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Effect of plant growth regulators on *in vitro* germination of coffee zygotic embryos

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Coffee propagation is performed by seeding. However, germination in coffee seed is slow and uneven. Indeed, the production of plants in field is affected by environmental factors, which retards the germination rates and, in consequence, the number of plants obtained. Plant tissue culture provides the possibility of applying different growing conditions, such as different types of media and using phytoregulators, to induce a response in order to shorten zygotic embryo germination times. Therefore, due to limited understanding of growing zygotic embryos of coffee, we evaluated the effect of certain plant growth regulators on zygotic embryo germination of *Coffea arabica*. To study germination times, we evaluated the effects of culture media with incubation either at photoperiod or darkness and with certain plant growth regulators [gibberellic acid (GA), abscisic acid (ABA), indole-3-butyric acid (IBA), naphthalene acetic acid (NAA), kinetin (KIN) and salicylic acid (SA), at concentrations of 0.1, 1, and 10 mg l^{-1} each]. Zygotic embryos were cultured on Murashige and Skoog (MS), and Gamborg media at different concentrations ($^1/_1$, ½, and ¼ of its ionic strength) under conditions of darkness and photoperiod. Germination times of zygotic embryos cultured in MS medium had a mean of 5.1 days, whereas zygotic embryos cultured in B5 medium germinated with a mean of 7.5 days. Among the plant growth regulators used, gibberellic acid at 0.1 mg l^{-1} proved to be the most efficient in germinating zygotic embryos, with 100% germination reached by the 5th day. Germination time of zygotic embryos was 12 days when ABA at 0.1 mg l^{-1} was used; however, ABA inhibited germination of embryos when it was used at concentrations of 1.0 and 10 mg l^{-1} since only 10% germination occurred. Regarding the germination times of zygotic embryos of coffee, the priority order of efficiency was GA > KIN > SA > IBA > NAA > ABA. Also, with the exception of SA, concentrations higher than 0.1 mg l^{-1} increased the zygotic embryo germination rates.

Key words: Zygotic embryos, coffee, germination, phytohormones.

INTRODUCTION

Coffee, one of the most widely traded commodities in international markets, is an agricultural crop of significant economic importance. Coffee is grown in 80 countries around the world with 70% being produced by smallholder farmers. Besides being a source of income for millions of people, it represents foreign currency generation. Commercially, only two of more than 100 species of the *Coffea* genus are cultured: *Coffea Arabica* L. (Arabica type coffee) and *Coffea canephora* p. former Fr. (Canephora or Robusta type coffee). The Arabica type

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coffee represents almost 75% of the world production, has a soft flavor, and possesses a content of caffeine of 1.5%; hence it is preferred among the consumers (De los Santos-Briones and Hernández-Sotomayor, 2006). Reproduction of coffee can happen in a sexual or asexual way. Although coffee can be vegetatively propagated either by grafting, stem-cutting, or *in vitro* propagation, thanks to its high rate of autogamy, the most appropriate and easiest method of propagation of Arabica coffee is by seeding propagation. Thus, Arabica plantations have been established from seed for many years (Wintgens and Zamarripa, 2004). Normally, Arabica seeds germinate by the following pattern: a) after 32 days if seeds have been fresh harvested, b) after 50 days if seeds have been stored for 8 weeks after harvesting, and c) after 42 to 70 days if seeds are older (Wintgens and Zamarripa, 2004).

In field, coffee seeds germinate slowly (Rena et al., 1986). *C. arabica* seedling emergence from the soil starts 50 to 60 days after sowing in the warmer periods of the year (Maestri and Vieira, 1961). When temperatures are lower, the emergence period may increase to 90 days (Went, 1957). Following germination, the coffee cotyledons grow and turn green by absorbing the endosperm (Wellman, 1961; Giorgini and Campos, 1992). The first seed parts to emerge from the soil are the cotyledons, characterizing epigeal seedling growth, and 3 to 4 weeks are required for the cotyledons to completely deplete the endosperm and be free from any residue of it (Huxley, 1965). Obviously, germination is faster under optimal conditions when environmental effects such as variations in day/night temperatures and soil water potential are absent. In addition, germination under field conditions is defined as seedling emergence from the soil; radicle protrusion has already been completed sometime before emergence, and germination *sensu stricto* is completed with radical emergence.

Little work has been done to understand the behavior of coffee seeds during germination, and there is a lack of information concerning the regulation of the germination process. *In vitro*, the germination of coffee seeds is influenced by several factors such as storage conditions (Bacchi, 1958), light (Valio, 1976), and endogenous and exogenous levels of hormones (Velasco and Gutierrez, 1974; Valio, 1976). Gibberellins (GAs) play an important role in seed germination. Exogenous gibberellic acid (GA3) inhibits coffee seed germination, the inhibition being greater in seeds placed in continuous light than in darkness (Valio, 1976). With GA3 treatment, there is an increase in peroxidase activity that is also detectable in untreated seeds at a later stage of germination (Takaki and Dietrich, 1979a). Takaki and Dietrich (1979b) observed that GA3-treated coffee seeds produced several droplets of exudate on their surface. Analysis of this exudate revealed the presence of large quantities of carbohydrates and amino acids, the former consisting largely of mannose, with traces of glucose, fructose, and galactose. The site of GA action has been proposed to be both in the endosperm and in the embryo (Karssen et al., 1989). According to Da Silva et al. (2005), coffee seed depends on the *de novo* synthesis of GAs. Endogenous GAs are required for embryo cell elongation and endosperm cap weakening during germination. The inhibition of germination by exogenous GAs is caused by factors that are released from the endosperm during or after its weakening, causing cell death in the embryo and leading to inhibition of radical protrusion. Contrary to many reports on the stimulatory effect of GA on seed germination and cell elongation, exogenous GA3 inhibits radicle protrusion and emergence in coffee seed (Maestri and Vieira, 1961; Valio, 1976; Takaki and Dietrich, 1979a,b; Takaki et al., 1979). This inhibition is proposed to be caused by a build up of mannose, as a degradation product of the hydrolysis of cell-wall mannans by endo-β-mannanase and β-mannosidase (Takaki and Dietrich, 1980). On the other hand, in *C. arabica* seeds, Valio (1976) found that endogenous ABA-like substances and exogenous ABA cause inhibition of germination by preventing embryo growth. Da Silva et al. (2004) reported a transient rise in endogenous ABA content during germination in the embryo cells, suggesting that ABA inhibits cell wall extensibility by not permitting an increase in cell turgor (Da Silva et al., 2004).

Most studies on coffee germination have dealt with preservation of seed viability (Santana-Buzzy, 2001; Ellis et al., 1990) and with mechanisms and regulation of coffee seed germination. These experiments were carried out by following compositional changes during the process, and the biological role of regulators was investigated using the whole coffee seed. Our goal was to investigate the influences that several phytoregulators might have upon zygotic embryos (without endosperm) during the time course of germination. To our knowledge, this is the first study concerning the relation between different phytoregulators directly on zygotic embryo germination of coffee. Since this species is fairly recalcitrant (as their seeds, in a relatively short time, dramatically reduces germination), the objectives of this study were to (1) evaluate a range of culture media for the response of zygotic embryo germination to different ionic strengths, photoperiod/dark conditions, and to media supplemented with different phytohormone concentrations; (2) compare germination rates of zygotic embryos with each different condition of culture media; and (3) establish the optimal conditions for promoting a short zygotic embryo germination and its conversion into plantlets. This method could be useful for seeds recovery from genetic material of interest that has been preserved for long periods of time.

MATERIALS AND METHODS

Plant materials

Coffee fruits were harvested from plants from the Botanical Garden

of CICY located in Merida, Yucatan, Mexico (21°01'48.72" N; 89°38'15.37" W). The fruits were manually depulped, and after 48 h, the seeds were rubbed against each other and rinsed with water several times until the mucilage adhered to the endocarp was removed. Then they were left to dry at room temperature, and the endocarp was manually removed and used immediately. Disinfection was achieved under aseptic conditions. Seeds were surface-sterilized in 10% sodium hypochlorite for 2 h followed by two rinses in sterilized, distilled, deionized water. Then 70% ethanol was added, and the seeds were imbibed for 5 min with continuous shaking, rinsed twice in sterile water, and soaked newly in 30% sodium hypochlorite for 10 min. Finally, seeds were rinsed in sterile water and left soaking for 48 h until zygotic embryo extraction.

Zygotic embryo extraction and germination tests

Under aseptic conditions, embryos from water-imbibed seeds were isolated by cutting the endosperm with a razor blade. Excised zygotic embryos were placed in 40 mm small glass bottles with 10 ml of imbibing medium which it corresponds to each treatment described in the experimental design. During imbibition, embryos were maintained at $25\pm1°C$ in photoperiod (198 µmole m^{-2} s^{-1}; 16 h light/8 h dark) or dark conditions until the hypocotyl stood upright. Emergence of the cotyledon was used as the criterion for estimating the germination time in culture. The germination time was recorded daily.

Culture media

Two different culture media were used: MS medium (Murashige and Skoog, 1962) and B5 medium (Gamborg et al., 1968). Complete MS and B5 media were prepared with both major and minor components at ¼ strength, ½ strength, and $^1/_1$ strength and supplemented with vitamins (100 mg l^{-1} myo-inositol, 10 mg l^{-1} thiamine, 25 mg l^{-1} cysteine, and 3% sucrose for MS; and 100 mg l^{-1} myo-inositol, 100 mg l^{-1} thiamine, 10 mg l^{-1} nicotinic acid, and 10 mg l^{-1} pyridoxine for B5). As a gelling agent, 0.22% gelrite was added to all media except for the ¼ strength B5 medium to which 0.9% agar was added.

Plant growth regulators

The plant growth regulators used in this study were gibberellic acid (GA), abscisic acid (ABA), indole-3-butyric acid (IBA), naphthalene acetic acid (NAA), kinetin (KIN), and salicylic acid (SA), in concentrations of 0.1, 1, and 10 mg l^{-1} each. All plant growth regulators were obtained from Sigma-Aldrich (Sigma, MO, USA) and prepared using water under alkaline conditions.

Tissue culture media preparation

After all salts were dissolved in water, the plant growth regulator was added to reach the required concentration. Subsequently, the pH of each medium was adjusted to 5.8, and the gelling agent (gelrite or agar) was added before autoclaving (18 min, 121°C, 15 psi). Only in the case of media including GA, the medium was autoclaved first, and then the phytoregulator was sterilized by filtration and added.

Experimental design

In an initial screening experiment, embryos were placed individually in both complete media (MS and B5), each at three different ionic

strengths: ¼ strength, ½ strength, and $^1/_1$ strength. For each treatment, embryos were then incubated at $25 \pm 1°C$ in photoperiod (16 h light/8 h dark) and in dark conditions. The combination of these parameters gave a total of 12 treatments. The germination time was determined daily.

Subsequently, the effects of six plant growth regulators (GA, ABA, IBA, NAA, KIN, and SA) were evaluated. Once the culture medium which produced better embryo germination (an optimal culture medium) was determined, we used it plus three concentrations of each plant growth regulator: 0.1 mg l^{-1}, 1 mg l^{-1}, and 10 mg l^{-1}. The combination of these parameters, in this second scheme, gave a total of 18 treatments. For each treatment, embryos were then incubated at $25 \pm 1°C$ in photoperiod (198 µmole m^{-2} s^{-1}; 16 h light/8 h dark) or in dark conditions (depending on the best condition). The germination time was recorded daily for each condition. These experiments were repeated 3 times.

Plantlet establishment in soil

Zygotic embryos were transferred to optimal germination medium contained in 40 mm small glass bottles and incubated at $25 \pm 1°C$ in photoperiod (198 µmole m^{-2} s^{-1}; 16 h light/8 h dark) until they have emerged cotyledons and produced radicles. Developed plantlets were transferred to fresh medium contained in magenta boxes and maintained under same incubation conditions. After 6 to 8 weeks, the plantlets were gently removed from the agar, placed individually in small pots containing sterilized agrolite moistened (1:1, v/v) with Hoagland medium (Hoagland and Arnon, 1950), and covered with transparent polyethylene bags with 1.5 cm slits to increase the atmospheric exchange. After 2 weeks, the transparent covers were removed and plantlets were left uncovered at the greenhouse (photoperiod at $28 \pm 2°C$). Two weeks later, plantlets were planted in black polyethylene bags or black pots containing soil for longer time than 6 months.

Statistical analysis

One sample of 10 embryos was used in each treatment. Multiple comparisons of germination percentages of zygotic embryos were carried out using the ANOVA and multiple range tests.

RESULTS

Screening of zygotic embryos to optimize germination in culture media

In order to determine an optimal germination time, and as an initial experimental scheme, extracted zygotic em-bryos were individually placed in small glass bottles with MS or B5 medium at different ionic strengths. Even though seed germination is established with the radicle protrusion, on this case we defined the embryo germination as the emergence of the cotyledon. Although zygotic embryos started germination at day 3 (for MS medium) and at day 5 (for B5 medium) (data not shown), in MS medium 100% germination was observed at days 5 to 6, there was no dependence on light conditions of the culture or ionic strength of the medium (Figure 1A). There were no statistical differences in germination times between the ¼, ½, and $^1/_1$ concentrations of MS medium and light conditions; however differences in color
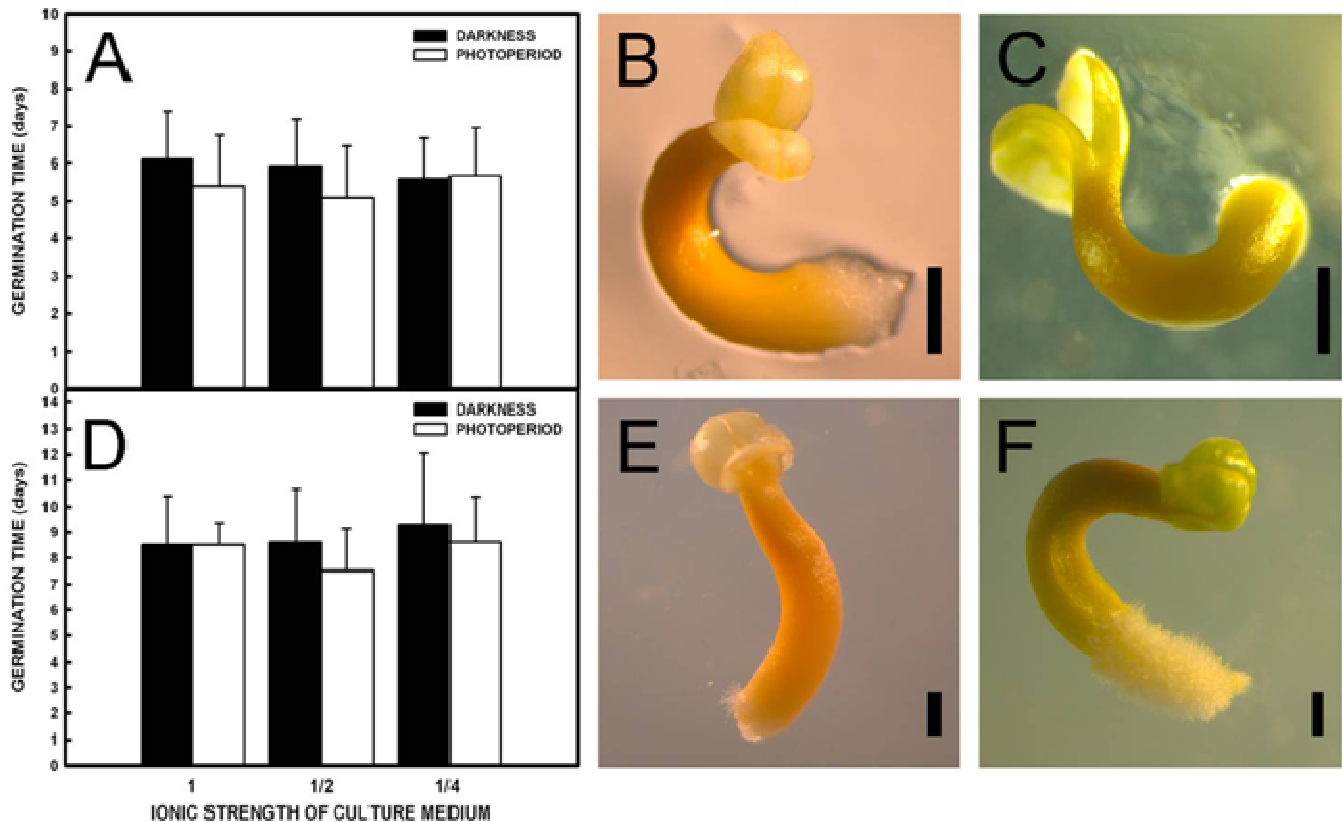
Figure 1. Germination times of *C. arabica* zygotic embryos cultured in different conditions. Extracted zygotic embryos were individually placed in small glass bottles with MS medium (A, B, C) or B5 medium (D, E, F), each at three different ionic strengths: ¼ strength, ½ strength, and $^1/_1$ strength. For each treatment, embryos were incubated at 25±1°C in dark conditions (B, E) and in photoperiod (198 µmole m^{-2} s^{-1}; 16 h light/8 h dark; C, F). The germination time was determined daily. One sample of 10 embryos was used in each treatment. Multiple comparisons of germination times of zygotic embryos were carried out using the ANOVA and multiple range tests. The experiment was repeated 3 times. Results are the mean ±SE. Bars on images represent 1 mm.

between zygotic embryos cultured in each light condition were observed: a pinkless coloration for embryos in darkness (Figure 1B) compared to a greenless coloration for embryos cultured in photoperiod (Figure 1C). Using $^1/_1$ MS medium, our results are similar to those obtained by Rambabu et al. (2005) where 100% germination was observed with zygotic embryos of another woody specie (*Givotia rottleriformis*, a forest tree) although they obtained 40% germination in ½ MS medium. In the B5 medium, zygotic embryos germinated at the 7th or 8th day without differences between light conditions and ionic strengths (Figure 1D). 100% germination was obtained at the 11th day of culture for either condition. Although there were no statistical differences in germination times between treatments, our data indicated a reduced time for reaching 100% germination when using a B5 medium compared to only 18% germination when using B5 with another plant genus (Rambabu et al., 2005). Also, a high percentage of zygotic embryos presented a radical protusion when cultured in both light conditions (Figures 1E and 1F). According to our results, we selected the medium to reduce the germination time of zygotic

embryos. Germination time of zygotic embryos cultured in MS medium had a mean of 5.1 days, whereas zygotic embryos cultured in B5 medium germinated with a mean of 7.5 days; also, we noted that embryos germinated in photoperiod presented a better appearance than those cultured in dark. Thus, for further experiments we used the MS medium with a half of its ionic strength (½ MS) to be cultured on photoperiod.

Effect of plant growth regulators on zygotic embryos germination

Once optimal conditions to germinate zygotic embryos were determined, and as a second experimental scheme, then the influence that a few phytohormones have upon germination times of zygotic embryos of coffee was evaluated. To do this, zygotic embryos were individually incubated in ½ MS medium with photoperiod containing 0.1, 1, or 10 mg l^{-1} of plant growth regulators (NAA, KIN, IBA, ABA, GA, SA; Figure 2), and the subsequent germination time was measured. With the exception of
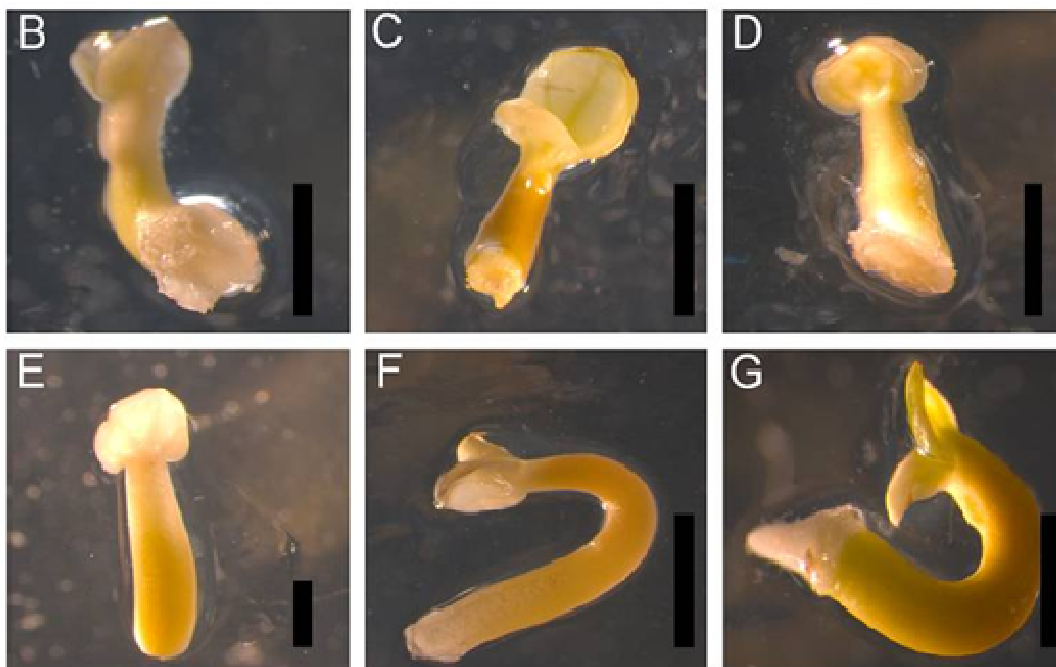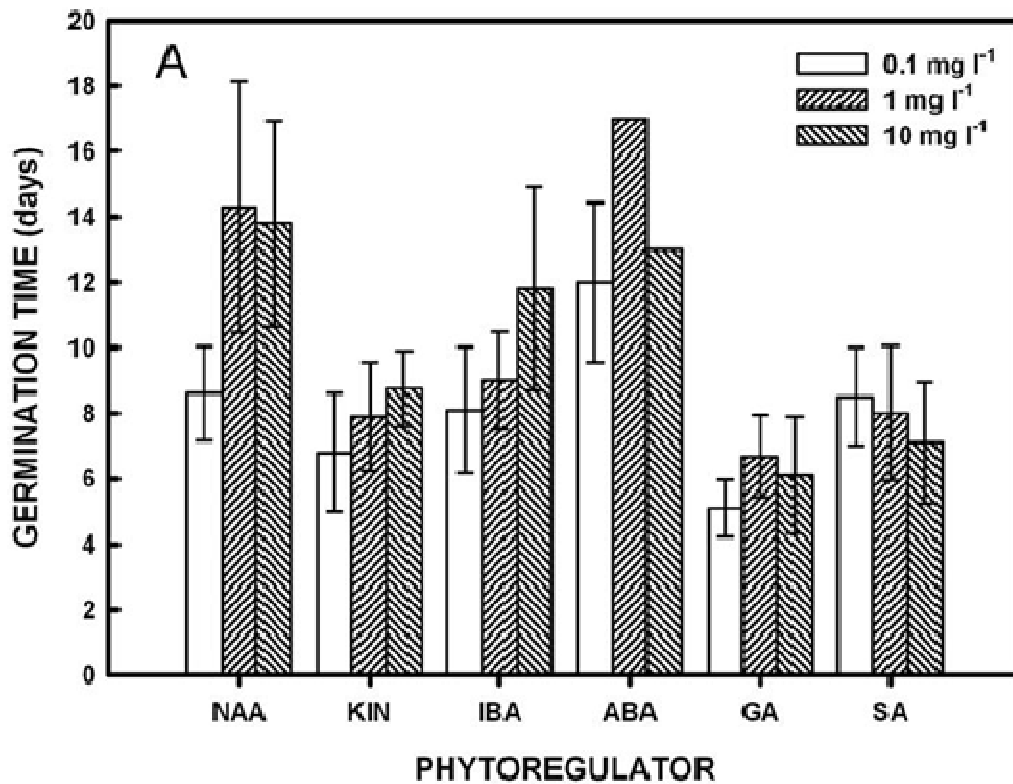
Figure 2. Effect of phytohormones on zygotic embryos germination of *C. arabica*. Extracted zygotic embryos were individually cultured in ½ MS medium containing 0.1, 1, or 10 mg l^{-1} of plant growth regulators **(A)**. The subsequent germination time was measured. For each treatment, embryos were incubated at 25±1°C in photoperiod (198 μmole m^{-2} s^{-1}; 16 h light/8 h dark). One sample of 10 embryos was used in each treatment. The experiment was repeated 3 times. Multiple comparisons of germination times of zygotic embryos were carried out using the ANOVA and multiple range tests. Results are the mean ±SE. Images correspond to extracted zygotic embryos individually cultured in ½ MS medium containing 1 mg l^{-1} of NAA (**B**), KIN **(C)**, IBA **(D)**, ABA **(E)**, GA **(F)**, and SA **(G)**. Bars on images represent 1 mm.

Table 1. Effect of certain phytoregulators on zygotic embryo germination of *Coffea arabica* L.

Plant growth regulator (mg l^{-1})	Germination day (mean ± SE)	Germination (%)
IBA		
0.1	8.1±1.9	100
1.0	9±1.49	100
10	11.8±3.1	50
NAA		
0.1	8.6±1.4	100
1.0	14.3±3.8	100
10	13.8±3.1	100
KIN		
0.1	6.8±1.8	100
1.0	7.9±1.6	100
10	8.7±1.6	80
SA		
0.1	8.5±1.5	100
1.0	8±2.0	100
10	7.1±1.8	100
GA		
0.1	5.1±0.8	100
1.0	6.7±1.2	100
10	6.1±1.8	100
ABA		
0.1	12.0	90
1.0	17.0	10
10	13.0	10

For each treatment, embryos were incubated at 25±1°C and photoperiod (198 µmole m^{-2} s^{-1}; 16 h light/8 h dark) in ½ MS medium containing 0.1 mg l^{-1}, 1 mg l^{-1}, or 10 mg l^{-1} of plant growth regulators (NAA, KIN, IBA, ABA, GA, and SA). The subsequent germination time was measured. Data were subjected to the ANOVA and multiple range tests. Results are the mean ± SE (n =10).

IBA at 10 mg l^{-1} (50% germination), KIN at 10 mg l^{-1} (80% germination), and ABA at 0.01, 1.0, and 10 mg l^{-1} (90, 10, and 10% germination, respectively) we observed 100% germination of zygotic embryos in any treatment after the 18th day (Table 1). Among the plant growth regulators used, GA at a concentration of 0.1 mg l^{-1} proved to be the most efficient in germinating zygotic embryos with 100% germination reached on the 5th day (Table 1; Figure 2A). When using GA, concentrations higher than 0.1 mg l^{-1} increased germination time by 2 or 3 days (Table 1; Figure 2A). Also, we observed an increased elongation in the hypocotyls of embryos when using GA at all three concentrations (Figure 2F). KIN and SA had a similar effect: germination occurred between the 7th and 10th day at any concentration used (Figure 2A). At a concentration of 0.1 mg l^{-1}, NAA and IBA presented a similar response since 100% germination was observed on the 8th day, but when concentrations are increased, 100% germination was not reached until the 14th day. Germination time of zygotic embryos was noted at the 12th day when ABA at 0.1 mg l^{-1} was used (Figure 2A); however, ABA inhibited germination of embryos when used at concentrations of 1.0 mg l^{-1} and 10 mg l^{-1} since only 10% germination occurred (Table 1).

A statistical analysis was done with all data without taking into account the concentrations used. As shown in Figure 3, there were differences between the plant growth regulators used. Independent of concentration, germination was earlier with GA, while KIN and SA had similar germination times. It is notable that ABA is a plant growth regulator which delays or inhibits germination; IBA and NAA had an intermediate effect between KIN, SA, and ABA (Figure 3A). On the other hand, when concentrations are considered but independently of phytohormone used, the statistical analysis revealed that concentrations higher than 0.1 mg l^{-1} delayed the germination time to 10 to 11 days. A concentration at 0.1 mg l^{-1} of any phytohormone was shown to reduce germination times to times ranging between 8 and 9 days (Figure 3B).
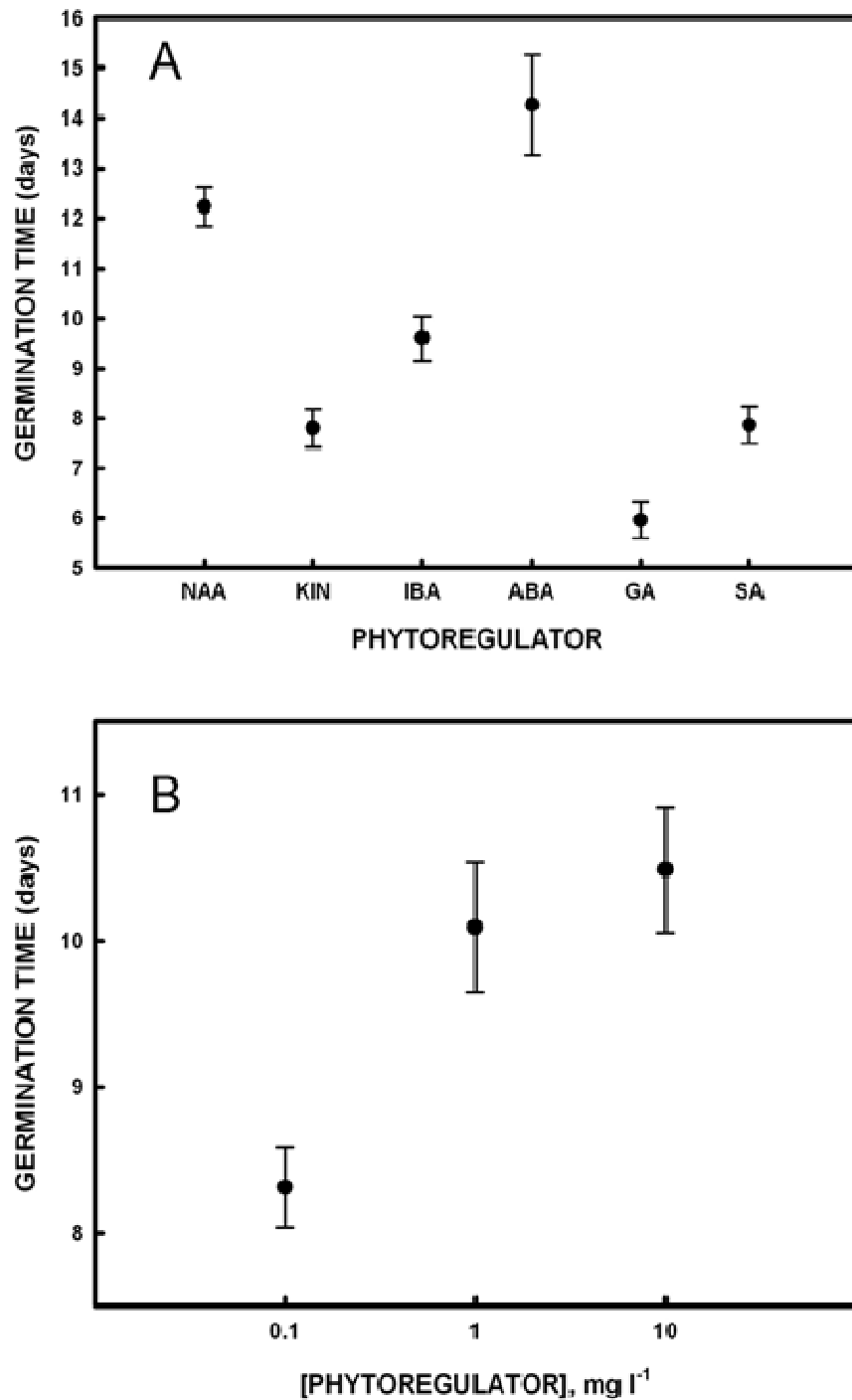
Figure 3. Effect of phytohormones concentration on zygotic embryos germination. A statistical analysis was done with all data of Figure 2A, without taking into account the concentrations used (A) or the phytohormone used (B). Multiple comparisons of germination times of zygotic embryos were carried out using the ANOVA and multiple range tests. Results are the mean ±SE

Plantlet establishment in soil

The optimal conditions to germinate zygotic embryos were determined to be on photoperiod in the MS medium

with a half of its ionic strength (½ MS). Zygotic embryos germinated at day 6 (Figure 4B). Shoots developed and were maintained for 3 weeks (Figure 4C). On the contrary, in field coffee seeds germinate slowly (Rena et
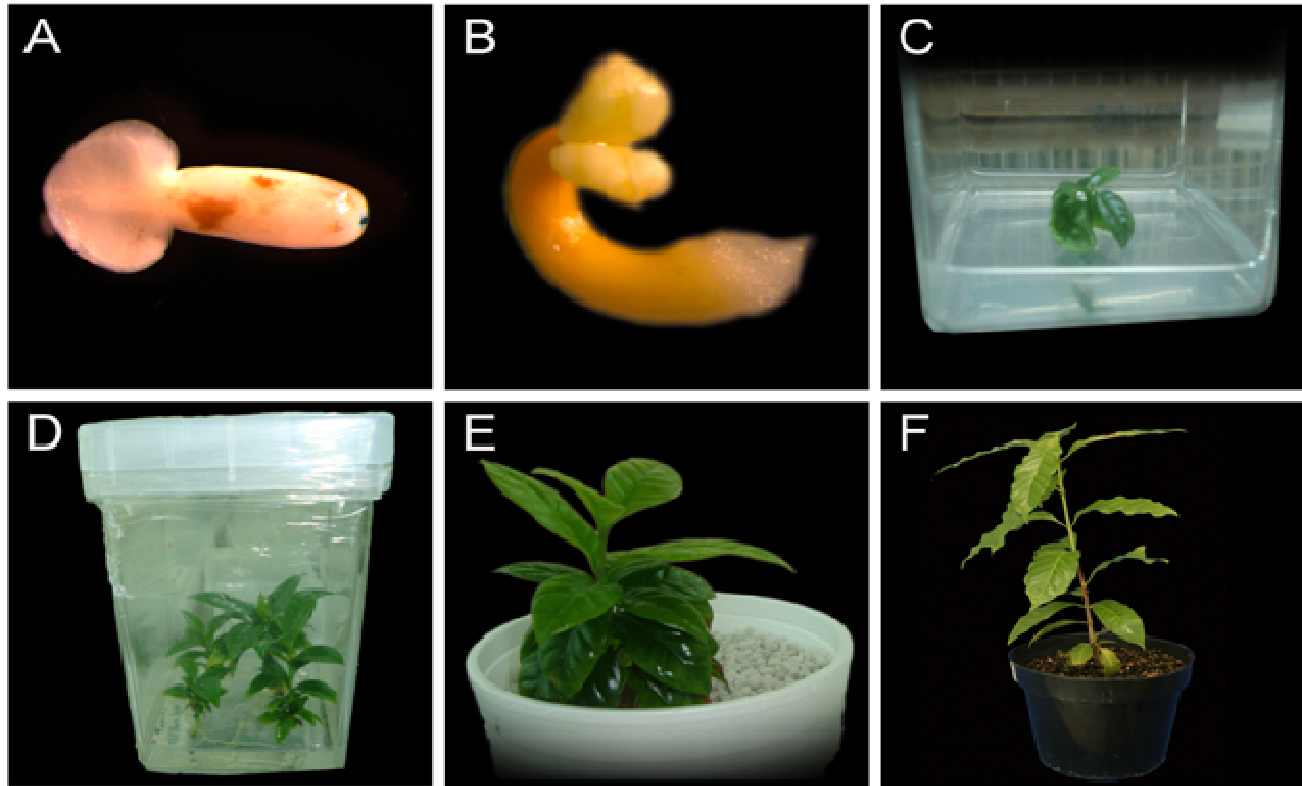
Figure 4. *In vitro* plantlet culture and establishment in soil. Extracted zygotic embryos (A) germinated in the MS medium with a half of its ionic strength (½ MS) at day 6 (B). Shoots developed and were maintained for 3 weeks (C). Plantlets were transferred to fresh medium and incubated until they were 8 weeks old (D). Subsequently, plantlets were placed individually in small pots containing sterilized agrolite, and left uncovered at the greenhouse (photoperiod at 28± 2 ℃) for two weeks more (E). Established plant which has grown in greenhouse for more than 1 year (F).

al., 1986). *C. arabica* seedling emergence from the soil starts 8 weeks after sowing in the warmer periods of the year (Maestri and Vieira, 1961). When temperatures are lower, the emergence period may increase to 12 weeks (Went, 1957). At the end of 3 weeks, plantlets produced radicles and were transferred to fresh medium and incubated under same conditions until they had 8 weeks old (Figure 4D). Plantlets were >5 cm height with a primary root at the time of transfer to *ex vitro* which was at 10 to 12 weeks (Figure 4E). They had already been in the greenhouse and continued their development for more than 30 weeks. Although we did not evaluate the percentage of plantlet survival *ex vitro*, plantlets eventually survived and have grew for more than 12 months (Figure 4F). Thus, we were able to establish plantlets in soil occupying a time of 11 to 13 weeks.

DISCUSSION

The coffee (*C. arabica*) fruit is a drupe containing two seeds. The coffee seed is comprised of an endosperm that envelops an embryo and a peripheral spermoderm or 'silver skin' (Krug and Carvalho, 1939; Mendes, 1941).

The coffee endosperm is composed of a hard greenish tissue and is divided into a hard external endosperm and a soft internal endosperm (Dedecca, 1957). The thickened cell walls of the endosperm are composed mainly of mannans with 2% of galactose (Wolfrom et al., 1961; Bewley and Black, 1994). The differentiated embryo lies inside an embryo cavity (Rena and Maestri, 1986). Seed germination 'begins with the water uptake by the seed and ends with the elongation of the embryonic axis, usually the 'radicle' (Bewley and Black, 1994). Under field conditions, coffee seed germination is relatively slow (Maestri and Vieira, 1961; Rena and Maestri, 1986). To solve this inconvenience, techniques are being researched to massively increase production of coffee plants, increase the capacity of plant multiplication, and produce specific agronomic traits. Plant tissue culture offers different opportunities to increase productivity, diversification, and production of different varieties with new properties in a relatively short time and in an efficient way. In this way, some reports about *in vitro* experiments have been developed and showed that endogenous ABA-like substances and exogenous ABA caused inhibition of coffee seed germination through inhibition of embryo growth (Valio, 1976). Da Silva et al.

(2004) showed that ABA is endogenously synthesized de novo in the embryo coffee seed, and ABA content in the embryos coincided with the cessation of growth (Da Silva et al., 2008). Here, we evaluated the effect on germination when phytohormones were applied directly to zygotic embryos. We show here that ABA has a similar effect when applied directly on zygotic embryos, increasing the germination time even when used without endosperm. Using ABA as the phytohormone, germination times ranged between 12 and 17 days (Figure 2A). On the other hand, contrary to reports on the stimulatory effect of GA during seed germination and tissue elongation, GA3 inhibited radicle protrusion (Valio, 1976; Takaki and Dietrich, 1979a; Takaki et al., 1979) and seedling emergence in coffee seed (Maestri and Vieira, 1961). This inhibition was proposed to be caused by mannose, a degradation product of the hydrolysis of mannans that are included in the cell walls of the endosperm (Takaki and Dietrich, 1980). Our results correlated with this proposal since GA was the plant growth regulator which caused earlier germination times by 4 to 7 days when the endosperm was not present (Figure 2A). For germination times of zygotic embryos of coffee, the priority order of efficiency is GA > KIN > SA > IBA > NAA > ABA (Figure 3A). Also, with the exception of SA, concentrations higher than 0.1 mg l^{-1} increased the zygotic embryo germination times (Figure 2A; Figure 3B). Similar results were reported concerning germination times that increased when higher concentrations of phytohormones were used (Valio, 1976; Pech-Ake et al., 2007). The experiments reported here confirmed the suggestion that some plant growth regulators that were previously detected in endosperm could control both growth and germination of embryos (Valio, 1976; Takaki and Dietrich, 1979a, b, 1980; Takaki et al., 1979; Da Silva et al., 2004; Da Silva et al., 2008). Finally, this study reveals a reduced germination time of zygotic embryos from *C. arabica* when treated with phytohormones. We also were able to establish plantlets in soil, in a reduced time compared with that of conventional germinated plantlets (Figure 4). It will aid in rapidly obtaining a research model to develop studies *in vitro*; although the data obtained also could be used to improve a propagation method as a major tool for both basic and practical investigation in agriculture. This *in vitro* technique will allow, by using the coffee plantlet as a 'model' studies at the cellular and molecular levels and has (among the woody species) great potential in molecular biology, biochemical, and biotechnological research.

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