

Effect of Photoperiod and Ascorbic Acid Concentration on Degree of Browning, Shoot Regeneration and Survival of Plantain (*Musa ssp.*) Cultivars Growing *in vitro*

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ABSTRACT

Plantain, an important crop in Sub-Saharan Africa, faces challenges such as low fertility and susceptibility to stresses. Micropropagation techniques offer disease-free planting materials, but success depends on various factors. This study explored the effects of ascorbic acid (AA) concentration and photoperiod on browning, shoot regeneration, and survival of explants in *in vitro* culture. Three plantain cultivars and four AA concentrations were examined, with explants subjected to darkness and a light/dark cycle. Browning, shoots/buds per explant, shoot height, and survival were measured. Results showed that darkness reduced browning compared to light, particularly in the French Clair and Batard cultivars. Preservation of AA in darkness prevented browning by inhibiting its decay and reducing enzyme activity related to phenolic compounds. Photoperiod did not significantly affect shoot characteristics or survival, while AA concentration had a significant impact on shoot regeneration. The Batard cultivar exhibited highest regeneration with 50mg/L AA, while higher concentrations had a negative effect. Therefore, incubating explants in darkness effectively reduces browning during *in vitro* culture. Optimal AA concentration (50mg/L) enhances shoot regeneration, while higher concentrations should be avoided. These findings have implications for improving micropropagation techniques, addressing plantain production challenges, and meeting the demand for high-quality planting materials in Sub-Saharan Africa.

Keywords: Plantain, micropropagation, browning, ascorbic acid, shoot regeneration.

INTRODUCTION

Plantain (*Musa* spp.) is an important food crop in Sub-Sahara Africa, providing approximately 25% of the food energy needed for people in the region [1]. It is considered a valuable source of nutrients and serves as a cash crop for rural farmers in Cameroon, contributing to both food security and economic sustainability [2]. However, plantain production faces challenges such as low reproductive fertility, slow propagation rate, and susceptibility to biotic and abiotic stresses [3].

Micropropagation techniques have been developed to overcome these challenges by providing healthy and disease-free planting materials [4]. However, the success of micropropagation is influenced by various factors, including explant type, nutrients, plant growth regulators, additives, temperature, light intensity, and duration [5].

During *in vitro* culture, plantain explants are prone to browning caused by the oxidation of phenolic compounds [6]. Browning reactions can lead to high mortality rates and hinder successful shoot regeneration [7]. Phenolic compounds, such as catechin, chlorogenic acid, and tannins, are abundant in plant tissues and are produced as a defence mechanism against injury [8, 9]. The oxidation of these compounds results in tissue browning and the production of toxic quinones, which negatively affect explant survival and growth [10].

To mitigate browning and promote successful *in vitro* culture, various approaches have been employed, including the use of antioxidants like ascorbic acid and pre-treatment of explants with substances such as potassium citrate and citrate [7, 11]. Activated charcoal has also been

utilized to control browning by adsorbing inhibitory substances, although it can affect the balance of medium components and influence plant regeneration [12].

Light intensity and duration are critical environmental factors in tissue culture that influence shoot regeneration, growth, and metabolite biosynthesis [13, 14]. Understanding the impact of light on phenolic oxidation and the efficacy of plant growth regulators is crucial for successful in vitro culture [5, 15].

This study aims to investigate the effect of ascorbic acid concentration and photoperiod on browning, shoot regeneration, and contamination of plantain explants during in vitro culture. By understanding these factors, improvements can be made to micropropagation techniques, addressing the challenges associated with plantain production and ensuring a steady supply of high-quality planting materials in Sub-Saharan Africa.

MATERIALS AND METHODS

Study Location

The experiment took place at the JP Johnson Biotechnology Laboratory, located in the Institute of Agricultural Research for Development (IRAD) in Ekona, Cameroon (4°16'44" N and 9°17'50" E) in the South West Region [16, 17].

Explant Preparation

Sword suckers from three cultivars (Ebanga, Batard, and French Clair) were collected from the collection plot at the IRAD Ekona Station. The suckers were carefully trimmed to remove roots and debris and transported to the laboratory's screen-house on the same day. After washing under running tap water, the suckers were further trimmed to have approximately five leaves and a base measuring about 5cm x 5cm x 5cm, resulting in explants. These explants were washed again under running water and then surface sterilized with 70% ethanol [2, 18].

Sterilization

In the laboratory, the explants were trimmed to a size of 3cm x 3cm x 3cm and rinsed with distilled water. Surface disinfection was performed by briefly flashing the explants with 70% alcohol for 1 minute, followed by three rinses with sterile distilled water (SDW). Subsequently, the explants were soaked in a disinfectant solution (30% sodium hypochlorite (NaOCl) mixed with two drops of Tween 20) for 20 minutes, and then rinsed three times with SDW. In the laminar flow hood, the explants were further trimmed to a size of approximately 1.5cm x 1.5cm x 1.5cm before inoculating them on the culture media [19] (Figure 1). While in the laminar flow hood, the explants were submerged in 1L of the disinfectant for 10 minutes. They were then rinsed in three changes of SDW before initiation [6, 20].

Culture Conditions and Media

The explants were cultured in jars containing 40 mL of Murashige and Skoog (MS) Basal Medium [21] supplemented with 30 g/L sucrose, 4 mg/L 6-benzylaminopurine (BAP), 0.8 mg/L indole-3-acetic acid (IAA), 2 g/L Gelrite, and 1 g/L ceftriaxone. The MS medium and all other components, except ceftriaxone, were weighed into a 1L glass bottle containing 700 mL of distilled water on a magnetic stirrer. After adding all the components, the water was topped up to the 1L mark, and the content was preheated while stirring and brought to a boil. The bottle

and its contents were then placed in a steam sterilizer and autoclaved at 121°C for 15 minutes at 1.5 pounds per square inch. After autoclaving, the medium was taken to the laminar flow hood and allowed to cool to 45°C before aseptically adding ceftriaxone with a sterile syringe. The medium was swirled, and 40 mL of the medium was poured aseptically into sterile jars. The medium was then allowed to cool and solidify overnight in the laminar flow hood before use.

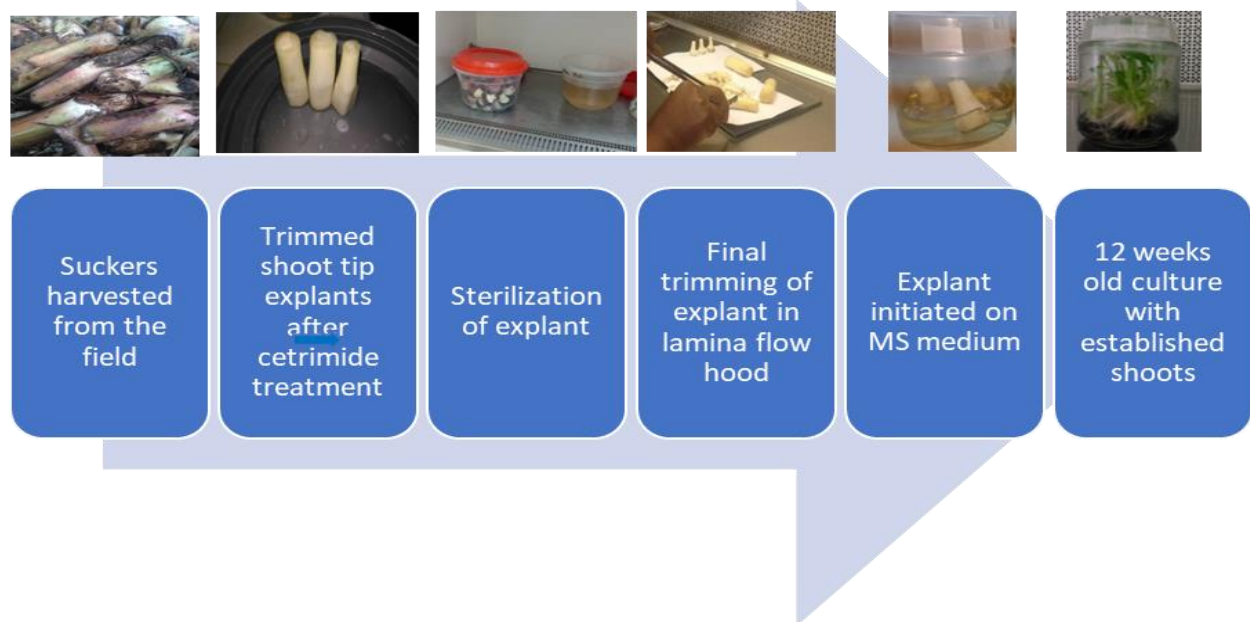


Figure 1: Steps in the establishment of in vitro culture of plantain

Before sterilization, ascorbic acid was added to the MS medium at four concentrations (0, 25, 50, 100 mg/L), resulting in four experimental treatments. Each treatment consisted of twenty culture vessels, with each vessel containing one explant. Each treatment was replicated three times. From each treatment, 10 culture vessels were incubated in 24 hours of darkness, while the other 10 vessels were subjected to a 16-hour light/8-hour darkness photoperiod for 12 weeks. All cultures were placed in a growth room maintained at $27 \pm 2^\circ\text{C}$. Lighting was provided by white cool fluorescent Philips lamps of 22 watts, placed 40 cm above the culture bottles. Each of the three plantain cultivars (Batard, Ebanga, and French Clair) was subjected to all ascorbic acid treatments and photoperiods.

Data Collection

Four weeks after initiation of culture, the degree of explant browning was evaluated using a scoring system ranging from 0 to 5, where 0 represented no browning and 5 represented intense browning [22]. The number of shoots and buds per explant was counted weekly, and the shoot height per plantlet was measured using a ruler after 12 weeks. The percentage of survived plantlets was determined by counting the total number of surviving plantlets and dividing it by the total number of plantlets, multiplied by 100 [23]. The percentage of survival was calculated using the following formula [23].

$$\% \text{ of survival} = (\text{Number of survived Plantlets}) / (\text{Total number of plantlets}) \times 100$$

Data Analysis

The data were analysed using a two-way general linear model analysis of variance (ANOVA) with a significance level set at $P = 0.05$, utilizing the Minitab statistical software package version 17. Treatment means were compared and separated using Tukey's method at a 5% probability level.

RESULTS AND DISCUSSION

The success of micropropagation depends on several factors, including the type of explant, culture conditions, and growth regulators used in the medium. In this study, we evaluated the effect of photoperiod and ascorbic acid concentration on the in vitro propagation of three plantain cultivars (Batard, Ebanga, and French Clair).

Effect of Photoperiod on Browning Degree and Bud Formation

The photoperiod had a significant effect on the degree of browning in the French Clair and Batard cultivars but did not have a significant effect on Ebanga (Table 1). Incubation in darkness resulted in significantly reduced browning compared to incubation in light (Table 1). This finding is consistent with the results obtained by other researchers [12]. It can be attributed to the rapid decay of ascorbic acid in the plant culture media, where light and pH accelerate this decay. Preserving ascorbic acid in darkness allows it to function more effectively as an antioxidant, preventing browning [10, 12]. Additionally, the activity of enzymes involved in the biosynthesis and oxidation of phenolic compounds is reduced in darkness [24].

The photoperiod did not have a significant effect on the number of buds formed in all the cultivars used in this study, although there was a tendency for more buds to develop when explants were incubated in light compared to darkness for the Ebanga and French Clair cultivars (Table 1).

Table 1: Mean ($\pm$ SD) degree of browning and number of buds produced by Batard, Ebanga and French Clair cultivars during light and darkness exposure

Photoperiod	Degree of Browning			No of buds		
	Batard	Ebanga	French Clair	Batard	Ebanga	French Clair
Light	4.04 $\pm$ 0.31 ^a	3.92 $\pm$ 0.55 ^a	4.20 $\pm$ 0.27 ^a	2.54 $\pm$ 1.05 ^a	3.34 $\pm$ 1.75 ^a	3.75 $\pm$ 2.77 ^a
Darkness	3.16 $\pm$ 0.56 ^b	3.84 $\pm$ 0.33 ^a	3.75 $\pm$ 0.31 ^b	3.46 $\pm$ 2.71 ^a	3.02 $\pm$ 2.00 ^a	2.21 $\pm$ 0.83 ^a

Values represent Means were separated using Tukey's HSD = 0.05.

Mean with similar letters within column indicate no significant differences among treatments

Effect of Photoperiod on Shoot Number and Length

The photoperiod had a significant influence on the number of shoots regenerated in all the cultivars used in this study. More shoots were developed under light conditions than in darkness (Table 2). This observation contrasts with the results obtained by other scientists, who showed that more shoots were produced when explants were incubated in darkness [12]. However, shoots grown in darkness exhibited better quality, with distinct elongation and ease of handling.

There were no significant differences in the average length of shoots between light and darkness conditions for all the cultivars in this study. However, the longest shoots were

observed when explants were incubated in darkness for both the French Clair and Ebanga cultivars (Table 2).

Table 2: (Mean ($\pm$ SD) number and length of shoots produced by Batard, Ebanga and French Clair cultivars during light and darkness exposure

Photoperiod	No of shoots			Length of shoots (cm)		
	Batard	Ebanga	French Clair	Batard	Ebanga	French Clair
Light	2.82 $\pm$ 0.34 ^a	2.32 $\pm$ 0.28 ^a	3.70 $\pm$ 0.15 ^a	2.67 $\pm$ 0.52 ^a	2.17 $\pm$ 0.95 ^a	2.19 $\pm$ 1.05 ^a
Darkness	2.32 $\pm$ 0.32 ^b	2.00 $\pm$ 0.27 ^b	3.21 $\pm$ 0.33 ^b	1.84 $\pm$ 1.64 ^a	2.60 $\pm$ 1.48 ^a	3.251 $\pm$ 1.49 ^a

Values represent Means were separated using Tukey's HSD = 0.05.

Mean with similar letters within column indicate no significant differences among treatments

Effect of Photoperiod on Percentage Survival of Plantain Explants

The survival percentage of explants under different photoperiods was evaluated as a parameter to assess efficacy. Overall, more than 85.00% of the plantlets survived the photoperiod phase, except for the Ebanga and French Clair cultivars, which recorded 82.50% under light and 80.00% under darkness, respectively (Figure 2). The results showed that the photoperiod did not have a significant effect on the survival percentage of the explants. This finding is consistent with previous studies on plantain micropropagation [25, 26]. It suggests that both darkness and light conditions can be used for the initiation and establishment of plantain cultures without significant differences in survival rates. Dehgahi and Joniyasa reported that the interaction of light rays with cellular molecules produces free radicals, which may form toxic substances contributing to cell destruction and plantlet death [27].

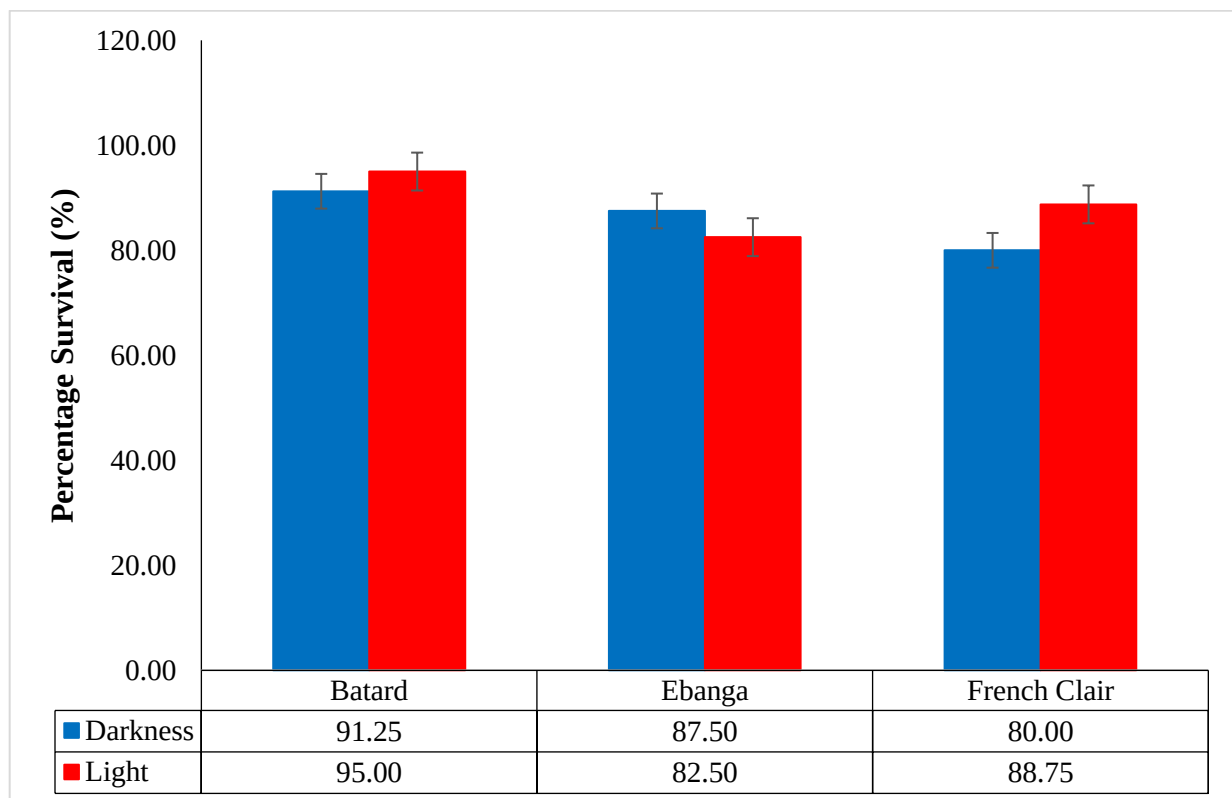


Figure 2: Effect of photoperiod on percentage survival on explant of plantain cultivars

Effect of Ascorbic Acid Concentration on Degree of Browning and Number of Buds

The concentration of ascorbic acid significantly affected the degree of browning in the Ebanga and Batard cultivars, but not in the French Clair cultivar (Table 3). For both the Batard and Ebanga cultivars, the least degree of browning was observed when explants were incubated in medium supplemented with 25 mg/L ascorbic acid (C1). In the case of French Clair, the least degree of browning was observed with 50 mg/L ascorbic acid (C2) (Table 3). The addition of ascorbic acid to the medium had a significant effect on the degree of browning in the explants. Ascorbic acid acts as a reducing agent and can scavenge oxygen radicals produced during tissue culture, thereby preventing oxidative browning [9, 18, 28-31]. The optimal concentration of ascorbic acid for reducing browning varied among the cultivars, with 25 mg/L being effective for Ebanga and Batard, and 50 mg/L for French Clair. These findings are consistent with previous reports indicating the role of ascorbic acid in controlling browning in tissue culture systems.

Table 3: Mean degree of browning and number of buds produced by Batard, Ebanga and French Clair cultivars when MS was substituted with different concentrations of Ascorbic acid

Conc. Levels	Degree of Browning			No of buds		
	Batard	Ebanga	French Clair	Batard	Ebanga	French Clair
C0	3.13±0.74 ^b	3.52±0.68 ^{ab}	4.04±0.69 ^a	2.60±0.55 ^a	2.75±1.17 ^a	2.53±0.93 ^a
C1	2.60±0.40 ^c	3.41±0.51 ^b	3.81±0.51 ^a	2.63±1.45 ^a	3.74±2.44 ^a	2.08±0.54 ^a
C2	3.42±0.43 ^{ab}	3.80±0.49 ^{ab}	3.62±0.45 ^a	2.70±0.95 ^a	3.77±3.17 ^a	3.33±2.91 ^a
C3	3.82±0.30 ^a	4.03±0.49 ^a	4.01±0.52 ^a	4.00±2.39 ^a	2.50±1.18 ^a	3.14±1.48 ^a

Values represent Means were separated using Tukey's HSD = 0.05.

Mean with similar letters within column indicate no significant differences among treatments

C0, 0mg/L; C1, 25mg/L; C2, 50mg/L; and C3, 100mg/L

The concentration of ascorbic acid did not significantly affect the number of buds formed in the three cultivars. However, the highest number of buds was generally observed at higher concentrations of ascorbic acid (50 mg/L for Ebanga and French Clair, and 100 mg/L for Batard). This suggests that higher concentrations of ascorbic acid may have a positive influence on bud formation in plantain micropropagation.

Effect of Ascorbic Acid Concentration on Number and Length of Shoots

Except for French Clair, the concentration of ascorbic acid did not significantly affect the number of shoots formed in both the Ebanga and Batard cultivars (Table 4). For both the French Clair and Batard cultivars, the highest number of shoots was observed when explants were incubated in medium supplemented with 100 mg/L ascorbic acid (C3). In all three cultivars, the lowest number of shoots was observed when explants were incubated in ascorbic acid-free medium (C0). These findings are consistent with previous studies demonstrating that maximum shoot regeneration is achieved when MS medium is supplemented with ascorbic acid [9, 31, 32]. However, it had earlier been reported in their studies that maximum shoot regeneration occurred in ascorbic acid-free medium [10, 12].

Table 4: Mean number and length of shoots produced by Batard, Ebanga and French Clair cultivars when MS was substituted with different concentrations of Ascorbic acid

Conc. Levels	No of shoots			Length of shoots (cm)		
	Batard	Ebanga	French Clair	Batard	Ebanga	French Clair
C0	1.67±2.44 ^a	1.19±1.19 ^a	1.83 ±1.94 ^b	0.73 ±1.07 ^c	1.68 ±1.65 ^a	1.79± 1.44 ^b
C1	2.13 ±1.80 ^a	2.73±2.15 ^a	3.03±1.96 ^{ab}	2.37±1.40 ^{ab}	2.84±1.29 ^a	2.11±0.98 ^b
C2	2.67±2.62 ^a	2.38 ±2.31 ^a	3.56±2.77 ^{ab}	1.87±1.40 ^b	1.97 ±1.46 ^a	2.12 ±1.38 ^b
C3	2.83±1.59 ^a	2.63 ±1.81 ^a	4.67± 1.23 ^a	3.17±0.53 ^a	2.02± 1.51 ^a	3.41 ±1.07 ^a

Values represent Means were separated using Tukey's HSD = 0.05.

Mean with similar letters within column indicate no significant differences among treatments

C0, 0mg/L; C1, 25mg/L; C2, 50mg/L; and C3, 100mg/L

There was no significant difference in the length of shoots produced under different ascorbic acid concentrations for the Ebanga cultivar. The shortest shoots were produced in the ascorbic acid-free medium for all three cultivars (Table 4). Batard and French Clair cultivars produced the longest shoots when incubated in medium supplemented with 100 mg/L ascorbic acid (C3), as shown in Table 4. This finding aligns with previous studies demonstrating the positive effect of ascorbic acid on shoot elongation [32].

Effect of Ascorbic Acid Concentration on Survival Percentage of Plantain Explants

Survival percentage is commonly used to assess the effectiveness of substances in in vitro cultivation. In this study, different concentrations of ascorbic acid (0, 25, 50, and 100 mg/L) were used in the media for all the plantain cultivars. More than 80% of the plantlets survived in all the treatments, except for the French Clair explant, which showed a lower survival percentage of 77.50% when exposed to 25 mg/L of ascorbic acid (Figure 3). The reduced survival percentage in French Clair at 25 mg/L of ascorbic acid can be attributed to tissue damage on the explant, leading to oxidation of polyphenolic compounds. This oxidative reaction formed a barrier around the tissues, impeding nutrient uptake and hindering growth [7, 33]. The survival percentages of the explants at 25 mg/L, 50 mg/L, and 100 mg/L concentrations of ascorbic acid were not significantly different ($P = 0.05$) from the survival of plantlets in the 0 mg/L concentration of ascorbic acid. Conversely, some studies have reported that the survival percentage increases with higher levels of ascorbic acid, which promotes a favourable germination response [34, 35].

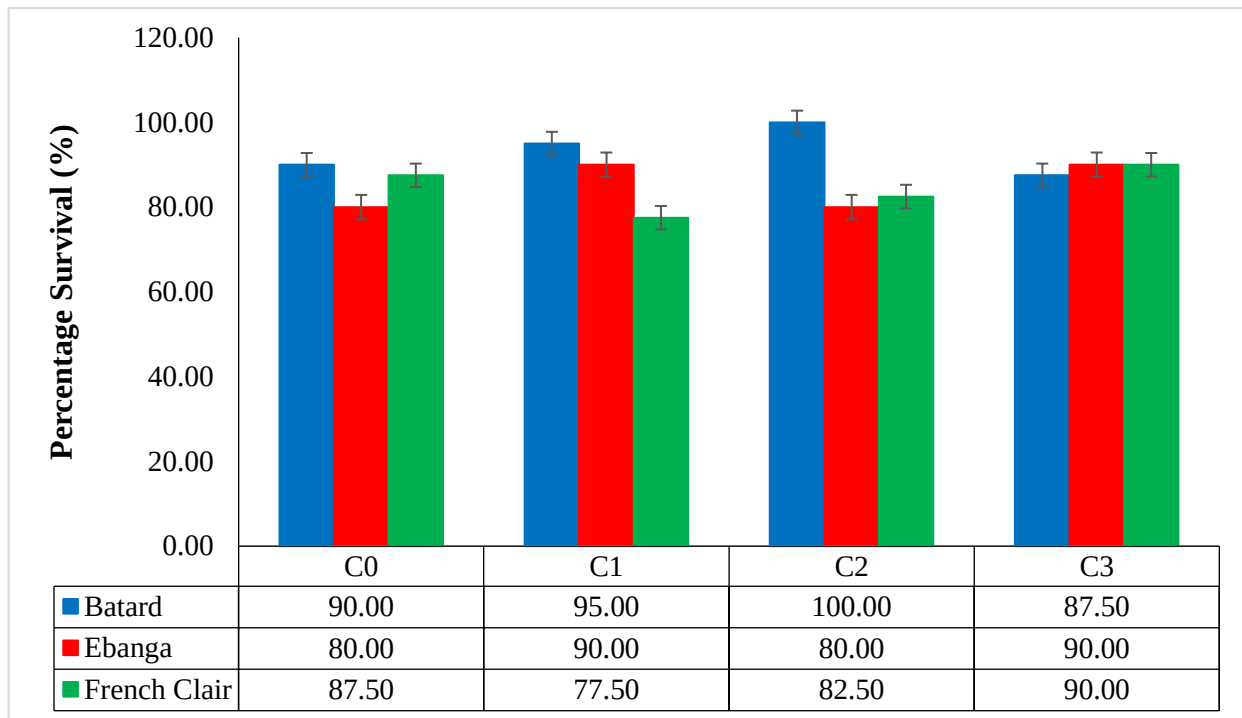


Figure 3: Effect of ascorbic acid on percentage survival on explant of plantain cultivars

CONCLUSION

Micropropagation is a promising technique for plantain production, but addressing the issue of explant browning is crucial for successful *in vitro* culture. This study investigated the influence of ascorbic acid and photoperiod on browning, shoot regeneration, and survival of plantain explants. The results revealed that incubation in darkness reduced browning compared to light, likely due to the preservation of ascorbic acid and reduced enzyme activity. It was also observed that more shoots were produced in light than in darkness for all cultivars. However, the concentration of ascorbic acid did not significantly affect browning or shoot growth. Survival rates varied among cultivars, with Batard showing the highest survival rate. These findings contribute to our understanding of the factors influencing successful micropropagation and provide valuable insights for optimizing plantain tissue culture protocols. Further research is needed to explore additional approaches to minimize browning and improve the efficiency of plantain micropropagation techniques.

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