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Hot Water-Treated Rhizome-Raised Banana Seedlings Revealed Improved *in vitro* Establishment

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ABSTRACT

Purpose: *Conventional banana suckers were unable to meet the demand of Ethiopian farmers since bananas produced 5 to 20 suckers per plant annually. Alternatively, in vitro propagation method provides adequate, uniform, and healthy planting materials. However, phenolic oxidation and high endogenous microbial contamination, which kill plants or retard their growth during the establishment phase, are the major challenges for this approach.*

Research Method: *Banana explants taken directly from the field and those grown in a greenhouse after hot water treatments were compared for their rate of phenolic oxidation, endogenous microbial contamination, and length of time to sprout using five released banana varieties.*

Findings: *Except for the Poyo variety from the field, which demonstrates a moderate survival and sprouting percentage, the use of banana explants from greenhouse significantly increased the survival and sprouting rate by 60% and 265%, respectively; and correspondingly decreased the phenolic oxidation and length of sprouting weeks by 76% and 22%, compared to the explants directly from the field. Banana explants sourced from the greenhouse could significantly reduce endogenous fungal and bacterial contamination and completely avoid endogenous yeast contamination.*

Research Limitations: *There was limited availability of facilities to study the detailed biology of contaminants and the chemical nature of phenolic oxidants.*

Originality/ Value: *The research output will help to increase the efficiency of banana in vitro establishment rate.*

Keywords: *Banana, Contamination, Phenolic oxidation, Sprouting, Survival, Tissue culture.*

INTRODUCTION

The edible banana (*Musa* spp.), both dessert and plantain, is an important food crop grown for home consumption and income in the tropical and subtropical countries of the world (Heslop-Harrison and Schwarzacher, 2007). Banana is the fourth most important food crop, next to rice, wheat, and maize. The annual world production of banana has increased by 70% in the past 40 years (Menon, 2016), which strengthens banana as one of the most important food-security fruit crops. Banana is also one of Ethiopia's most important economic fruit crops (Alemu, 2017; Amen and Desalegn, 2018; Benyam and Abatneh, 2019). Among all the fruits grown in Ethiopia, banana alone covered 56.79%, and its production increased from 539 thousand tons from an area of 66.8 thousand ha to 898.4 thousand tons from an area of 96 thousand ha in the years 2019/20 and 2020/21, respectively (CSA, 2021). The presence of an increasing demand for the fruit crops in the community, and becoming the major income source for the producers were the driving forces for its expansion

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Both the cooking type and dessert banana are grown in Ethiopia, although the dessert type is the most common. Bananas are mainly propagated asexually via suckers, which normally remain true-to-type. Furthermore, suckers are cheap, easy to obtain, transport, and manage, and little care is required for plants in the field. However, the scarcity of healthy planting materials, low multiplication rate, and risk of variety mix-up have restricted their use (Eckstein and Robinson, 1995; Bohra *et al.*, 2013). In vitro micropropagation of banana has been a promising tool used to overcome the limitations of asexual propagation via suckers (Bower and Fraser, 1982; Swamy *et al.*, 1983; Cronauer and Krikorian, 1984; Hwang *et al.*, 1984; Wong, 1986). In Ethiopia, Asmare *et al.* (2012) developed micropropagation protocols for three dessert banana varieties (Dwarf Cavendish, Giant Cavendish, and Poyo). In addition, protocol optimization was conducted for three other dessert banana varieties (Williams-1, Grand Nain, and Butuzua) (Surafel, 2015; MARC, 2016).

The establishment phase is the most challenging during in vitro propagation of bananas, where a large number of cultures are discarded due to systemic pathogen contamination or death from phenolic oxidation (Drew *et al.*, 1989; Hamill *et al.*, 1993; Ko *et al.*, 2009; Safwat *et al.*, 2015). The causes of lethal necrosis could be from the secretion of phenolic compounds, which may be due to calcium deficiency (Alderson *et al.*, 1987; Abousalim and Mantell, 1994), extended gaps between subculturing (Alderson *et al.*, 1987), imbalance of the NO_3^-/NH_4^+ ratio in the medium, altered cytokinin concentrations (Vieitez *et al.*, 1989; Amo-Marco and Lledo, 1996), or low medium pH (De Block, 1990). The necrosis in initiated banana plants may result in shoot tip dieback, total death, or retarded growth, typically correlated with the severity of necrotic tissue.

The addition of ascorbic acid, citric acid, and activated charcoal (Safwat *et al.*, 2015), increased calcium chloride concentration (Martin *et al.*, 2007), frequent subculturing (Hamill *et al.*, 1993), immersing explants in 0.125% potassium citrate and/or 0.1–0.5 mg/mL potassium citrate, and/or incorporating 1.2 g/mL ascorbic acid (Permadi *et al.*, 2023) were reported to reduce the intensity of phenolic oxidation in micro propagation of banana. *In vitro* Endogenous contamination, that comes from explants, is also a major challenge during the establishment phase (Habiba *et al.*, 2002). Plant tissue culture medium usually contains nutritionally important minerals, sugar and organic components (Leifert *et al.*, 1994). The presence of sugar increases the rapid establishment of the saprophytic microbes, as it supplements a continuous source of energy. Even though these saprophytic microbes are a challenge in in vitro conditions, they have minimal importance in the field (Leifert and Waites, 1994), as all heterotrophic microorganisms need adequate amount of mono- and oligosaccharides and organic acids for growth. When saprophytic microbes grow on such medium, they compete with the plant; and produce phytotoxic chemicals that suppress the growth of the plant which result in the death of the plant (Leifert and Waites, 1994).

The use of small suckers (Dangi *et al.*, 2009; Waman *et al.*, 2015), establishment of the culture during the dry season, and a lower sucrose concentration medium (Waman *et al.*, 2015); addition of antibiotic, nanoparticles such as zinc, zinc oxide, or silver in the media (Permadi *et al.*, 2023), surface sterilization using 30% hydrogen peroxide (Hassen *et al.*, 2022) were reported to decreased both endogenous contamination and lethal necrosis with a better establishment percentage. Furthermore, hot-water treatment prior to hypochlorite sterilization was reported to reduce the contamination rate significantly (Moutia and Dookun, 1999; Roldan and Icalla, 2003). In this paper, the influence of two sources of banana explants, which were taken from field grown banana, and from seedlings raised after hot water treatment of rhizomes was studied for in vitro establishment to evaluate their difference in the reduction of phenolic oxidation, endogenous contamination, and, ultimately, improving the establishment rate.

MATERIALS AND METHODS

The experiment was conducted from June to October, 2021 at Melkassa Agricultural Research Center, Plant Tissue Culture and Molecular Laboratory. Five released dessert banana varieties in Ethiopia, namely Dwarf Cavendish, Giant Cavendish, Poyo, Williams-1, and Grand Nain, were used for the experiment. Twenty to thirty sword suckers of 70–120 cm height were collected from each variety from the fruit orchard of the Melkassa Agricultural Research Center (Figure 01a). The suckers were thoroughly washed with tap water, and the shoot tips used for the in vitro initiation were carefully excised without damaging the visible buds available around the rhizome of the suckers, which is designated as "explant from field". The remaining rhizomes were divided into sections based on visible buds, treated with 50 °C hot-water for 10 minutes, and planted in the greenhouse. Every agronomic practice was done accordingly. When the grown plant reached a size of 30–40 cm (Figure 01b), they became ready to be used for in vitro establishment, after carefully excising

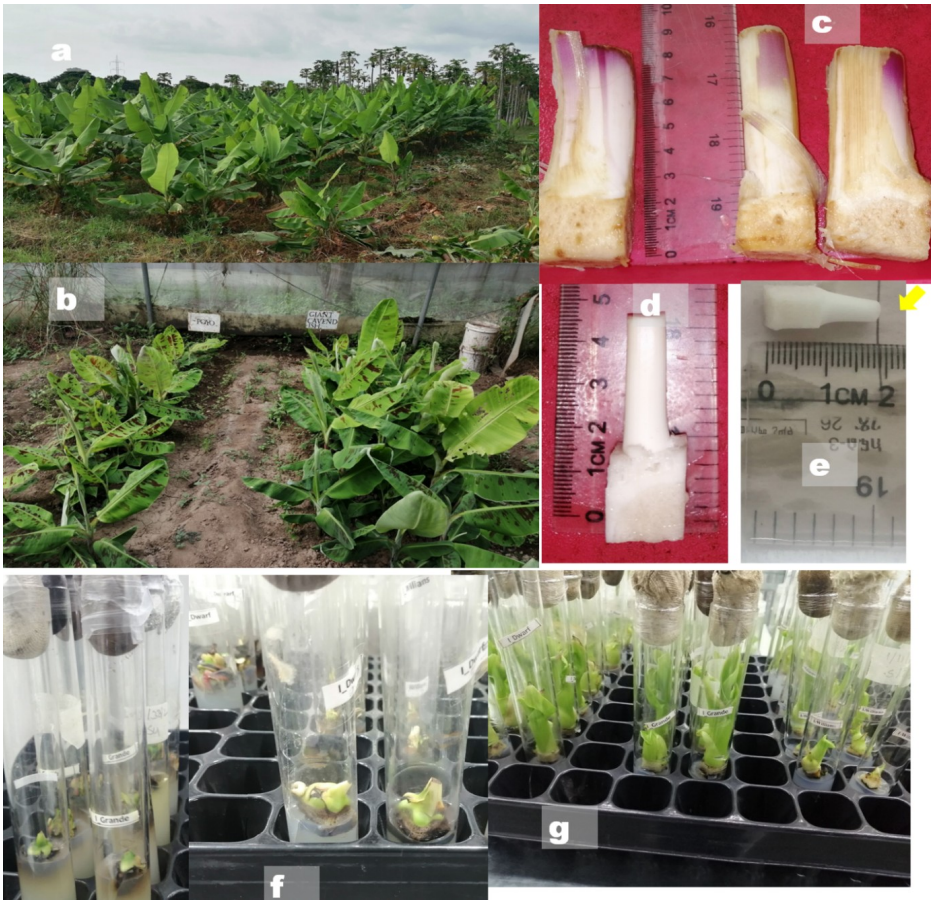

Figure 1: Banana explants used for *in vitro* establishment: a. Explant from field; b. explant from greenhouse; c. the size of explants used for the first step surface sterilization; d. the size of explant used for the second step surface sterilization; e. the size of explant cultured on the initiation media; f. banana explant initiated from field (three weeks after establishment); and g. banana explant from the greenhouse (five weeks after establishment).

the shoot tip from the rhizome; this source was referred as an "explant from greenhouse".

The collected suckers, either from the field or greenhouse, were thoroughly washed in tap water containing detergent with a soft brush. The shoot tips were trimmed to a size of 2–3 cm at the bottom, 5–8 cm at the top, and 3–4 cm in width (Figure 01c), then thoroughly washed with tap water containing detergent using a soft brush, followed by surface sterilized with a 1% hypochlorite solution and droplets of Tween-20 for 10 minutes. After rinsing three times with sterilized distilled water, the shoot tips were further excised to 1.0–1.5 cm at the basal, 3.0–4.0 cm at the top, and 1.0–1.5 cm in width (Figure 01d); and separately placed in Falcon™ 50 mL conical centrifuge tubes.

The final surface sterilization was conducted under aseptic conditions inside the laminar flow hood cabinet in the transfer room. Similarly, the shoot tips were sterilized with 1% hypochlorite solution and droplets of Tween-20 for 10 minutes, followed by three-time rinse with sterilized distilled water. The final size of the shoot tip used for the initiation phase was excised to 0.5–1.0 cm at the basal, 1.0–1.5 cm at the top and approximately 1 cm of width (Figure 01e), and cultured on sterilized medium consists of MS salt (Murashige and Skoog, 1962), 1 mg L^{-1} thiamine–HCl, 100 mg L^{-1} myo-inositol, 30 g L^{-1} of sucrose supplemented with growth hormone of 3.0 mg L^{-1} BAP for Dwarf Cavendish, Giant Cavendish, and Grand Nain varieties; and 2.0 mg L^{-1} BAP for Poyo and Williams-1 varieties (Asmare *et al.*, 2012; Surafel, 2015; MARC, 2016). The pH of the medium was adjusted to 5.7–5.8 with KOH or HCl and solidified with 7 g of agar per liter. Ten mL of medium was dispensed into a 15–20 cm long test tube of 55–70 mL volume and stem-sterilized for 20 min at 121 °C at 105 kPa. Cultured explants were grown in a controlled growth room with a temperature of 24–27 °C and a photoperiod of 16/8 h day/night under a white light-emitting diode of approximately 1000 lux.

The experiment was conducted using a completely randomized design (CRD) with two factors: namely source of explant and variety, with 10 replications. Data

Table 1: Analysis of variance of the two sources of explants for survival, sprout and sprouting weeks

Source of variation	Survival percentage		Sprout percentage		Sprouting time in weeks	
	Df	F value	Df	F value	Df	F value
Total						
Plant source	1	31.56***	1	111.80***	1	133.90***
Residuals	193		193		193	
Total						
Plant source	9	7.85***	9	18.83***	9	25.24***
Variety						
Residuals	185		185		185	
Dwarf Cavendish						
Plant source	1	28.00***	1	37.33***	1	43.59***
Residuals	28		28		28	
Williams1						
Plant source	1	20.01***	1	119.97***	1	119.90***
Residuals	44		44		44	
Poyo						
Plant source	1	1.36 ns	1	3.54*	1	13.78***
Residuals	34		34		34	
Grand Nain						
Plant source	1	3.87 ns	1	24.00**	1	29.31***
Residuals	43		43		43	
Giant Cavendish						
Plant source	1	0.52 ns	1	20.16**	1	41.55***
Residuals	36		36		36	

Note: ns, ***, ** are non-significance, significant at *0.001*, *0.001*, and *0.01*, respectively.

related to the survival of the explants and the sprouting of explants was recorded weekly; additionally, the intensity of phenolic oxidation was assessed weekly as 0, 1, 2, and 3 (representing no, mild, medium, and high phenolic oxidation, respectively). Before being subcultured to multiplication media, the explants were evaluated for ten weeks in a row. The multiplication medium consisted of 0.4 mg L^{-1} IAA in combination with 3.0 mg L^{-1} BAP and 4.0 mg L^{-1} BAP for Dwarf Cavendish and Giant Cavendish, respectively. For Poyo a combination of 0.2 mg L^{-1} IAA and 3.0 mg L^{-1} BAP was used. For Grand Nain and Williams-1, a single hormone at 4.0 mg L^{-1} BAP was used (Asmare et al., 2012; Surafel, 2015; MARC, 2016). The explants were cultured by splitting the shoots into 2–4 parts according to their size. After 4 weeks, each culture was evaluated for its multiplication rate.

The homogeneity of the collected data of each variable across each variety was confirmed by using Bartlett's homogeneity test in R using 'onewaytests' package (Dag *et al.*, 2018; R Core Team, 2021). As the replications of each treatment were not equal general liner model (GLM) were used to compare the statistical difference between the treatments. Moreover, GLM was also the most appropriate method to analyze the response data. The data were analyzed using R-software (Vallejo *et al.*, 2010; R Core Team, 2021).

RESULTS AND DISCUSSION

The GLM statistical analysis result indicated the presence of an overall significant difference for total survival, sprouts and length of time to sprout. However, significant differences at $p < 0.05$ between the two sources of explants, were observed for Dwarf Cavendish and Williams-1 for survival percentage; for all varieties except Poyo for the sprout percentage; and, for all varieties sprouting time (Table 01).

The total average survival percentage for the explant directly taken from the field was 58.7%, whereas for the explant taken from a greenhouse-grown plant was 94.2%. After 10 weeks, 23.8% of the explants taken from the field were sprouted; on the contrary, 87.0% of the explants taken from greenhouse-grown plants were sprouted (Table 02). When the length of time to sprout was compared, average of 5.45 and 4.28 weeks were obtained for the explants taken from the field and the greenhouse-grown plants, respectively. Overall, improvements in survival and sprout percentages and reductions in phenol oxidation and time taken to sprout (Table 02) were observed for explants sourced from the greenhouse-grown plants. In this study, the highest death rate was recorded for the explants taken from

Table 2: Average survival, and sprout percentages, and phenol of the two sources of explants

Source	Parameter	Dwarf Cavendish	Williams-1	Poyo	Grand Nain	Giant Cavendish	Total mean	Improvement++
Explant from field	Survival percentage	25.00	43.33	75.00	62.96	84.00	58.73	
	Sprout percentage	20.00	3.33	62.50	22.22	16.00	23.81	
	Average Phenol*	2.00	2.53	1.71	0.88	0.48	1.31	
	Sprouting time in weeks+	5.00	7.00	4.40	6.33	7.75	5.45	
Explant from greenhouse	Survival percentage	100.00	100.00	91.67	83.89	92.31	94.20	**60.4%**
	Sprout percentage	100.00	87.50	91.67	83.33	76.92	87.69	**265.3%**
	Average Phenol*	0.20	0.19	0.25	0.75	0.25	0.33	**-76.9%**
	Sprouting time in weeks+	4.00	4.14	3.09	5.07	4.00	4.28	**-21.5%**

Note: * Average phenol: the scale of phenolic oxidation recorded only for survived explant at 10th and 6th weeks for explants taken directly from field and explants taken from preconditioned seedling respectively; + Sprouting time in weeks: the length of weeks taken to sprout for survived explants (the data were calculated for average weeks of sprouted explants); ++ Improvement = ((total mean from greenhouse – total mean from field) / total mean from field) * 100.

the field, and the presence of a significant difference at $p<0.001$ was observed among the five varieties for total survival, sprouting percentage, and length of sprouting time (Table 03). From the five varieties, the least survival percentage of 50% was recorded for the Dwarf Cavendish variety in two weeks after initiation, of which 20% were eliminated due to bacterial contamination and the rest 30% were due to phenolic oxidation (Figure 02a and Table 04). After 10 weeks, only 25% of the explants survived, and later on in the process of multiplication, 25% of the cultures died due to phenolic oxidation (Table 04). Conversely, the highest survival percentage was exhibited for Giant Cavendish, for which 84% of the cultures survived after 10 weeks; however, only 16% of the survived explants sprouted in 10 weeks (Table 02). The highest death rate was observed for all varieties on the second week after initiation (Figure 02a).

The rate of lethal necrosis was reported to vary based on the varietal difference, in vitro growth stage, and the concentration of calcium chloride (Martin *et al.*, 2007). Whereas the modification in the strength of calcium chloride (Martin *et al.*, 2007), addition of compounds, such as polyvinylpyrrolidone, activated charcoal, or ascorbic acid (Ko *et al.*, 2009) were reported to improve phenolic oxidation. In our study, preconditioned explants, i.e., hot-water treated rhizome at 50 ºC for 10 min and then grown in greenhouses to use as explant sources, not only significantly reduced lethal phenolic oxidation, but also significantly reduced the endogenous contamination. Moreover, surface sterilization at 50 ºC in sugarcane was also reported to reduce contamination by 25 to 75 percent in Moutia and Dookun (1999).

Leifert and Waites (1994) reported that suckers with diameter of 10-12 cm exhibited less phenolic oxidation and maximum proliferation compared with smaller suckers. In our study, the diameter of the suckers taken from the greenhouse was much smaller than those of taken from the field (Figure 03), and they demonstrated less intensity of phenolic oxidation with a maximum rate of proliferation within a short period of time than the field source. According to Roy *et al.* (2010); and Waman *et al.* (2015), brown tissue formed as a result of phenolic oxidation either delays or hinders their establishment and elongation.

Hamill*et al.* (1993) reported that the size of suckers

Table 3: Analysis of variance between varieties across the two sources of explants

Source of Explant	Source of Variation	Df	F value	Pr(>F)
Explant from field	Total Survival – Variety	4	6.304	0.000121***
	Total Survival – Residuals	121		
	Total Sprout – Variety	4	8.554	4.1e-06***
	Total Sprout – Residuals	121		
	Sprouting weeks length – Variety	4	11.55	5.62e-08***
	Sprouting weeks length – Residuals	121		
Explant from greenhouse	Total Survival – Variety	4	0.666	0.618
	Total Survival – Residuals	64		
	Total Sprout – Variety	4	0.753	0.56
	Total Sprout – Residuals	64		
	Sprouting weeks length – Variety	4	3.15	0.0199*
	Sprouting weeks length – Residuals	64		

Note:* significant at 0.05, *** highly significant at 0.001.

has direct association with reduction of endogenous contamination, as bigger suckers are always prone to contamination. The size of suckers from greenhouse were smaller than from the field, in addition to their size, hot-water treatment of rhizomes before planting may also contributed for the reduction, as briefly stated in the materials and methods part. Previous studies revealed that hot-water treatment on banana rhizomes at 60–70 °C for 1 minute only improved the maximum sprouting of plants from rhizomes (Saraswathi *et al.*, 2024), and treatment of rhizomes at 53–55 °C for 20 minutes significantly reduced the infestation of nematodes (Speijer *et al.*, 2001). The study revealed that the percentage of systemic bacterial and fungal contamination was significantly reduced for the explants taken from greenhouse grown plants as compared with those taken directly from the field (Table 4 and 5).

In agreement with the results of the present study smaller suckers (less than 7 cm) were reported to be useful to minimize the rate of fungal and bacterial contamination (Waman *et al.*, 2015). In the present study, the explants taken from greenhouse were small in size (Figure 03) and had the least percentage of contamination compared to explants taken from the field. Although explants taken during the drier season were reported to reduce the rate of fungal and bacterial contamination significantly compared to the explants taken during the wet season (Waman *et al.*, 2015), in our study, the explants taken from the field were collected at the end of the dry season in Ethiopia (June 2nd to June 6th, 2021), on which a higher rate of contamination was observed compared to explant sources taken from preconditioned greenhouse-grown plants (Table 5).

The highest sprouting performances of 58% and 62.5% were observed at the end of 5 and 10 weeks of establishment, respectively, for explants taken directly from the field for the Poyo variety (Figure 02c). The least sprouting performance was exhibited for Williams-1, for which only 3.3% of the explants were sprouted after 10 weeks. For the remaining three varieties, the sprouting performance was very poor, with a range of 16–22% (Figure 02c). This delayed sprouting may be related to the report of Saraswathi *et al.* (2024), which demonstrated

Table 4: Weekly progression of survival and contamination (percentage) from field explants (Part 1)

Explant Source	Variety	Status	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8	Wk9	Wk10
Field	Dwarf Cavendish	Survival	90.0	50.0	45.0	45.0	40.0	35.0	30.0	30.0	25.0	25.0
		Bacteria	5.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
		Fungus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Yeast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Phenol	5.0	30.0	35.0	35.0	40.0	45.0	50.0	50.0	55.0	55.0
Field	Williams-1	Survival	93.3	83.3	83.3	83.3	83.3	83.3	83.3	70.0	50.0	43.3
		Bacteria	6.7	10.0	10.0	10.0	10.0	10.0	10.0	20.0	33.3	33.3
		Fungus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	3.3
		Yeast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Phenol	0.0	6.7	6.7	6.7	6.7	6.7	6.7	10.0	13.3	20.0
Field	Grand Nain	Survival	70.4	66.7	66.7	66.7	63.0	63.0	63.0	63.0	63.0	63.0
		Bacteria	14.8	18.5	18.5	18.5	22.2	22.2	22.2	22.2	22.2	22.2
		Fungus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Yeast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Phenol	14.8	14.8	14.8	14.8	14.8	14.8	14.8	14.8	14.8	14.8
Field	Poyo	Survival	100.0	87.5	87.5	79.2	79.2	79.2	79.2	79.2	75.0	75.0
		Bacteria	0.0	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2
		Fungus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Yeast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Phenol	0.0	8.3	8.3	16.7	16.7	16.7	16.7	16.7	20.8	20.8
Field	Giant Cavendish	Survival	100.0	84.0	84.0	84.0	84.0	84.0	84.0	84.0	84.0	84.0
		Bacteria	0.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
		Fungus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Yeast	0.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
		Phenol	0.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0

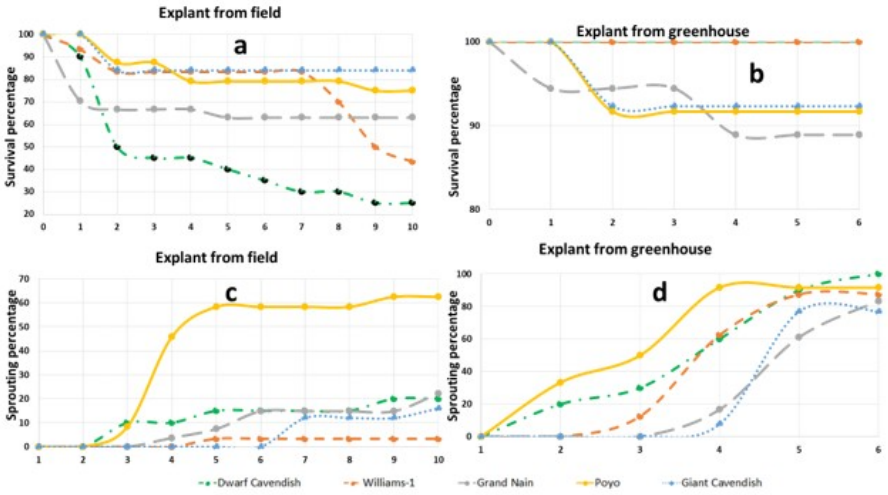

Figure 2: Weekly survival and sprouting difference between explants from field and greenhouse a. and c. explants from field; b. and d. explants from greenhouse.

Table 5: Weekly progression of survival and contamination (percentage) from greenhouse explants (Part 2)

Explant Source	Variety	Status	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8	Wk9	Wk10
Greenhouse	Dwarf Cavendish	Survival	100	100	100	100	100	100	–	–	–	–
		Bacteria	0	0	0	0	0	0	–	–	–	–
		Fungus	0	0	0	0	0	0	–	–	–	–
		Yeast	0	0	0	0	0	0	–	–	–	–
		Phenol	0	0	0	0	0	0	–	–	–	–
Greenhouse	Williams-1	Survival	100	100	100	100	100	100	–	–	–	–
		Bacteria	0	0	0	0	0	0	–	–	–	–
		Fungus	0	0	0	0	0	0	–	–	–	–
		Yeast	0	0	0	0	0	0	–	–	–	–
		Phenol	0	0	0	0	0	0	–	–	–	–
Greenhouse	Grand Nain	Survival	94.4	94.4	94.4	88.9	88.9	88.9	–	–	–	–
		Bacteria	0	0	0	5.56	5.56	5.56	–	–	–	–
		Fungus	5.56	5.56	5.56	5.56	5.56	5.56	–	–	–	–
		Yeast	0	0	0	0	0	0	–	–	–	–
		Phenol	0	0	0	0	0	0	–	–	–	–
Greenhouse	Poyo	Survival	100	91.7	91.7	91.7	91.7	91.7	–	–	–	–
		Bacteria	0	8.33	8.33	8.33	8.33	8.33	–	–	–	–
		Fungus	0	0	0	0	0	0	–	–	–	–
		Yeast	0	0	0	0	0	0	–	–	–	–
		Phenol	0	0	0	0	0	0	–	–	–	–
Greenhouse	Giant Cavendish	Survival	100	92.3	92.3	92.3	92.3	92.3	–	–	–	–
		Bacteria	0	0	0	0	0	0	–	–	–	–
		Fungus	0	0	0	0	0	0	–	–	–	–
		Yeast	0	0	0	0	0	0	–	–	–	–
		Phenol	0	7.69	7.69	7.69	7.69	7.69	–	–	–	–

Figure 3: The diameter of sucker used for initiation: a. explant source directly taken from field; b. explant source taken from greenhouse preconditioned seedlings

Table 6: Information collected during the first subculture (S1) and the second subculture (S2) of multiplication stage for the two sources of explants

Explant Source	Records	Dwarf Cavendish	Williams-1	Grand Nain	Poyo	Giant Cavendish
Explant from field	Number of Jars to S1	5	14	17	17	21
	Total Number of Explants	10	29	34	38	42
	Jars discarded due to bacterial contamination	0	4	1	5	2
	Jars with mild bacterial contamination but without inhibiting growth	0	8	0	2	0
	Clean culture jars / Explant	5/10	2/4	16/32	12/24	19/38
	Clean culture transferred to S2 (Jars / Explant)	10/33	5/18	34/118	26/93	26/92
	Multiplication rate	3.3	4.5	3.7	3.9	2.4
	Establishment (sprout: survival ratio)	0.80	0.08	0.35	0.83	0.19
Explant from greenhouse	Number of Jars to S1	10	16	14	11	12
	Total Number of Explants	12	32	28	22	24
	Jars discarded due to bacterial contamination	0	1	2	5	0
	Jars with mild bacterial contamination but without inhibiting growth	0	0	0	0	0
	Clean culture jars / Explant	10/12	15/30	12/24	6/12	12/24
	Clean culture transferred to S2 (Jars / Explant)	20/56	25/99	20/67	11/31	21/68
	Multiplication rate	4.7	3.3	2.8	2.6	2.8
	Establishment (sprout: survival ratio)	1.00	0.88	0.94	1.00	0.83

maximum growth axillary buds from hot-water treated rhizomes compared to untreated rhizomes during the precondition stage.

The cause for the death of explants for Giant Cavendish and Grand Nain was endogenous bacterial contamination, whereas for Poyo, Williams-1, and Dwarf Cavendish, phenolic oxidation was the main cause for the mortality of the explants (Table 5). Moreover, 8% of the explants for the Giant Cavendish variety died due to lethal phenolic oxidation (Table 04). The contribution of explant death due to endogenous fungal and yeast contamination was very small; only 5–7% mortality of explants from Williams-1 and Grand Nain varieties were recoded.

When greenhouse-grown plants were used as explant source; the total survival percentage, sprouting percentage, length of sprouting time, and intensity of phenolic oxidation were all improved significantly. The best improvement was observed in the percentage of sprouting seedlings; the rate of explants sprouted from greenhouse-grown plants was 3.65 times higher than that of explants taken directly from the field (Table 02). The intensity of phenolic oxidation that caused lethal death decreased to zero for all the varieties except for Giant Cavendish, for which 7.69% of the explants died due to phenolic oxidation (Table 4 and 5).

Explants obtained directly from the field were survived for ten weeks, but without sprouting, and then transferred to multiplication media for multiple shoot regeneration. The presence of low or high sprout or survival ratio could not guarantee for better regeneration rate in the next multiplication stage. For instance, Williams-1 had 3.3% of sprouting; however, it exhibited the highest shoot regeneration rate (4.5); in contrast, Giant Cavendish from the greenhouse had 76.9% of sprouting, but, exhibited the least multiplication rate (2.4) (Table 02 and Table 06). Therefore, sprouting and elongation might not

be necessary for the best multiplication rate in the subsequent stages.

According to Waman *et al.* (2015), the size of the sucker affects the response of shoot elongation due to the formation of callusing and a swollen base at the base of the explant. Additionally, they suggested that small sucker-derived explants were more responsive than larger suckers and explained that even if callusing and a swollen base were observed in both the dry and rainy seasons, those explants initiated in the rainy season exhibited 54.94% shoot elongation. In contrast to Waman *et al.* (2015), the response of elongation appears to correlate with varietal difference rather than sucker size and season of initiation. In our study Poyo variety responded better in both sources of explants, whereas Giant Cavendish showed less response in both sources of explants, irrespective of sucker size and season of initiation (Figure 02).

Explants from the field of Williams-1 variety's showed higher levels of endogenous bacterial contamination in the succeeding multiplication stage than did the other varieties. Out of the 14 subcultured cultures, 4 were eliminated because of bacterial contamination, 8 showed mild bacterial contaminations, and only 2 showed clean culture (Table 06). Cultures with mild bacterial contamination did not exhibit any discernible delay of plant growth.

CONCLUSION

Phenolic oxidation and endogenous contamination are the major challenges for the establishment of banana in vitro culture. In our study, explants taken directly from the field showed up to 40% mortality, 23% of which was due to lethal phenolic oxidation. On the contrary, explants taken from greenhouse grown plants obtained from hot water-treated rhizomes significantly improved the survival rate by 60%, and reduced lethal phenolic oxidation to 2%. Such improvement at the initial stage of establishment could be either due to the use of hot-water treatment of rhizomes that could potentially kill all in vitro contaminants of field grown suckers or the small size of the explants taken from the greenhouse and their age during the establishment stage. However, to determine the exact causes that lead to a better establishment rate, they require comprehensive and thorough investigation.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the author(s).

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