Disinfection of *Guadua angustifolia* Kunth explants for *in vitro* propagation

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ABSTRACT

Objective: To evaluate the effect of different disinfectants and concentrations on *Guadua angustifolia* explants collected during rainy and dry seasons for *in vitro* propagation, and to determine their impact on shoot emergence and survival.

Design/Methodology/Approach: Nodal segments were collected and subjected to six disinfection treatments combining 3% NaClO, the fungicide azoxystrobin (1.5 g L^{-1}), and antibiotics (gentamicin, streptomycin, kanamycin, 5 ml) in Murashige and Skoog (MS) and Yasuda culture media supplemented with Gamborg vitamins, adjusted to pH 5.0, solidified with phytagel, and sterilized at 15 PSI for 20 minutes.

Results: The Yasuda medium presented lower contamination rates across treatments and improved explant survival during the dry season. Disinfection with fungicide alone resulted in 20 surviving explants, while the combination of antibiotics, fungicide, and 3% NaClO resulted in 19 surviving explants. No statistical differences were found between culture media regarding survival. Disinfection and oxidation were reduced when combining azoxystrobin, 3% NaClO, and fungicide, although survival improved in both treatments. Explants collected during the dry season exhibited lower contamination, higher survival, and greater shoot formation.

Limitations/Implications: Genetic variability among parent plants may influence explant response, potentially affecting propagation success and in vitro culture outcomes.

Findings/Conclusions: Explant survival improved with the combined use of azoxystrobin and 3% NaClO, as well as with azoxystrobin alone. Collection during the dry season reduced contamination and enhanced survival and shoot development, making it a more suitable period for explant procurement.

Keywords: *Guadua angustifolia* Kunth, *in vitro* culture, explant disinfection.

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INTRODUCTION

Bamboo is a fast-growing forest resource (Leão *et al.*, 2020) that has become a sustainable alternative with economic, social, and cultural relevance (Aguirre-Cadena *et al.*, 2018). It has a wide range of uses, including housing construction, charcoal production, paper and cellulose manufacturing, and applications in the pharmaceutical and food sectors, among others (Leão *et al.*, 2020).

In Mexico, the highest diversity of woody bamboo species is found in Chiapas, Veracruz, and Oaxaca, which together harbor 70% of endemic species (Aguirre-Cadena *et al.*, 2018). *Guadua angustifolia* Kunth, native to Venezuela, Colombia, and Ecuador, has been introduced into Mexico and other American countries (Jiménez *et al.*, 2006). It is considered an efficient carbon sink, and more recently, tar oil has been extracted from it with reported antibiotic and antioxidant properties (Nadha *et al.*, 2012). In Mexico, its demand has increased as part of crop diversification strategies to improve farmers' income in marginalized areas, such as the northeastern region of Puebla (Aguirre-Cadena *et al.*, 2018). However, propagation by seed is difficult due to its irregular flowering patterns (Koshy & Gopakumar, 2005; Leão *et al.*, 2020). Vegetative propagation through cuttings is also practiced, but expansion is limited by the availability of propagules (Koshy & Gopakumar, 2005), requires intensive labor (Nadha *et al.*, 2012), and shows low survival rates due to poor sprouting and root emergence (Lárraga Sánchez *et al.*, 2011). In this context, *in vitro* culture methods represent an alternative for the mass propagation of *G. angustifolia*, offering advantages such as reduced propagation times and the production of disease-free plants (Luna, 2002; Sánchez *et al.*, 2002). However, fungal and bacterial contamination in *in vitro* culture of woody species is a frequent problem. *G. angustifolia* is no exception, showing a high level of bacterial contamination in culture media (Nadha *et al.*, 2012), which results in significant material loss and increased production costs (Digonzelli *et al.*, 2005; Das & Pal, 2005). Moreover, seasonal environmental changes have been shown to influence explant performance (González *et al.*, 2005). For this reason, it is essential to determine the optimal season for explant collection in *G. angustifolia* Kunth that minimizes contamination, as well as to identify effective disinfection methods for successful *in vitro* establishment.

MATERIALS AND METHODS

Nodal segments with latent buds (explants) of *G. angustifolia* Kunth, one year old, were collected during the rainy season (May-November) and the dry season (January-April).

Disinfection in the field and laboratory

In the field, the fungicide azoxystrobin was applied three days prior to explant collection. Once collected, explants were disinfected in the laboratory with water and soap, and subsequently immersed for 10 minutes in an antioxidant solution containing citric acid (100 mg L^{-1}), ascorbic acid (150 mg L^{-1}), sucrose (30 g), and two drops of Tween 80.

Under aseptic conditions in a laminar flow hood, explants were dissected into 3 cm^2 segments and placed in Murashige and Skoog medium (Murashige & Skoog, 1962) (MS) and Yasuda medium (Yasuda *et al.*, 1985), both supplemented with Gamborg vitamins (Gamborg, 2002). Using these combinations, the disinfection treatments were established (Table 1).

Sowing in the growing media

The MS and Yasuda culture media supplemented with Gamborg vitamins, 30 g L^{-1} sucrose, 1.125 mg L^{-1} BAP, 8 g L^{-1} Phytagel®, adjusted to pH 5.0, plus 5 ml of the

Table 1. Disinfection treatments applied to the nodal segments of *G. angustifolia* Kunt.

Season	Culture medium	Disinfection of explants
Rains	MS	NaClO 3%
		NaClO 3% + azoxystrobin
		Gentamicin, Streptomycin, and Kanamycin + NaClO 3%
		Gentamicin, Streptomycin, and Kanamycin + azoxystrobin and NaClO 3%
		Gentamicin, Streptomycin, and Kanamycin
		azoxystrobin
	Yasuda	NaClO 3%
		NaClO 3% + azoxystrobin
		Gentamicin, Streptomycin, and Kanamycin +NaClO 3%
		Gentamicin, Streptomycin, and Kanamycin + azoxystrobin and NaClO 3%
		Gentamicin, Streptomycin, and Kanamycin
		azoxystrobin
Dry	MS	NaClO 3%
		NaClO 3% + azoxystrobin
		Gentamicin, Streptomycin, and Kanamycin + NaClO 3%
		Gentamicin, Streptomycin, and Kanamycin + azoxystrobin and NaClO 3%
		Gentamicin, Streptomycin, and Kanamycin
		azoxystrobin
	Yasuda	NaClO 3%
		NaClO 3% + azoxystrobin
		Gentamicin, Streptomycin, and Kanamycin + NaClO 3%
		Gentamicin, Streptomycin, and Kanamycin + azoxystrobin and NaClO 3%
		Gentamicin, Streptomycin, and Kanamycin
		azoxystrobin

bactericides gentamicin, streptomycin, and kanamycin, and indole-3-acetic acid (IAA) as a growth regulator at 2 mg L^{-1}, were sterilized at 15 PSI and 120 °C for 20 minutes.

Environmental incubation conditions

The explants were placed in test tubes (150 mm × 20 mm) containing 10 ml of sterilized culture medium. They were then transferred to an incubation chamber at 26±1 °C, 60% relative humidity, with a light intensity of 45 μE m^{-2}, under a photoperiod of 16 hours light and 8 hours dark.

Evaluation for response variables

Seventy-two hours after inoculation, the presence of fungi and bacteria was evaluated as indicators of explant contamination. Oxidation percentage was also visually assessed by the degree of tissue darkening (0, 25, 50, 75, 100%). After four weeks, survival was evaluated by assigning a value of 0 (dead) or 1 (alive) to each explant. In addition, the number of shoots emerging from new explant tissue was recorded. All variables were monitored every eight days following explant inoculation.

Data analysis

The data were analyzed using a completely randomized design with a factorial arrangement and 20 replications.

Data were processed in Excel to obtain simple statistics such as means and percentages. Values were plotted with Sigma Plot software (version 10.0, Jandel Scientific) ± standard error.

RESULTS AND DISCUSSION

Contamination percentage

On the third day after explant inoculation, contamination by bacteria and fungi was observed in both culture media. Contamination was identified by turbidity and a whitish appearance in the medium, as well as white and gray spores around the nodal segment.

Explants collected during the dry season showed lower contamination levels in both media when treated with azoxystrobin at 1.5 g L^{-1} (30%) in MS, and when supplemented with gentamicin, streptomycin, and kanamycin (26%) in Yasuda. However, explants placed in MS medium treated with antibiotics (gentamicin, streptomycin, and kanamycin), combined with 3% NaClO and azoxystrobin, exhibited the highest contamination level, reaching 93% (Figure 1).

In this regard, Nadha *et al.* (2012) reported that the use of kanamycin and streptomycin sulfate were the most effective antibiotics against bacteria, specifically *Pantoea agglomerans* and *Pantoea ananatis*. They also noted that kanamycin exhibited lower phytotoxic effects in the *in vitro* culture of this species.

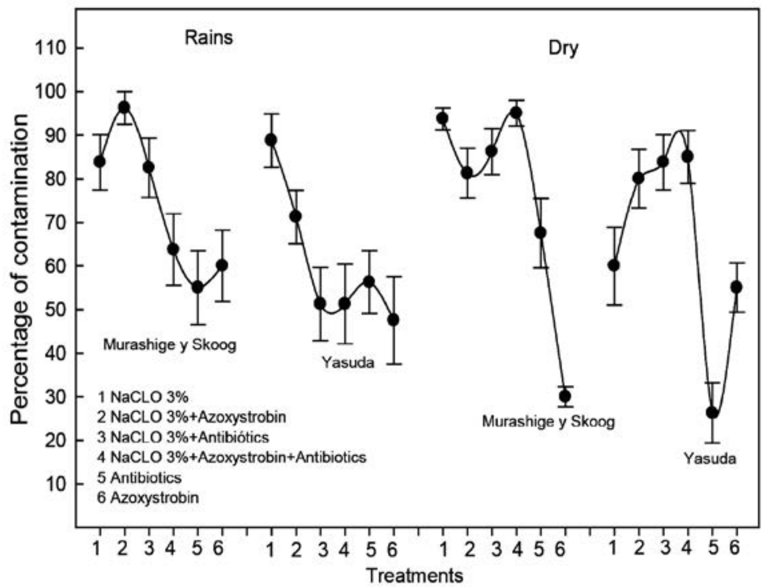

Figure 1. Contamination dynamics in nodal segments of *G. angustifolia* Kunth explants under the interaction of two culture media and two collection seasons. Values represent means of 20 replications ± standard error.

Explants obtained during the rainy season and cultured in MS medium showed the highest contamination level (95%) with the treatment of 3% NaClO plus azoxystrobin, while the lowest level (58%) was recorded under the antibiotic treatment.

In Yasuda medium, the highest contamination occurred with 3% NaClO alone (90%), whereas the lowest was observed with azoxystrobin (48%). For explants collected in the dry season and cultured in MS medium, the application of fungicide was the most efficient treatment to prevent contamination. Conversely, for explants collected during the rainy season and established in Yasuda medium supplemented with Gamborg vitamins, the addition of azoxystrobin (1.5 g L^{-1}) was the most effective treatment to control contamination levels.

Other authors have tested different approaches to reduce explant contamination. Jiménez *et al.* (2006) proposed using 10% NaClO for 10 minutes as an alternative for disinfecting nodal explants. Borges-García *et al.* (2004) recommended lowering NaClO concentration to 2% for 5 minutes, followed by a second treatment after 24 hours, achieving favorable results in the *in vitro* establishment of *G. angustifolia* explants. Meanwhile, Ramírez-Correa *et al.* (2014) reported that the most effective treatment for disinfection and sprouting in this species was 2% NaClO for 15 minutes. Overall, explant contamination varied according to the concentration and type of disinfectant used on *G. angustifolia* nodal segments, with harvest season exerting a decisive influence rainy season explants showing the highest contamination rates. This trend has also been observed in similar studies. Gielis & Oprins (2002) reported reduced microbial contamination during low rainfall periods in the *in vitro* establishment of bamboos. Similarly, Pérez-Alonso *et al.* (2015) found that, in the *in vitro* establishment of *Aloe vera* L. explants, dry-season collections combined with 2% NaClO yielded the highest number of contamination-free explants. In their study, microbial contamination rates ranged from 6.6% to 33.3% in the dry season, while in the rainy season contamination varied between 65% and 100%. Likewise, Ahmadpoor *et al.* (2022), working with *Melia azedarach* L., reported lower contamination levels when explants were treated with 2% NaClO (without pH adjustment) for 12 minutes, in combination with benomyl (2 g L^{-1}) for 2 hours and sterilization with 7% H_2O_2 for 10 minutes.

Percentage of oxidation

Explants collected during the dry season and cultured in MS medium exhibited oxidation levels between 51% and 58% across treatments, with the lowest value obtained using the combination of 3% NaClO plus azoxystrobin. In Yasuda medium, similar oxidation levels were observed among treatments with 3% NaClO, averaging 52%. The lowest oxidation levels of the entire study occurred when antibiotics were added to Yasuda medium, with an average of 45%, and further decreased to 33% when azoxystrobin was included. These results suggest that the combined use of antimicrobial agents and fungicides such as azoxystrobin enhances explant viability *in vitro*.

The reduction in oxidation can be explained by the lower presence of endogenous microorganisms, which typically induce the synthesis of phenolic compounds responsible for tissue browning (Ahmadpoor *et al.*, 2022; Nadha *et al.*, 2012). The accumulation of such compounds has been identified as one of the main limiting factors in the *in vitro* propagation of woody species (Lambardi *et al.*, 2006). Furthermore, the effectiveness of Yasuda medium may be linked to its balanced mineral composition, which promotes physiological stability in plant tissues and reduces oxidative responses to disinfection-induced stress (Yasuda *et al.*, 1985). These findings are consistent with research conducted on other woody species (Espinosa-Leal *et al.*, 2018).

For explants collected during the rainy season and cultured in MS medium, the lowest oxidation level was observed with azoxystrobin alone (51%), while the highest (60%) occurred with 3% NaClO plus azoxystrobin. This difference suggests that the exclusive use of azoxystrobin may exert less phytotoxicity and act more specifically against fungal contaminants, thereby preserving tissue integrity. Under conditions of high precipitation, such as during the rainy season, explant sensitivity increases due to heightened metabolic activity, making them more susceptible to oxidation (Espinosa-Leal *et al.*, 2018). In the case of explants collected during the rainy season and cultured in Yasuda medium, the lowest oxidation was obtained with 3% NaClO plus azoxystrobin, whereas the highest occurred with 3% NaClO plus antibiotics (Figure 2). This effect may be attributed to the nutritional composition of Yasuda medium, which has proven more favorable for woody species by reducing susceptibility to oxidation in the presence of certain disinfectants (Yasuda *et al.*, 1985). Moreover, several studies have reported that antibiotics may cause secondary effects on uncontaminated tissues, inducing necrosis or oxidation through the alteration of key cellular functions (Ahmadpoor *et al.*, 2022; Nadha *et al.*, 2012).

Comparatively, studies conducted on *G. angustifolia* K. also show that combining antibiotics with NaClO can be counterproductive when no active bacterial infection is present, and instead recommend alternatives such as the use of PPM™ a mixture of isothiazolone compounds (*e.g.*, methyl-isothiazolinone) in aqueous solution designed to

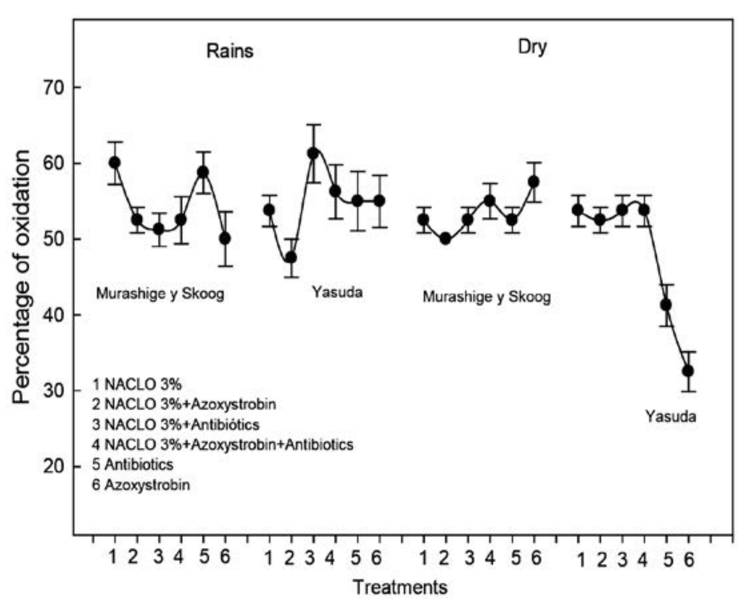

Figure 2. Oxidation dynamics in nodal segments of *G. angustifolia* Kunth under the interaction of two culture media and two different explant collection seasons. Values represent means ± standard error, with 20 replications per treatment.

inhibit microbial growth without affecting plant tissues. This broad-spectrum commercial preservative has been shown to reduce contamination without increasing oxidation levels (Rodríguez & Rojas, 2020). Similarly, in species such as *Melia azedarach* and *Bambusa vulgaris*, azoxystrobin use has been associated with significant reductions in phenolic oxidation and explant necrosis (Ahmadpoor *et al.*, 2022; Singh *et al.*, 2015). These findings confirm that no single disinfection protocol is universally effective across species or environmental conditions. The choice of disinfectants must take into account the collection season, explant physiology, culture medium, and the predominant microorganisms, prioritizing treatments that minimize oxidation while ensuring effective decontamination. These results suggest that both culture medium selection and disinfectant agents are critical factors in minimizing oxidation during *in vitro* establishment of explants, particularly under high-humidity conditions. According to Azofeifa (2009), oxidation is the most frequent problem that can inhibit *in vitro* culture progression and even lead to tissue death, especially during the establishment phase of woody explants, due to metabolic reactions triggered by multiple factors. Such effects can be mitigated or prevented through the use of antioxidants or adsorbents of the released compounds.

Survival

During the dry season, the highest explant survival was observed in MS medium with the treatment that included azoxystrobin, and in Yasuda medium when either azoxystrobin alone or antibiotics were applied. In both media, the number of surviving plants ranged between 19 and 20. The lowest survival rate was recorded when combining 3% NaClO + antibiotics + azoxystrobin (Figure 3).

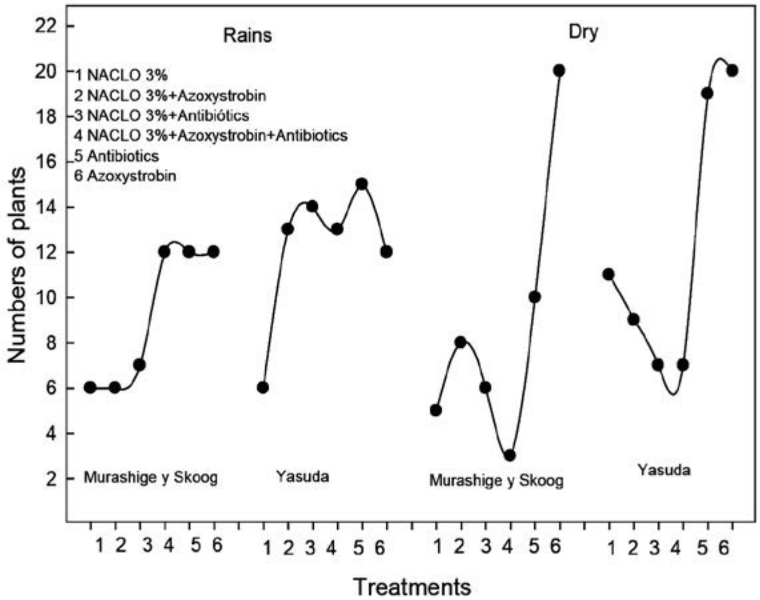

Figure 3. Survival dynamics of seedlings from nodal segments of *G. angustifolia* Kunth under the interaction of two culture media and three explant collection periods. Values represent means of 20 replications ± standard error.

During the rainy season in MS medium, survival increased to 12 explants with the simple treatment of 3% NaClO and when this was separately combined with azoxystrobin and/or antibiotics. However, the combined treatments showed the lowest survival values in this medium. In contrast, in Yasuda medium during the same rainy season, all treatments produced between 12 and 15 surviving explants, except for the treatment with 3% NaClO alone, which yielded the lowest value of 6 explants. No statistical differences ($p \leq 0.05$) were found between MS and Yasuda media across treatments. Survival of explants was observed to depend primarily on contamination levels. According to Sierra *et al.* (1999), in their study on apical bud disinfection of *Philodendron xanadu*, explant survival reached 35% using calcium hypochlorite (2.0%) for 35 minutes. Similarly, Fajardo (2006) tested different disinfection times (5, 10, and 20 minutes) with 1.0% sodium hypochlorite in the in vitro establishment of *G. angustifolia* Kunth and found no statistical differences among treatments for explant contamination percentage or survival.

Number of plant shoots

The number of shoots increased in MS medium compared to Yasuda, and among explant collection periods, the dry season showed higher values.

Within treatments, MS medium with dry-season explants produced 17 shoots under 3% NaClO treatment, which decreased to 11 when combined with 3% NaClO, antibiotics, and azoxystrobin. The number of shoots was further reduced to 9 with 3% NaClO plus antibiotics, and to 7 when antibiotics were replaced by azoxystrobin. In Yasuda medium, the highest value was six shoots in explants treated with 3% NaClO and in those treated with 3% NaClO combined with azoxystrobin and antibiotics. Similarly, in the *in vitro* culture of *M. azedarach*, improved callus induction was obtained using MS medium supplemented with 5 mg L^{-1} 2,4-D and 5 mg L^{-1} kinetin (Ahmadpoor *et al.*, 2022). The presence of shoots

Table 2. Number of shoots in nodal explants of *Guadua angustifolia* Kunth.

Culture medium	Treatments	Explants cut during the rainy season	Explants cut in dry seasons
		Number of plant shoots	Number of plant shoots
MS	NACLO 3%	2	17
	NaCLO 3% + azoxystrobin	5	7
	NaCLO 3% + antibiotics	3	9
	NaCLO 3% + azoxystrobin	2	11
	+ antibiotics	2	2
	antibiotics	1	0
Yasuda	azoxystrobin	2	6
	NACLO 3%	0	3
	NaCLO 3% + azoxystrobin	0	0
	NaCLO 3% + antibiotics	2	1
	NaCLO 3% + azoxystrobin	3	3
	+ antibiotics	1	0

MS medium yielded the highest number of shoots from explants (Table 2).

in explants was influenced by the type and concentration of disinfectants. Contamination can be a limiting factor that inhibits shoot growth or emergence. Although shoot emission was recorded even in contaminated explants, growth was deficient and in some cases shoots died. Fajardo (2006), using different disinfection times of 5, 10, and 20 minutes with 1.0% sodium hypochlorite in the in vitro establishment of *G. angustifolia* Kunth, reported that five minutes of exposure to the disinfectant yielded the highest number of sprouted buds per explant, differing statistically from the other treatments.

CONCLUSIONS

Explant collection season influences contamination, oxidation, regrowth, and survival. Contamination levels during both rainy and dry seasons decreased in both culture media when treatments included antibiotics and/or the fungicide azoxystrobin. Oxidation was reduced in explants collected during the dry season and cultured in Yasuda medium supplemented with antibiotics and/or azoxystrobin, which also improved survival.

Shoot regeneration was favored in explants collected during the dry season and cultured in MS medium with 3% NaClO plus antibiotics.

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