), dry matter (%), leaf length (cm), leaf width (cm) and SPAD were recorded.

Antioxidant enzyme activities: Fresh plant samples (300 mg) were collected from the rooting treatments and placed in a porcelain mortar; they were then ground in liquid nitrogen to a fine powder and homogenized in 2 mL of 0.1 M phosphate buffer containing 0.1 mM ethylene diamine tetra acetic acid. The mixture containing the samples was centrifuged at 15,000 rpm for about 20 min at 4°C. The supernatant was collected and stored under cold conditions (4°C) for about 24 hrs to determine the antioxidant activity of the enzymes.

All physiological and biochemical measurements were performed in at least three biological replicates to ensure the reliability of the results. The data obtained from the enzyme assays (SOD, CAT, APX) and antioxidant tests (DPPH, total phenolics) were subjected to a One-way Analysis of Variance (ANOVA) using SPSS statistical software. Means were compared using LSD or Duncan's multiple range test at a significance level of $p<0.05$. All results are presented as the mean standard deviation (SD) and figures were generated to visualize the enzymatic response variations across the different rooting treatments.

The SOD activity was determined by measuring its ability to inhibit the auto-oxidation of pyrogallol, a process monitored by the change in absorbance at 320 nm[15]. The CAT activity was determined by measuring the decomposition of H_2O_2 through the formation of a yellow ammonium molybdate complex, which was quantified spectrophotometrically at 405 nm[16]. APX activity was quantified by measuring the decline in absorbance at 290 nm due to ascorbate oxidation over a three-minute interval[17]. In addition, the antioxidant capacity was measured by monitoring the colour change of the DPPH radical from purple to yellow, with the reduction in absorbance used to calculate the percentage of radical scavenging activity[18]. Total phenolic content was determined using the Folin-Ciocalteu colorimetric method, where the resulting shift from yellow to dark green was measured against a gallic acid standard curve[19].

Abnormal phenomena of plantlets under gelling agent and aerated conditions: The 8-week-old plantlets of the rooting treatments were collected and observed under a stereomicroscope (Olympus Corporation, SZX2-ILLK, Tokyo, 163-0914, Japan) to obtain the percentage of abnormal phenomena (%) including callus at the root, yellowing of leaf and plant morphology.

Change of medium in plantlet vessel: The culture media were recorded before inoculation (medium preparation) and after 8 weeks of inoculation to assess the variation in medium volume and the percentage of medium used (%).

Endogenous hormone extraction and HPLC analysis: The profiling of endogenous hormones, including auxins-AUX (IAA), cytokinins-CK (2iP, KIN, zeatin), GA3, ABA, SA, JA and melatonin, was conducted using a dual-extraction method followed by HPLC-UV quantification. Fresh tissue (0.1 g) was homogenized in Bieleski's solution and extracted at -30°C for 4 hrs, with internal standards added to account for recovery losses. Following centrifugation, the resulting supernatant was purified via Sep-Pak C18 cartridges, vacuum-dried and reconstituted in acidified water (pH 2). Chromatographic separation was achieved on a Thermo-Ultimate 3000 HPLC system using a BDS Hypersil C18 column and a gradient mobile phase of acetonitrile and acidified Milli-Q water. Hormones were detected at 280 nm and quantified by comparing peak area ratios against specific calibration curves derived from the internal standards. Optimal treatments for growth potential and a control (agar without filter paper) were used to analyze the content and ratio of endogenous hormones.

Statistical processing: Each treatment was repeated 3 times with 15 culture vessels/treatment. All data after collection corresponding to each indicator are processed by Microsoft Excel 2016 and SPSS 20.0 software according to Duncan test with $p<0.05$[20].

RESULTS AND DISCUSSION

***In vitro* growth under a gelling agent and aerated conditions:** The results showed that the gelling agent and aerated conditions affected the rooting and growth of *Ananas* sp. plantlets after 8 weeks of culture Table 1 and Fig. 1(a-c). In particular, the aerated condition gave a higher *in vitro* rooting (100%) than the non-aerated condition on agar and gelrite

Fig. 1(a-c): *In vitro* growth performance of *Ananas* sp. plantlets after 8 weeks of culture under different gelling agents and aeration conditions, (a) Plantlets grown in culture vessels under semi-closed (aerated) conditions, (b) Corresponding plantlets after removal from vessels, showing variation in shoot proliferation and vigor and (c) Individual plantlets arranged to compare morphological traits (shoot length, leaf development and root formation) under four treatments: (1) Agar without filter paper, (2) Gelrite without filter paper, (3) Agar with filter paper and (4) Gelrite with filter paper (arranged from left to right in each row)

Table 1: *In vitro* growth of *Ananas* sp. plantlets under gelling agent and aerated condition after 8 weeks of culture

Gelling agent	Aerated condition	Rooting (%)	No. of roots	Root length (cm)	No. of leaves	Plantlet height (cm)	Fresh weight (mg)	Dry weight (mg)	Dry matter (%)	Leaf length (cm)	Leaf width (cm)	SPAD
Agar	Without filter	80^{b}	5.00^{c}	2.50^{b}	22.33^{a}	4.67^{c}	480.00^{d}	39.67^{c}	8.20^{b}	4.00^{b}	0.43^{b}	27.70^{c}
Gelrite	paper	-	-	-	21.00^{a}	3.73^{c}	1363.33^{b}	93.33^{b}	6.91^{b}	1.70^{c}	0.37^{b}	38.43^{b}
Agar	With filter	100^{a}	9.00^{b}	3.50^{b}	10.00^{b}	7.50^{b}	996.67^{c}	110.67^{b}	11.08^{a}	6.17^{b}	0.60^{ab}	48.77^{a}
Gelrite	paper	100^{a}	12.33^{a}	5.17^{a}	11.33^{b}	11.17^{a}	2170.00^{a}	257.33^{a}	11.88^{a}	9.43^{a}	0.77^{a}	52.07^{a}

*Different letters (a, b, ⋯) within column indicate significant differences ($p \leq 0.05$) between treatments according to Duncan's test-No recorded data

Table 2: Antioxidant enzyme activities of *Ananas* sp. plantlets under gelling agent and aerated condition after 8 weeks of culture

Gelling agent	Aerated condition	SOD (U/g)	CAT (U/g)	APX (U/g)	DPPH (µMTE/100 g)	Phenolic (mg GAE/100 g)
Agar	Without filter paper	184.57^{a}	367.94^{a}	0.44^{b}	51.17^{a}	56.16^{a}
Gelrite		189.88^{a}	378.37^{a}	0.46^{ab}	54.10^{a}	45.88^{b}
Agar	With filter paper	151.18^{b}	377.70^{a}	0.54^{a}	38.69^{b}	42.03^{c}
Gelrite		115.49^{c}	267.51^{b}	0.39^{b}	26.77^{c}	34.33^{d}

Values represent mean ± SD of three replicates. Different letters within each column indicate significant differences at $p \leq 0.05$. SOD: Superoxide dismutase, CAT: Catalase, APX: Ascorbate peroxidase, DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity and GAE: Gallic acid equivalents

substrates (80%) and the number of roots and root length were optimal in the agar treatment combined with aerated conditions (12.33 roots and 5.17 cm). In addition, growth indicators such as plantlet height (11.17 cm), fresh weight (2170 mg), dry weight (257.33 mg), dry matter (11.88%), leaf length (9.43 cm) and SPAD (52.07) obtained in gelrite and aerated condition treatments were also higher than those of plants in other treatments (Table 1). In contrast, in the non-aerated condition, the number of leaves (22.33 and 21.00 leaves) tended to be more numerous and the leaves were smaller (Table 1).

The experimental results demonstrate that the morphogenesis and physiological vigour of *Ananas* sp. plantlets are significantly modulated by the interplay between the physical state of the substrate and the gaseous microenvironment of the culture vessel. In addition, the achievement of a 100% rooting and optimal root growth (12.33 roots; 5.17 cm) in the aerated treatments suggests that oxygen availability is a primary limiting factor for rhizogenesis in *Ananas* sp. micropropagation. In hermetically sealed (non-aerated) systems, the depletion of O_2 and the concomitant accumulation of ethylene often act as potent inhibitors of root primordia initiation and elongation. Furthermore, the superior SPAD values (52.07) and dry matter content (11.88%) observed in the aerated and gelrite treatment indicate enhanced photosynthetic capacity and carbon sequestration. This suggests that passive or active gas exchange facilitates a transition from heterotrophic to photo-mixotrophic metabolism. This shift is critical; increased CO_2 availability within the vessel likely promotes Rubisco activity, leading to the observed increase in plantlet height (11.17 cm) and fresh weight (2170 mg).

Besides, the data indicate that while gelling agents provide structural support, their chemical composition and matric potential influence nutrient bioavailability. Gelrite is frequently utilized for its high transparency and its combination with aeration yielded superior vegetative growth. This suggests that the water-holding capacity and impurity profile of gelrite may provide a more stable osmotic environment for *Ananas* sp. when gas exchange is not a limiting factor. The lower biomass observed in without aerated treatments may be a result of subtle hyperhydricity, a common physiological disorder in solidified media that impairs leaf function and biomass accumulation[21].

A notable observation was the higher number of leaf (22.33) in non-aerated conditions, despite a reduction in individual leaf size and overall plantlet weight. This serves as a classic example of stress-induced morphogenic plasticity. Under conditions of high relative humidity and restricted gas exchange, plantlets may prioritize the initiation of new leaf primordia as a survival mechanism, though these tissues remain underdeveloped and lack the robust cuticle and mechanical strength found in aerated treatments. From a micropropagation perspective, the gelrite-aerated protocol is the most viable for commercial scaling. The significant increases in root length, biomass and SPAD values are reliable indicators of plantlet "hardness." Robust root systems and higher chlorophyll density are vital for minimizing transplant shock during the transition to *ex vitro* conditions, as they allow for immediate autotrophic competence and efficient water regulation upon transfer to soil.

Antioxidant enzyme activities: The antioxidant enzyme activities of *Ananas* sp. plantlets also changed and were recorded after 8 weeks of culture (Table 2). The results showed that without aerated conditions with agar and gelrite substrates, the indicators such as SOD (184.57 and 189.88 U/g), CAT (367.94 and 378.37 U/g), APX (0.44 and 0.46 U/g), DPPH (51.17 and 54.10 µMTE/100 g) and Phenolic (56.16 and 45.88 mg GAE/100 g) were all higher than those in aerated conditions (using filter paper) (Table 2). This showed that without aerated conditions, antioxidant enzyme activities of *Ananas* sp. plantlets increased compared to those in aerated conditions. Corresponding to the optimal growth in agar and aerated condition treatment, indices such as SOD (115.49 U/g), CAT (267.51 U/g), APX (0.39 U/g), DPPH (26.77 µMTE/100 g) and Phenolic (34.33 mg GAE/100 g) were the lowest in aerated condition with gelrite substrate (Table 2).

A critical finding is the significant elevation of antioxidant enzyme activities-SOD, CAT and APX-in non-aerated conditions. The SOD levels reached up to 189.88 U/g in non-aerated vessels, compared to the baseline of 115.49 U/g in the optimized aerated medium. This upregulation of the

Fig. 2(a-c): Abnormal phenomena of *Ananas* sp. plantlets under gelling agent and aerated conditions after 8 weeks of culture, (a) Plantlet with callus at the root, (b) Plantlet with yellowing of the leaves and (c) Yellowing of the leaf

Table 3: Abnormal phenomena of *Ananas* sp. plantlets under gelling agent and aerated condition after 8 weeks of culture

Gelling agent	Aerated condition	Yellowing of leaf (%)	Callus at the root (%)	Normal (%)
Agar	Without filter paper	80.00^{a}	20.00^{b}	-
Gelrite		51.10^{b}	42.22^{a}	6.67^{c}
Agar	With filter paper	20.00^{c}	48.89^{a}	31.11^{b}
Gelrite		2.23^{d}	6.67^{c}	91.10^{a}

*Different letters (a, b, ⋯) within column indicate significant differences ($p \leq 0.05$) between treatments according to Duncan's test-No recorded data

antioxidant defense system indicates the accumulation of reactive oxygen species (ROS). In restricted environments, high relative humidity and CO_2 depletion induce physiological hypoxia[22]. To mitigate oxidative damage, plantlets must divert metabolic energy toward the synthesis of protective enzymes and secondary metabolites, such as Phenolics (56.16 mg GAE/100 g) and antioxidants measured by DPPH (54.10 µMTE/100 g). This "growth-defense trade-off" explains why non-aerated plantlets produced more leaves (22.33) but remained smaller and less robust; the energy was redirected from structural growth to biochemical detoxification.

Abnormal phenomena: Abnormal phenomena are often recorded in micropropagation in many different species and this study was also recorded on *Ananas* sp. plantlets. The results showed that two phenomena, yellowing of the leaf and callus at the root, were recorded at different rates in culture conditions with media (agar and gelrite) and aerated conditions (with/without filter paper) Fig. 2(a-c). Aerated conditions with filter paper recorded lower yellowing of leaf and callus at the root than non-aerated conditions on both media types (Table 3). The highest percentage of abnormal phenomena recorded was 80% yellowing of leaf and 20% callus at the root in the agar treatment and without aerated condition and vice versa, the lowest was 2.23% yellowing of leaf and 6.67% callus at the root (91.10% normal plantlets) in the geltite treatment and with aerated condition (Table 3).

The occurrence of physiological abnormalities, specifically leaf yellowing and callus at the root, highlights the critical role of the medium in *Ananas* sp. micropropagation. Our results demonstrate that the physical state of the medium and the gas exchange capacity of the culture vessel are paramount in determining plantlet quality.

The significantly higher rates of leaf yellowing (80%) observed in non-aerated conditions suggest a build-up of ethylene and excessive humidity within the vessel. In closed systems, ethylene accumulation often leads to chlorophyll degradation and senescence. By introducing filter paper for aeration, these gases are vented and the internal relative humidity is slightly reduced. This result aligns with findings in other bromeliads, where improved gas exchange enhances photosynthetic enzyme activity and prevents the physiological "suffocation" of the plantlet. In addition, the superiority of Gelrite over Agar (achieving 91.10% normal plantlets when aerated) can be attributed to the chemical purity and the water potential of the medium. Gelrite is a highly purified gellan gum that often allows for better diffusion of nutrients compared to the more complex structure of traditional agar. However, the high incidence of abnormalities in non-aerated agar treatments suggests that agar may exacerbate poor drainage or oxygen deficiency at the root zone when ventilation is absent[23]. The formation of callus at the root base (up to 20% in agar/non-aerated treatments) is often a stress response to high levels of endogenous auxins or poor oxygen availability. The reduction of callus in aerated Gelrite treatments indicates that a more porous or better-oxygenated medium prevents the undifferentiated cell division that characterizes callusing, instead favouring the development of functional, vascularized root systems.

Table 4: Change volume medium of *Ananas* sp. plantlet vessel under gelling agent and aerated condition after 8 weeks of culture

		Weight of medium (g)		Percentage of medium (%)	
Gelling agent	Aerated condition	Initial	Remained	Used	Remained
Agar	Without filter paper	125.33[a]	97.00[b]	22.60[c]	77.40[b]
Gelrite		114.67[b]	106.00[a]	7.54[d]	92.46[a]
Agar	With filter paper	125.33[a]	12.33[d]	90.16[a]	9.84[d]
Gelrite		116.33[b]	86.33[c]	25.79[b]	74.21[c]

*Different letters (a, b, ⋯) within column indicate significant differences ($p \leq 0.05$) between treatments according to Duncan's test

Table 5: Endogenous hormone concentrations of *Ananas* sp. plantlets under gelling agent and aerated condition after 8 weeks of culture

		Content (ug/g fresh weight)							
Gelling agent	Aerated condition	GA3	IAA	ABA	Zeatin	Kinetin	2iP	Salicylic acid	Melatonin
Agar	Without filter paper	16.16±0.15	1.12±0.01	0.46±0.01	0.18±0.01	0.19±0.01	0.07±0.01	1.11±0.01	1.25±0.00
Gelrite	With filter paper	26.74±0.15	2.21±0.02	0.47±0.01	0.41±0.01	0.26±0.01	0.06±0.00	1.36±0.01	1.39±0.01

Values are expressed as mean±SD (n = 3). GA3: Gibberellic acid, IAA: Indole-3-acetic acid, ABA: Abscisic acid, 2iP: N6-(Δ2-isopentenyl) adenine and SA: Salicylic acid

Table 6: Ratio of endogenous hormone of *Annas* sp. plantlets under gelling agent and aerated condition after 8 weeks of culture

Gelling agent	Aerated condition	CKs (ug/g fresh weight)	IAA/CK	GA3/CK	IAA/ABA	CK/ABA	GA3/ ABA
Agar	Without filter paper	0.44±0.01	2.53±0.05	36.46±0.54	2.41±0.02	0.96±0.02	34.89±0.28
Gelrite	With filter paper	0.74±0.01	3.00±0.03	36.30±0.58	4.68±0.09	1.56±0.04	56.50±1.12

Values are expressed as mean±SD (n = 3). CKs: Cytokinins, IAA: Indole-3-acetic acid, ABA: Abscisic acid and GA3: Gibberellic acid

Change of medium during the period of culture: A notable result in this study that we recorded was the change in the volume of the medium after 8 weeks of culture (Table 4). The initial volume of the medium of the treatment containing agar was about 125 g/culture vessel and the medium without gelrite was about 115 g/culture vessel and there was a change when the explants were cultured under conditions of with/without filter paper after 8 weeks of culture. For the treatments using agar as a gelling agent, the remaining medium weight was 97 g (without filter paper) compared to 12.33 g (filter paper) equivalent to the amount of used medium of 22.60% and 90.16%. Meanwhile, for the experiments using gelrite as a gelling agent, the amount of remaining medium was higher for the experiment without filter paper (106 g) and the filter paper experiment (86.33 g) equivalent to the percentage of used medium of 7.54% and 25.97% (Table 4). This result shows that the condition without filter paper gives a higher possibility of medium loss than filter paper and agar also give a higher possibility of medium loss than gelrite. In the current medium where agar is used as an aerated substrate with filter paper, the rate of water evaporation of the medium is faster than that of gelrite; Therefore, the percentage of medium used and lost is more.

Content and ratio of endogenous hormones: The endogenous hormone concentrations of the explants obtained in the treatments with agar and without filter paper and gelrite with filter paper also showed differences in endogenous hormones such as GA3, IAA, KIN, SA and melatonin (Table 5); in which ABA, zeatin and 2iP did not show any difference (Table 5). Endogenous hormones such as GA3, IAA, KIN, SA and melatonin of the explants in the treatments with agar and without filter paper were higher than those of the explants in the treatments with gelrite with filter paper after 8 weeks of culture.

The interplay between AUX and CKs is a fundamental determinant of plant development, with their ratio governing cell differentiation and tissue distribution[24,25]. In plant biotechnology, the AUX/CKs ratio is a critical factor for optimizing regeneration; typically, elevated ratios stimulate cell division and root initiation, while lower ratios favour shoot organogenesis[19]. In the present study of *Ananas* sp., we observed a distinct hormonal gradient where the AUX/CKs ratio increased under agar and without filter paper as compared to with gelrite and filter paper treatment. In addition, when comparing the ratio of endogenous hormones of *Ananas* sp. plantlets under gelling agent and aerated conditions after 8 weeks of culture, there were also differences (Table 6). The gelrite treatment with filter paper recorded higher CKs (0.74 ug/g fresh weight), the IAA/CK (3.00), IAA/ABA (4.68), CK/ABA (1.56) and GA3/ABA (56.50) ratios than the agar and without filter paper treatments (0.44, 2.53, 2.41, 0.96 and 34.89; respectively). The results showed that there was no significant difference in GA3/CK between the two treatments (36.46 and 36.30). These results highlight how endogenous hormone profiles at specific internode positions dictate explant fate, offering a biological basis for selecting the most effective tissues for *in vitro* regeneration.

CONCLUSION

The integration of gelrite with aerated culture conditions significantly enhanced the micropropagation efficiency of *Ananas* sp., achieving 100% rooting, maximum plantlet height (11.17 cm) and fresh weight (2170 mg). Aeration reduced oxidative stress, as indicated by lower SOD (115.49 U/g) and CAT (267.51 U/g) activities and minimized abnormalities with only 2.23% leaf yellowing and 6.67% basal callus formation. This optimized system produced uniform, physiologically stable plantlets. Therefore, it offers a reliable protocol for large-scale propagation and successful field establishment.

SIGNIFICANCE STATEMENT

This study highlights that integrating aerated culture systems with Gelrite significantly improves pineapple micropropagation. Unlike traditional agar, this combination enhances nutrient uptake and biomass while maintaining hormonal homeostasis (auxins, gibberellins and melatonin). Proper gas exchange prevents oxidative stress, which otherwise causes leaf yellowing and enzyme. By reducing basal callus formation and enhancing growth uniformity, this optimized system provides a physiological foundation for the large-scale production of high-quality pineapple clones optimized for subsequent field acclimatization.

REFERENCES

1. Gennari, P., J. Rosero-Moncayo and F.N. Tubiello, 2019. The FAO contribution to monitoring SDGs for food and agriculture. Nat. Plants, 5: 1196-1197.
2. Lakho, M.A., M.A. Jatoi, N. Solangi, A.A. Abul-Soad, M.A. Qazi and G. Abdi, 2023. Optimizing *in vitro* nutrient and *ex vitro* soil mediums-driven responses for multiplication, rooting, and acclimatization of pineapple. Sci. Rep., Vol. 13. 10.1038/s41598-023-28359-9.
3. Francisco-Rodríguez, J.A., M. Chalchi-Martínez, H. López-Arjona, E. Pérez-Molphe-Balch, J.A. Morales-Serna and E. Villalobos-Amador, 2025. A pilot-scale micropropagation plant for two commercial varieties of pineapple *Ananas comosus* (L.) Merr. reveals shortcuts in a complex system through the recycling of fruit. Plant Cell Tissue Organ Cult., Vol. 160. 10.1007/s11240-024-02962-8.
4. Alavijeh, M.K. and A. Zarei 2025. Introducing an efficient protocol for micropropagation high quality 'MD_2' pineapple; novel insights into iron form effects on *in vitro* plantlet quality. Biocatal. Agric. Biotechnol., Vol. 68. 10.1016/j.bcab.2025.103720.
5. Nhut, D.T., H.T. Tung and E.C.T. Yeung, 2022. Plant Tissue Culture: New Techniques and Application in Horticultural Species of Tropical Region. 1st Edn., Springer, Singapore, ISBN: 978-981-16-6498-4, Pages: 397.
6. Hawanis, H.S.N., R.A. Ilyas, M.H. Ahmad, C.H. Xin and N.K.I. Mahadi *et al*., 2025. Recent developments in pineapple (*Ananas comosus* L.) based biocomposites and their potential industrial applications: A review. Int. J. Biol. Macromol., Vol. 328. 10.1016/j.ijbiomac.2025.147531.
7. Silue, O., O.K.S. Kouadio, K.H. Koffi, E. Zran, M. Toure and M.K. Kouassi, 2024. Efficient and rapid *in vitro* micropropagation of MD2 and H4 pineapple varieties. Aust. J. Crop Sci., 18: 591-597.
8. Kessel-Domini, A., D. Pérez-Brito, A. Guzmán-Antonio, F.A. Barredo-Pool and J.O. Mijangos-Cortés *et al*., 2022. Indirect somatic embryogenesis: An efficient and genetically reliable clonal propagation system for *Ananas comosus* L. Merr. Hybrid "MD2". Agriculture, Vol. 12. 10.3390/agriculture12050713.
9. Rosmaina, A. Mufadillah, S.I. Zam and Zulfahmi 2024. Low-cost of shoot propagation of pineapple (*Ananas comosus* l. Merr) cv queen using gandasil-D and AB-mix. IOP Conf. Ser.: Earth Environ. Sci., Vol. 1362. 10.1088/1755-1315/1362/1/012014.
10. He, F., Q. Zhang, G. Deng, G. Li and B. Yan *et al*., 2024. Research status and development trend of key technologies for pineapple harvesting equipment: A review. Agriculture, Vol. 14. 10.3390/agriculture14070975.
11. Shu, H., R. Zhan, M.A. Eissa, F. Vafadar and Z. Ding *et al*., 2024. Propagation of pineapple (*Ananas comosus* L.) embryogenic cell suspension is regulated by LEAFY COTYLEDON1 gene AcoLEC1-1. Sci. Hortic., Vol. 332. 10.1016/j.scienta.2024.113173.
12. de Souza Marchi, M., T.S. Ornellas, Y. Fritshe, M.P. Guerra and V.M. Stefenon, 2022. Morphometric parameters and photosynthetic performance of *in vitro* propagated pineapple. Revista Facultad Agronomía, Vol. 121. 10.24215/16699513e098.
13. Cacaï, G.H.T., B.A.M. Ahokpossi, S.S. Houédjissin, J.A. Houngue, B.T. Badou and C. Ahanhanzo, 2023. Plant regeneration through indirect organogenesis in two cultivars of pineapple (*Ananas comosus* L.). Open J. Appl. Sci., 13: 1039-1058.
14. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant., 15: 473-497.
15. Marklund, S. and G. Marklund, 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem., 47: 469-474.

16. Góth, L., 1991. A simple method for determination of serum catalase activity and revision of reference range. Clin. Chim. Acta, 196: 143-151.
17. Nakano, Y. and K. Asada, 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in Spinach chloroplasts. Plant Cell Physiol., 22: 867-880.
18. Desmarchelier, C., M.J.N. Bermudez, J. Coussio, G. Ciccia and A. Boveris, 1997. Antioxidant and prooxidant activities in aqueous extracts of Argentine plants. Int. J. Pharmacogn., 35: 116-120.
19. Wu, G., X. Wei, X. Wang and Y. Wei, 2021. Changes in biochemistry and histochemical characteristics during somatic embryogenesis in *Ormosia henryi* Prain. Plant Cell Tissue Organ Cult., 144: 505-517.
20. Duncan, D.B., 1955. Multiple range and multiple *F* tests. Biometrics, 11: 1-42.
21. Ayenew, B., A. Mengesha, T. Tadesse and E. GebreMariam, 2012. *Ensete ventricosum* (Welw.) Cheesman: A cheap and alternative gelling agent for pineapple (*Ananas comosus* var. smooth cayenne) *in vitro* propagation. J. Microbiol. Biotechnol. Food Sci., 2: 640-652.
22. Huang, C. and C. Chen, 2005. Physical properties of culture vessels for plant tissue culture. Biosyst. Eng., 91: 501-511.
23. Bairu, M.W. and M.E. Kane, 2011. Physiological and developmental problems encountered by *in vitro* cultured plants. Plant Growth Regul., 63: 101-103.
24. Li, G., C. Zhang, G. Zhang, W. Fu and B. Feng *et al*., 2020. Abscisic acid negatively modulates heat tolerance in rolled leaf rice by increasing leaf temperature and regulating energy homeostasis. Rice, Vol. 13. 10.1186/s12284-020-00379-3.
25. Saeedpour, A., S.J. Godehkahriz, T. Lohrasebi, K. Esfahani, A.H. Salmanian and K. Razavi, 2021. The effect of endogenous hormones, total antioxidant and total phenol changes on regeneration of barley cultivars. Iran. J. Biotechnol., 19: 30-39.