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Optimization of bud disinfection technique and influence of growth regulators on micropropagation in ginger (*Zingiber officinale* Rosc.)

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ABSTRACT

Ginger (*Zingiber officinale* Rosc.) is a spice, considered as a food medicine because of its numerous beneficial actions on health. However, its production faces several constraints, including the lack of efficient planting material. This is a limiting factor for industrial ginger production. The present study therefore aimed at developing an effective *in vitro* regeneration protocol for ginger. First, three disinfecting agents (sodium hypochlorite, calcium hypochlorite and mercury chloride) were tested. Then, different combinations of naphthalene acetic acid (0.5 mg/L NAA) and/or benzyl amino purine (1, 3 and 5 mg/L BAP) were evaluated on *in vitro* shoots regeneration. The results revealed that 3.6% sodium hypochlorite, used for 20 min, induced the best disinfection rates (100%) and healthy buds (84.66%). Furthermore, this study showed that the tested hormonal combinations significantly influenced shoots proliferation in ginger. However, the Proliferation Medium 4 (PM_4) [Murashige and Skoog medium including vitamin B_5 (MSB) + 0.5 mg/L of Naphthalene Acetic Acid (ANA) + 5 mg/L of Benzyl Amino Purine (BAP)] was the most effective. It induced the highest average number of shoots (22.83 shoots) with an induction rate of 80.50%. As a result of this study, 3.6% sodium hypochlorite used for 20 min and MP4 medium (MSB + 0.5 mg/L ANA + 5 mg/L BAP) were selected for *in vitro* ginger regeneration.

Keywords: *Zingiber officinale*, sterilization, BAP, NAA, *in vitro* propagation, seed production.

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) is an herbaceous, rhizomatous monocot plant in the zingiberaceae family. It is cultivated mainly for its rhizomes, which are generally used for human and animal consumption (Butin, 2017; Soma Massieke et al., 2017; Mba Tene et al., 2020). Ginger is receiving increasing attention around the world, due to its various healing properties (Agbebi et al., 2013) and its use in different culinary and gastronomic types (Meinertzhagen, 2007; Valenzuela, 2011). In

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several West African countries, most of the ginger produced is used to make a cold beverage "*gnamankoudji*", which is highly prized and served in almost every celebratory ceremonies (Nandkangre et al., 2015). Another part is also used in the pharmacopoeia (Bashige et al., 2020). Although this activity is not widely popularized, it is an important source of income. The price of a kilogram of ginger can vary from 0.83 to 1.09 dollars (OCPV-CI, 2022).

Ginger production in Côte d'Ivoire, which is 21,249 tons (Wamucii, 2019), could be higher if ginger farmers were not faced with the unavailability of efficient planting materials. Indeed, a no less important part of their harvest, about 2000 kg of ginger per hectare, is used as seed for the next planting (Valenzuela, 2011). This use of a large quantity of the production as seed reduces the farmers' profit margin. In addition, huge losses in ginger production due to diseases and pests have been recorded when ginger rhizomes are used as seed (Tadesse et al., 2015). Nematodes, for example, can cause a general loss of agricultural production of about 11% (Agrios, 2005) and of ginger specifically up to 50% (Assih et al., 2018). In this context, several authors have proposed micropropagation to significantly improve production through the provision of healthy, rejuvenated and efficient planting materials (Seran, 2013; Nirmal et al., 2016). Furthermore, *in vitro* culture, in addition to producing a large quantity of seedlings through micropropagation methods, could be used to create new varieties through mutagenesis, somaclonal variations, transgenesis (Quashie and Kokou, 2009).

In Côte d'Ivoire, very little work has been done on ginger seed production. Also, none of these works to date has mentioned data allowing to appreciate the effectiveness of micropropagation protocols of this plant. However, many works have shown that it is possible to induce bud regeneration in various plant species, by adding growth substances (Nigar and Mohammad, 2012; Mirrofanova et al., 2021). However, bud proliferation depends on the hormones used (Giovanni and Marcello, 2008).

The present work contributes to the improvement of the living conditions of peasant populations and to the reduction of poverty through subsequent income. Thus, this study aimed at developing an efficient disinfection protocol before *in vitro* cultivation of ginger buds, in order to meet the farmers needs in plant material. To do this, the determination of the effects of disinfectants, the concentration and time of the best disinfectant agent through the budding rates of ginger buds were tested. The influence of several hormonal formulations on the proliferation of ginger buds was also evaluated.

MATERIALS AND METHODS

Plant material

The plant material consisted of ginger (*Zingiber officinale* var. roscoe) rhizomes produced in Koun Fao (Bondoukou-Côte d'Ivoire) and marketed at the "*Gouro*" market in Adjamé (Abidjan-Côte d'Ivoire).

Preparation of plant material

Once the ginger rhizomes arrived at the laboratory, they were washed thoroughly with tap water to remove some surface impurities (mud, sand etc.). Then, they were soaked in a liquid soap bath for 5 to 10 min for surface disinfection. Bagging with a black polyethylene bag was performed for one week to promote rapid budding (Figure 1A). Under a laminar flow hood, the buds were removed using a blade mounted on a scalpel (Figure 1B). These buds, trimmed and reduced to approximately 0.5 cm, were washed abundantly with sterile distilled water (Figure 1C). They constituted the explants to be cultured.

Research of an efficient disinfection protocol of ginger rhizomes

Preparation of in vitro budding medium

The *in vitro* budding medium used was MS medium (Murashige and Skoog, 1962) supplemented with vitamin B_5 (Gamborg et al., 1968) to which 30 g/L sucrose was added (MSB medium). This medium was solidified by adding 0.75 g/L MgCl2 and 2.5 g/L gelrite. The pH of the medium was adjusted to 5.8 with HCl (0.1 N) or NaOH (0.1 N). Sterilization of the medium was performed in an autoclave at 121°C for 30 min under a pressure of 1 bar.

Explant disinfection

Effect of the nature of the disinfecting agent

In this study, the effects of three disinfecting agents (sodium hypochlorite, calcium hypochlorite and mercury chloride) were tested at the 1% concentration on ginger explants. Under a laminar flow hood, the explants underwent a rapid 1 min soak in 70% ethanol, followed by a 10 min immersion in one of the disinfecting agents. After each soaking time, the explants were washed three times with sterile distilled water to remove traces of the disinfectant. Then, the ginger explants were placed in test tubes containing 10 mL of budding medium (MSB medium) for 30 days. One explant was placed per test tube. Each treatment was repeated three times. A total of 27 experimental units were tested. A count was carried out to enumerate the explants that developed buds and those that were infected (budding or not). It should be noted that only budded and healthy explants were considered as budded explants. The following formulas were used to determine budding rate (BR) and infection rate (IR):

$$BR = \frac{\text{Total number of budded healthy explants}}{\text{Total number of explants cultured}} \times 100$$

$$IR = \frac{\text{Number of infected explants}}{\text{Number of explants cultured}} \times 100$$

Ten explants were used for each disinfecting agent and each experiment was repeated three times. The best disinfecting agent was selected for further work.

Effect of disinfecting agent concentration

Five different concentrations (1, 2, 2.5, 3 and 3.6%) of the best disinfecting agent were tested for 30 days on the budding medium (solid MSB without hormone) as before. Five explants were used for each concentration and each experiment was repeated three times. A total of 45 experimental units were tested. The best concentration of the disinfecting agent was retained.

Effect of disinfection time

The best concentration of the disinfecting agent selected was tested at different bud soaking times (5, 10, 15, 20, 25 and 30 min) on the same budding medium. Ten explants were used for each soaking time and each experiment was repeated three times. A total of 54 experimental units were tested.

Micropropagation of ginger shoots

Induction of ginger shoots

Previously disinfected buds were cultured at the rate of one explant per Pyrex test tube of size 2.1 x 15 (L x ø cm) containing 10 mL of induction medium. This shoot induction medium (MI) consisted of MS base medium supplemented with vitamin B_5 (MSB) to which different concentrations of BAP were added as follows:

MI_0 (control): MSB (Murashige and Skoog, 1962 + vitamins B_5 from Gamborg et al. (1968) + 30 g/L sucrose)

MI_1: MSB + 0.25 mg/L BAP

MI_2: MSB + 0.50 mg/L BAP

MI_3: MSB + 1.00 mg/L BAP

After 30 days of culture, the bud break rate (BR) of the explants, expressed as a percentage, was calculated:

$$PR = \frac{NBP}{NDB} \times 100$$

$$BR = \frac{NBD}{NTB} \times 100$$

NBD = number of buds budded

NTB = total number of explants cultured

NBP = number of proliferated buds

NDB = number of debudded buds cultured

Proliferation or multiplication of ginger shoots

Under a laminar flow hood, the debudded explants from the shoot induction medium were scarified at the base. Then, each explant was transferred to a 250 mL jar containing 50 mL of shoot proliferation medium. The different proliferation media (PM) were prepared from MSB medium to which NAA (0.5 mg/L) and/or BAP (1, 3 and 5 mg/L) were added. Four different treatments were carried out to test the effect of hormonal combinations on shoot induction:

PM_1: MSB + 0,5 mg/L NAA

PM_2: MSB + 0,5 mg/L NAA + 1 mg/L BAP

PM_3: MSB + 0,5 mg/L NAA + 3 mg/L BAP

PM_4: MSB + 0,5 mg/L NAA + 5 mg/L BAP

The jars were hermetically closed by their caps and then sealed with stretch film paper. After 60 days of culture in the culture room, the proliferation rate (PR) of the shoots, expressed as a percentage, was calculated by the following formula:

Culture conditions

The different cultures were carried out in a 20m²culture room, under a 16 h photoperiod, at a temperature of 24 ± 2°C, with an illumination of 2000 lux provided by 60 cm long fluorescent tubes (Cool White Phillips).

Statistical analysis

Statistical analyses were performed with Statistica 6.0 software. Single-criteria analysis of variance was used to test the difference between the effects of disinfecting agents, concentration, disinfection time, bud break rate, number of shoots per explant and shoot induction rate according to the hormonal combinations. Comparison of means was performed using Tukey's test at 5% risk.

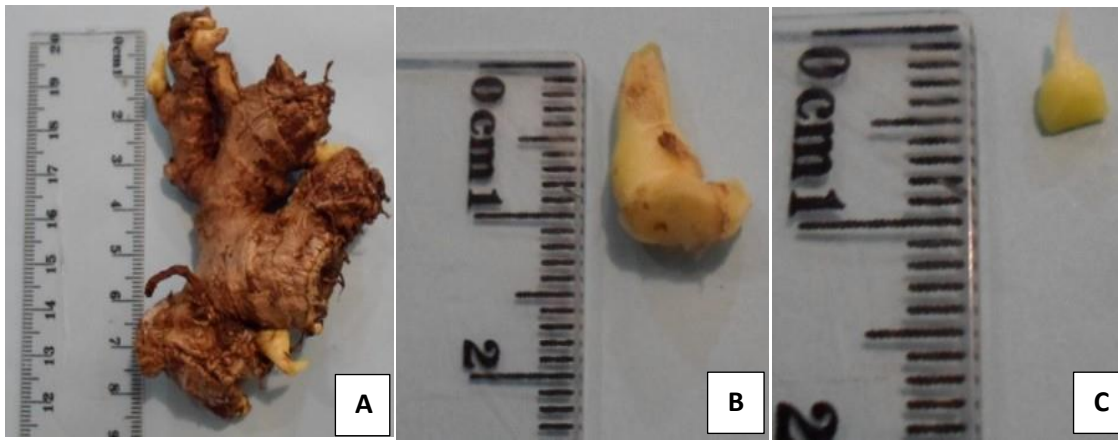

Figure 1: Preparation of explants for shoot initiation from a ginger rhizome.
A: Ginger rhizome; B: Bud taken from the rhizome; C: Explant.

RESULTS

Effect of disinfecting agents

Three disinfecting agents (sodium hypochlorite, calcium hypochlorite and mercury chloride) were used, each at the concentration of 1% for 10 min during this study. The results show that all disinfecting agents resulted in leafy shoot budding (Figure 2). However, explants treated with sodium hypochlorite recorded the best rate of healthy budded explants (23.33%), followed by explants treated with mercury chloride (6.66%) and calcium hypochlorite (3.33%). On the other hand, the infection rate of mercury chloride-treated explants was the lowest (05.24%), but with the most significant rate of healthy non-budded explants (88.1%). As for the highest infection rate of buds (92.55%), it was obtained with calcium hypochlorite (Table 1). Sodium hypochlorite, which induced the best rate of healthy buds, was therefore retained for further work.

Effect of sodium hypochlorite concentration

The results of the effect of sodium hypochlorite concentration on bud disinfection are recorded in Table 2. The highest rate of healthy budded explants (68.66%) was obtained with 3.6% sodium hypochlorite. In addition, the analysis of variances also revealed that sodium hypochlorite with 3.6% active chlorine caused the lowest infection rate (15.58%) in cultured explants. Moreover, only 15.76% of healthy explants did not induce buds. The 1% concentration of sodium hypochlorite recorded the highest infection rate (51.66%). Thus, 3.6% sodium hypochlorite induced the best budding and disinfection rates and was selected for further work.

Effect of disinfection time

Table 3 shows the effect of explant soaking time in sodium hypochlorite during bud disinfection in ginger. The results show that the best rates of healthy budded explants were obtained at 15 min (80.66%) and 20 min (84.66%). However, sodium hypochlorite with 3.6% active chlorine used for 20 min resulted in no infection of the explants, while at 15 min, 10.66% infection was recorded. Furthermore, the results revealed that prolonging the soaking time in sodium hypochlorite beyond 20 min inhibited budding and resulted in the death of some explants cultured, although no infection was present. Thus, the analysis of variances retained the soaking time of 20 min as the best disinfection time for explants in ginger.

Shoot induction

All the induction media allowed explants to bud after 30 days of culture (Table 4). However, the MI_3 medium (MSB + 1 mg/L BAP) induced the best bud break rate of (93.33%), with vigorous seedlings. MI_2 (MSB + 0.50 mg/L BAP) and MI_1 (MSB + 0.25 mg/L BAP) media, with 76.66 and 51.33% bud break rates respectively, were very significantly better than MI_0 medium (medium without BAP) which induced 13.90% bud break. From this analysis, it is evident that the addition of BAP significantly stimulated shoot induction in ginger.

Shoot multiplication

The results show that all tested hormonal combinations induced shoot proliferation (Figure 3). However, shoot induction (mean number and proliferation rate) was significantly influenced by BAP concentration (Table 5). The shoot proliferation rate varies between 13.90 and 80.50%. As for the average number of shoots produced per explant, it changed from 1.39 to 22.83 shoots with increasing BAP concentration. Thus, the PM_4 medium (MSB + 0.5 mg/L NAA + 5 mg/L BAP) induced the highest average number of shoots per explant (22.83 shoots). Consequently, the best percentage of shoot proliferation (80.50%) was recorded with the PM_4 medium. It is followed by the PM_3 (57.37%) and PM_2 (35.80%) media which performed better than the control medium, PM_1 (18.84%), containing no BAP.

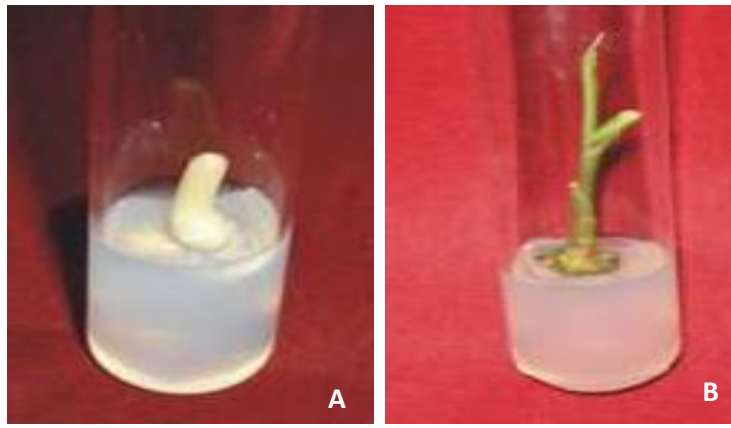

Figure 2: Induction of leafy shoots of ginger on MSB medium.
MSB: MS culture medium + vitamin B_5 + 30 g/L of sucrose; A: Starting explant; B: explant having budded a leafy shoot after 30 days of culture.

Table 1: Rate of healthy and infected buds according to disinfectants.

Disinfectants	Rate of healthy budded explants (%)	Infection rate (%)	Rate of healthy non-budded explants (%)
Calcium hypochlorite	3,33 ± 0,06**c**	92,55 ± 0,12**a**	4,12 ± 0,19**c**
Mercury chloride	6,66 ±0,05**b**	05,24 ± 0,08**c**	88,10 ± 0,28**a**
Sodium hypochlorite	23,33± 0,07**a**	52,61 ± 0,14**b**	24,06 ± 0,15**b**

±S: Standard error; In the same column, the means followed by the same letter are not significantly different at the 5% threshold (Tuckey's test). The values represent the mean of three repetitions.

Table 2: Effect of sodium hypochlorite concentrations on explant disinfection.

Concentration of sodium hypochlorite (%)	Rate of healthy budded explants (%)	Infection rate (%)	Rate of healthy non-budded explants (%)
1	22,33 ± 0,13**a**	51,66 ± 0,14**d**	26,01 ± 0,21**b**
2	31,66 ± 0,11**b**	28,66 ± 0,08**c**	39,68 ± 0,19**d**
2,5	42,66 ± 0,11**c**	19,33 ± 0,09**b**	38,01 ± 0,25**d**
3	53,33 ± 0,07**d**	17,66 ± 0,10**b**	29,01 ± 0,17**c**
3,6	68,66 ± 0,08**e**	15,58 ± 0,16**a**	15,76 ± 0,27**a**

±S: Standard error; In the same column, the means followed by the same letter are not significantly different at the 5% threshold (Tuckey's test). The values represent the mean of three repetitions.

Table 3: Rate of healthy and infected buds as a function of disinfection time with 3.6 % sodium hypochlorite.

Time to disinfection (min)	Rate of healthy budded explants (%)	Infection rate (%)	Rate of healthy non-budded explants (%)
5	37,66 ± 0,19 **a**	56,66 ± 0,18 **d**	05,68 ± **a**
10	65,33 ± 0,12 **b**	16,33 ± 0,14 **c**	18,34 ± **b**
15	80,66 ± 0,22 **c**	10,66 ± 0,16 **b**	08,68 ± **c**
20	84,66 ± 0,47 **c**	00,00 ± 0,00 **a**	15,34 ± **d**
25	67,33 ± 0,25 **b**	00,00 ± 0,00 **a**	32,67 ± **e**
30	33,33 ± 0,19 **a**	00,00 ± 0,00 **a**	66,67 ± **f**

±S: Standard error; In the same column, the means followed by the same letter are not significantly different at the 5% threshold (Tukey's test). The values represent the mean of three repetitions.

Table 4: Ginger explant bud burst assessment.

Shoot induction medium	Bud burst rate (%)
MI_0: MSB	13,90 ± 0,09 **a**
MI_1: MSB + 0,25 mg/L BAP	51,33 ± 1,19 **b**
MI_2: MSB + 0,50 mg/L BAP	76,66 ± 0,97 **c**
MI_3: MSB + 1,00 mg/L BAP	93,66 ± 1,70 **d**

±S: Standard error; In the same column, the means followed by the same letter are not significantly different at the 5% level (Tukey's test). The values represent the mean of three repetitions.

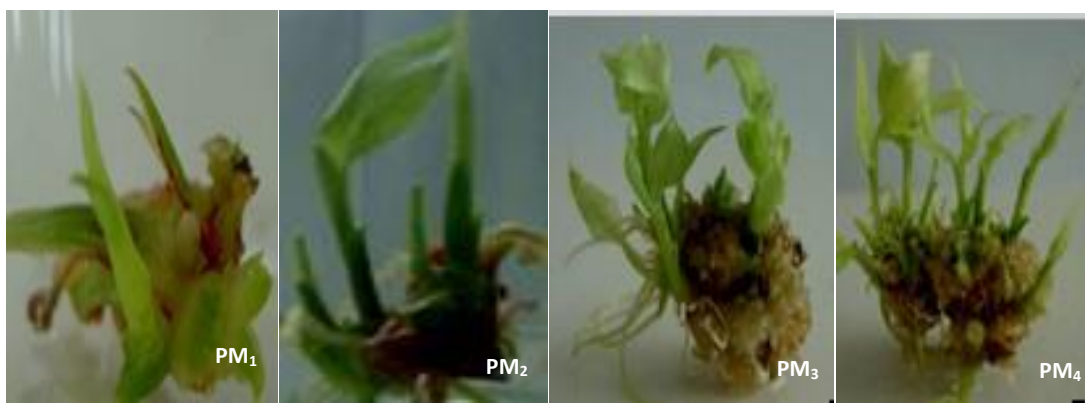

Figure 3: proliferation of ginger shoots under the influence of the hormonal diet.
PM_1: MSB + 0.5 mg/L NAA, PM_2: MSB + 0.5 mg/L NAA + 1 mg/L BAP,
PM_3: MSB + 0.5 mg/L NAA + 3 mg/L BAP, PM_4: MSB + 0.5 mg/L NAA + 5 mg/L BAP.

Table 5: Effect of hormonal combination on the proliferation of ginger shoots.

Proliferation medium	Average number of shoots per explant	Proliferation rate shoots (%)
PM_1 (MSB + 0,5 mg/L NAA)	03,56 ± 0,14 **a**	18,84 **a**
PM_2 (MSB + 0,5 mg/L NAA + 1 mg/L BAP)	10,83 ± 0,14 **b**	48,30 **b**
PM_3 (MSB + 0,5 mg/L NAA + 3 mg/L BAP)	16,89 ± 0,09 **c**	60,91 **c**
PM_4 (MSB + 0,5 mg/L NAA+ 5 mg/L BAP)	22,83 ± 0,14 **d**	80,50 **d**

±S: Standard error; In the same column, the means followed by the same letter are not significantly different at the 5% threshold (Tukey's test). The values represent the mean of three repetitions.

DISCUSSION

The results of the ginger rhizome disinfection study show that the rate of healthy budded explants and the rate of infection were significantly influenced by the disinfecting agent used. Thus, treatment with 1% sodium hypochlorite gave the best disinfection percentage, with 23.33% healthy budded explants, followed by mercury chloride (6.66%) and calcium hypochlorite (3.33%). Indeed, sodium hypochlorite seems to penetrate inside the cells and therefore would act more efficiently on the endogenous pathogens of ginger cells. These results are contrary to those reported by Rajani (2006) who showed that disinfection of ginger explants with mercury chloride is better compared to sodium hypochlorite. This author used 0.1% mercury chloride and 0.5% for sodium hypochlorite. Mercury chloride is extremely toxic because of the strong corrosive action of its two chlorine atoms and also its mercury ion (Courtois et al., 2012). This could explain the low rate of budding of explants in our study where it was used at 1%. Certainly, at this concentration, mercury chloride induced ginger cell death. In contrast, the concentration of sodium hypochlorite (0.5%) used by this author was too low to destroy endogenous cell pathogens, which explains its low disinfectant activity. In our study, sodium hypochlorite was used at 1%, hence its greater disinfecting action on ginger explants. Wondyfraw and Surawit (2006) reported a very good disinfection rate of Korarima (Zingiberaceae) buds with sodium hypochlorite. Our work is in agreement with Zahid et al. (2021) who mentioned that sodium hypochlorite is the ideal disinfecting agent in ginger. According to Renaudeau and Ronchi (2020), because of its ability to capture electrons, sodium hypochlorite is an oxidant capable of attacking the proteins that make up the cell membrane of microorganisms (bacteria, spores, molds and viruses), giving it its bactericidal, sporicidal, fungicidal and virucidal properties.

On the other hand, the high contamination rate (92.55%) recorded with calcium hypochlorite in this study could be explained by a non-significant disinfectant activity related to the low concentration used (1%). Indeed, Koné et al (2009b) were able to obtain healthy rhizomes of *Erwinia carotovora* using calcium hypochlorite at 7%. Also, it should be noted that calcium hypochlorite is a surface disinfectant. This would suggest that endogenous pathogens in ginger cells would not be attacked by calcium hypochlorite as mentioned by Koné et al. (2009a) in voandzou.

The results of the effect of sodium hypochlorite concentration on bud disinfection showed that 3.6% sodium hypochlorite gave the best healthy bud rate (68.66%) while 1% sodium hypochlorite concentration gave a healthy bud rate of 22.33% with 51.66% infection rate. According to Estrela et al. (2002), hypochlorous acid, a substance present in the sodium hypochlorite solution, when in contact with organic tissues, acts as a solvent and releases chlorine which, combined with the protein amino group, forms chloramines that interfere with cellular metabolism. Chlorine (a powerful oxidant) has an antimicrobial action inhibiting bacterial enzymes leading to an irreversible oxidation of SH groups (sulfhydryl group) of essential bacterial enzymes. Thus, the high concentration of sodium hypochlorite (3.6%) would cause a higher release of chlorine and thus a better disinfection of the buds in ginger. Moreover, this concentration of sodium hypochlorite would not be toxic for the explants, hence the high rate of healthy buds recorded. These results are in agreement with those of Yapo et al. (2011) who obtained healthy pineapple buds using 3.6% sodium hypochlorite. With sodium hypochlorite concentrations below 3.6% (1, 2, 2.5 and 3%), infections were observed and healthy buds were obtained. However, the significance of the events is inversely proportional to the concentration of sodium hypochlorite tested. These concentrations do not appear to be sufficient to eliminate all pathogens present in ginger cells. This finding has already been reported by Yede (2006) in cotton. Thus, the concentration would be an important parameter

in the ability of sodium hypochlorite to disinfect explants in plants. Also, the disinfection time seems to play an important role in the disinfection of buds as mentioned by Doukouré (2000) in yam. In our study, sodium hypochlorite with 3.6 % active chlorine used for 20 min showed the best disinfection (100 %) and the best budding (84.66 %) rate in ginger. This result suggests that soaking for 20 min appears to be sufficient for complete and effective disinfection of ginger buds, eliminating all pathogens present in the explants. In contrast, soaking times of 5 to 10 min recorded high infection rates because they are insufficient to destroy all microorganisms present in the explants. Zero infection rates were also obtained with bud soaking times of 25 and 30 min. However, the healthy buds rates were 67.33 and 33.33%, respectively. This decrease in the rate of healthy buds observed with soaking times greater than 20 min seems to indicate a destruction of the buds by sodium hypochlorite. Indeed, according to Al-Taha et al. (2020), soaking of explants for more than 20 min would be harmful. In contrast, Yapo et al. (2011) reported soaking times of 30 min in sodium hypochlorite in pineapple. Similarly, Tilkat et al. (2013) recommended a soaking time of 30 min in 10% sodium hypochlorite for disinfection of pistachio (*Pistacia vera* L). The soaking time thus seems to depend on the plant material used.

Concerning the effect of the composition of the culture medium on budding, the results showed that it significantly influenced the bud break of the shoots. Indeed, all the tested media induced bud break of the cultured buds. However, it appears from this study that the addition of BAP increasingly stimulated shoot induction in ginger. For example, explants from MI_3 medium (MSB + 1 mg/L BAP) recorded the highest rate of bud break. This result suggests that BAP positively influences explant bud break as reported by Magyar-Tabori et al. (2010) in apple. This result is in agreement with that reported by Beena et al. (2003) in *Ceropegia candelabrum* and Kouakou (2010) in *Laccosperma secundiflorum* who showed that BAP is essential for explant bud break. Indeed, the high rate of explant bud break observed on BAP- rich media would be due to an active stimulation of cell division and morphogenesis by the BAP molecule (Van Staden et al., 2008).

Otherwise, the addition of 0.5 mg/L NAA to media containing BAP stimulated shoot development on budded explants. The number of shoots induced per explant steadily increased with increasing BAP concentration. These results are in agreement with those of Kambaska and Santilata (2009) who reported that the addition of auxin (NAA) to a medium containing cytokinin enhances bud multiplication. Indeed, cytokinins stimulate cell division more actively in the presence of auxins (Kouadio et al., 2017**)**. Thus, the number of shoots obtained per explant with the PM_4 medium (MSB + 0.5 mg/L NAA + 5 mg/L BAP) increased to 22.83 shoots per explant against 3.56 shoots for PM_1 (medium without BAP). These results differ from those of Raju et al. (2005) who recorded 4.5 shoots per explant using the NAA/BAP combination in *Curcuma zedoria* (Zingiberaceae). The control medium, containing NAA alone, recorded the lowest rate of shoots, suggesting that the increase in the number of shoots in the other media containing the NAA/BAP combination would therefore be a function of the concentration of BAP used. This shows the important role of the NAA/BAP combination on shoot proliferation in ginger. This study therefore revealed that in ginger, increasing cytokinin concentrations in a medium in the presence of auxin would promote shoot proliferation. Thus, according to the results of this study, the best hormonal combination (MSB + 0.5 mg/L NAA + 5 mg/L BAP) would allow to obtain from a single explant more than one million ginger buds in one year. This would help to overcome the lack of quality planting material that many farmers around the world are facing. This quality planting material would increase production and consequently improve the living conditions of millions of people living from this crop.

Conclusion

At the end of this study, it can be said that the disinfection of explants and culture media is essential for the success of *in vitro* culture in ginger. Sodium hypochlorite with 3.6% active chlorine used for 20 min provides better disinfection of ginger explants. Regarding the proliferation of ginger shoots, the study showed that naphthalene acetic acid acted as a catalyst on 6-benzyl-aminopurine. Thus, the ideal cytokinin and auxin combination during this study for the multiplication of ginger explants is PM_4 medium (MSB + 0.5 mg/L NAA + 5 mg/L BAP). This culture medium would allow large-scale production of healthy planting materials for yield improvement of ginger producers.

COMPETING INTERESTS

The authors declare no competing interests.

AUTHORS' CONTRIBUTIONS

The development and design of the protocol for this study was done by DK. NY carried out the field and laboratory activities accompanied by ARN. The statistical analyzes of the results were made with the contribution of OKSK and OS. THK supervised the work. DK, OKSK and OS actively participated in the writing of this manuscript. All authors have given their consent to the final version of the manuscript and its publication.

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