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ORIGINAL ARTICLE

The effects of colchicine on banana stem apex

Efeitos da colchicina em ápices caulinares de bananeira

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ABSTRACT: The cultivation of traditional varieties of banana in Brazil has suffered from major pests and diseases. The breeding of new strains of banana based on the duplication of chromosomes via treatment with antimitotic agents has been proposed as a way of introducing disease resistance into the hybrids produced by banana breeding programs. The aim of this study was to evaluate the action of colchicine in the apex of diploid banana using an antimitotic at different concentrations and exposure times. Apexes of diploid banana 1318-01 were used as plant material. Colchicine was used at concentrations of 0 (control), 2.5, 5.0, 7.5, 10.0 and 12.5 mM under agitation (20 rpm) for periods of 24 and 48 h. We assessed the survival rate of plants subjected to colchicine. The highest survival rate of the explants (91.7%) in 2.5 mM colchicine was found for 24 h of exposure to the antimitotic, and a survival rate of 50% was found at a concentration of 7.5 mM colchicine for 48 h of exposure. The study showed that the survival of the explants was relatively high after the exposure of diploid 1318-01 to colchicine for a period of 24 h.

RESUMO: *O plantio de variedades de bananeira tradicionais no Brasil tem sido um fator de vulnerabilidade às principais pragas e doenças que afetam a cultura. Diante disso, estratégias alternativas de melhoramento genético, fundamentadas na duplicação de cromossomos induzida por tratamentos com agente antimitótico, têm sido propostas como forma de introduzir resistência a doenças nos híbridos gerados pelos programas de melhoramento genético da bananeira. O objetivo deste trabalho foi verificar a ação da colchicina em ápices caulinares de bananeira diplóide, utilizando-se diferentes concentrações e tempos de exposição ao antimitótico. Como material vegetal, foram utilizados ápices caulinares de bananeira do diplóide 1318-01. A colchicina foi utilizada nas concentrações de zero (tratamento controle); 2,5; 5,0; 7,5; 10,0; 12,5 mM, em solução, sob agitação (20 rpm), por períodos de 24 e 48 h. Foi avaliada a taxa de sobrevivência de plantas submetidas à colchicina. Verificou-se maior sobrevivência dos explantes (91,7%) em 2,5 mM de colchicina por 24 horas de exposição ao antimitótico e 50% na concentração de 7,5 mM de colchicina por 48 horas de exposição. Conclui-se que a exposição do diplóide 1318-01 à colchicina por um período de 24 h possibilita maior taxa de sobrevivência dos explantes.*

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1 Introduction

Banana cultivation is socially and economically important in more than 80 countries. Bananas are grown primarily on small holdings. Bananas are among the leading tropical fruit products. Brazil is the fifth largest producer of bananas in the world, behind India, the Philippines, China and Ecuador (ANUÁRIO BRASILEIRO DE FRUTICULTURA, 2011). In Brazil, banana cultivation extends from the North to the South. The planting of traditional varieties of banana has been associated with vulnerability to major pests and diseases that affect banana culture, such as black sigatoka (*Mycosphaerella fijiensis* Morelet), yellow sigatoka (*Mycosphaerella musicola* Leach) and panama disease (*Fusarium oxysporum* f. sp. *cubense*) (CORDEIRO; MATOS; KIMATH, 2005).

The great vigor of plants of the genus *Musa* is directly associated with ploidy, as triploid and tetraploid varieties are more vigorous than diploid varieties (STOVER; SIMMONDS, 1987). Although tetraploid hybrids generally perform less well than triploids in standard tests involving resistance to damage by a falling object and resistance to bending, it is possible to improve this performance through the use of a triploid (AAA) cultivar after a cross with an elite diploid. This procedure is expected to yield new banana cultivars (DANTAS et al., 1999).

Alternative strategies for the genetic improvement of banana have been based on the duplication of chromosomes, which can be induced by treatment with antimitotic agents. This approach has been proposed as a way of introducing disease resistance into hybrids generated by breeding programs.

Colchicine is often used to induce polyploidy in breeding programs aimed at the improvement of agricultural crops, forest species and ornamental plants. Its mechanism of action is known. It reversibly binds to tubulin dimer, causing a conformational change that prevents the polymerization of the mitotic spindle and, therefore, blocks the cell in metaphase. At lower concentrations, colchicine promotes the depolymerization of the mitotic spindle, leading to the accumulation of metaphase chromosomes. In this phase of mitosis, the chromatids are condensed, and their arms are separated as a result of their natural repulsion in the absence of centromere division. This technique, among others, can be used for karyotyping (MONDIN; DOCHA NETO, 2006).

Despite its widespread use, colchicine has high toxicity human, high phytotoxicity in vitro and when used in high concentrations may increase the frequency of the regenerated plants mixoploidy (VAN DUREN et al., 1996; GANGA; CHEZHIYAN, 2002; COSTA et al., 2011).

However, colchicine also causes the regenerated plants to show sterility and abnormalities (WAN; PETOLINO; WIDHOLM, 1989). Therefore, various procedures for inducing polyploidy have been applied to attain greater treatment efficiency. These procedures involve various concentrations, exposure times and forms of application of substances that induce polyploidy. These procedures have become indispensable in breeding programs aimed at chromosome duplication.

The aim of this study was to investigate the action of colchicine in diploid banana shoot tips using various concentrations and times of exposure to this antimitotic agent.

2 Materials and Methods

This work was performed at the Laboratory of Tissue Culture, located in the Department of Agriculture, Federal University of Lavras, Lavras/MG, using an improved diploid banana cultivar developed by the breeding program of Embrapa Cassava and Tropical Fruits.

Shoot apexes of diploid banana 1318-01 (Malaccensis FHIA x Sinwobogi), pre-established in vitro, were used as plant material. This material was then multiplied on MS medium (MURASHIGE; SKOOG, 1962) supplemented with 30 g L^{-1} sucrose and 4 mg L^{-1} 6-benzylaminopurine (BAP) to allow the proliferation of shoots treated with colchicine.

Colchicine was used at concentrations of 2.5, 5.0, 7.5, 10.0 and 12.5 mM. The containers in which the plant material was exposed to colchicine were stirred (20 rpm) for periods of 24 and 48 h. Additional plant material was kept in vitro to serve as an untreated control. After 24 and 48 h, the apexes exposed to the antimitotic were removed from the container, washed three times in distilled water and autoclaved. They were then transferred to test tubes containing 15 mL of MS medium and subsequently maintained in a growth room with artificial lighting provided by specialized daylight fluorescent bulbs (OSRAM 20 W), with an average irradiance of 42 W m^{-2}, a 16 h photoperiod and a temperature of 25±2 °C.

The experimental design was a completely randomized 5 × 2 factorial layout (five concentrations x two periods of exposure). A total of 12 plants were used per treatment, i.e., four replicates with three plants each. The survival rate of plants exposed to colchicine after a 60 day period *in vitro* was evaluated. The data were analyzed with a polynomial regression using SISVAR software (FERREIRA, 2011).

3 Results and Discussion

In a study of the effects of colchicine on orange embryos, Gmitter Junior and Ling (1991) found high embryonic mortality in the orange varieties Hamlin and Pineapple Ridge. These results showed that the embryos were highly sensitive to colchicine. Additionally, Yang et al. (2006) observed a decrease in the survival of *Vitis vinifera* with increasing concentrations of colchicine and exposure time.

During an exposure of 24 h, higher concentrations of colchicine caused higher explant mortality (Figure 1a). Tissue necrosis was evident. It is possible that this necrosis represented a toxic effect of the antimitotic. Similar observations have been reported by Hamill, Smith and Dodd (1992) who found that explants initially treated with colchicine had a lower growth rate than controls and that mortality increased from 0 to 70% as the concentration of the antimitotic increased to 25 mM.

In the current study, a relatively high survival rate was observed at concentrations of 2.5 and 5.0 mM colchicine for 24 h of exposure (Figure 2a). These results are consistent with the findings of Van Duren et al. (1996), who observed that colchicine treatment generally caused a lower rate of regeneration relative to the control. Viehmannová et al. (2009), working with yacon (*Smallanthus sonchifolius*), observed a relatively high explant survival rate at colchicine concentrations of 5.0 mM over 24 h. In diploid banana, Rodrigues et al. (2011) observed that the survival rate

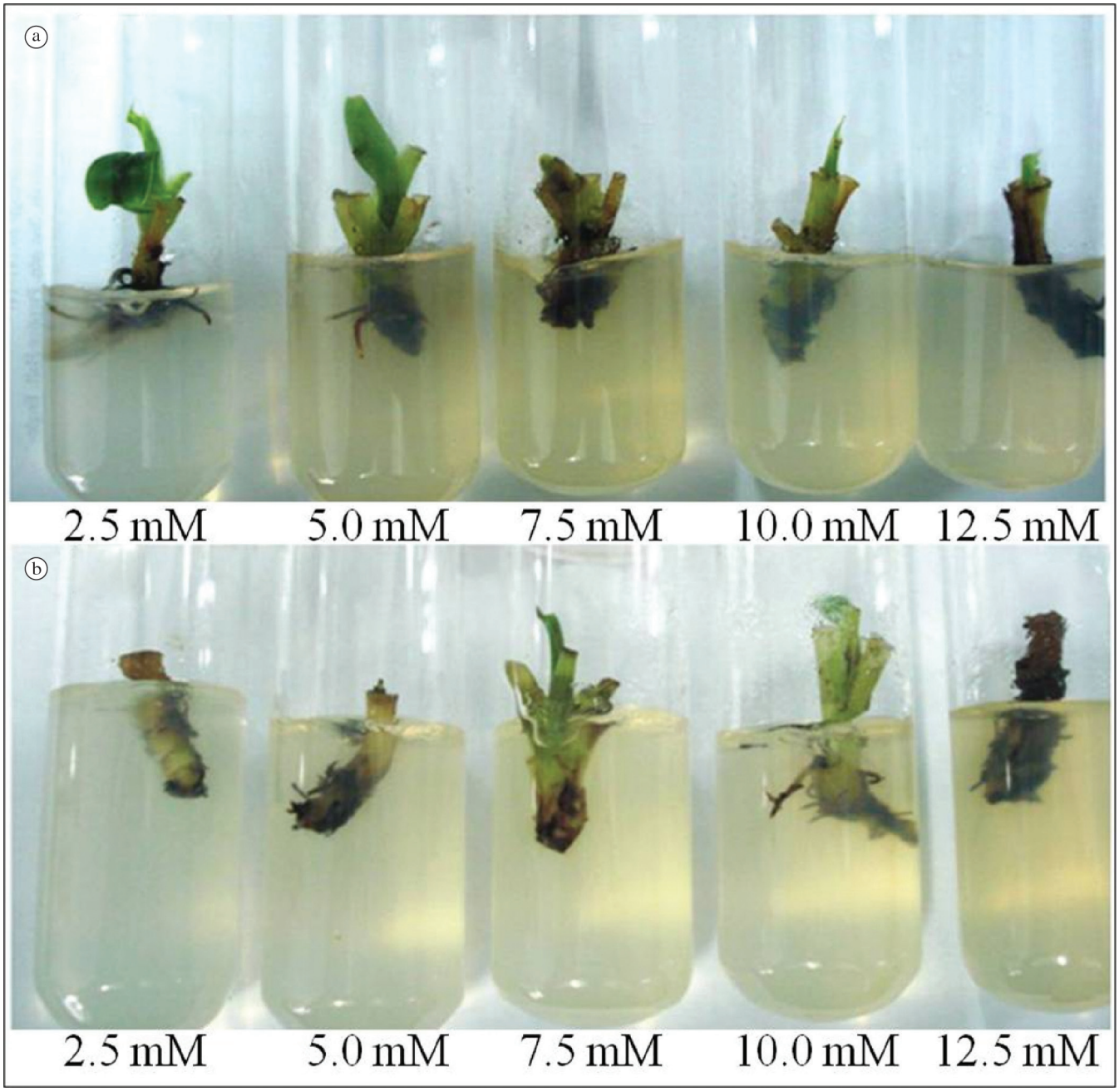

Figure 1. Diploid banana shoot apices subjected to various concentrations of colchicine for 24 h (a) and 48 h (b).

of explants was relatively low after exposure to 5.0 mM colchicine for 48 or 24 h. Unemoto et al. (2009) have stated that the duration of exposure to colchicine is an important factor that affects both the survival and the vegetative development of the plants.

Exposure of banana explants to 2.5 and 5.0 mM colchicine for 48 h (Figure 2b) produced a relatively low survival rate. A similar trend was observed by Barbosa, Davide and Pereira (2007), who found that colchicine drastically affected the development of meristems and stem segments.

According to Thao et al. (2003), the survival of explants treated with colchicine depends on the concentration and the duration of the treatment. Generally, higher concentrations and longer periods of exposure to the inducing agent decrease the survival of seedlings. Depending on the species, colchicine may have a greater cytotoxic effect than other antimitotic substances, producing sterility, abnormal growth and morphology and the loss or rearrangement of chromosomes. In contrast, Vichiato et al. (2007), working with *Dendrobium nobile* Lindl, showed that immersion of the plants in a 1.25 mM or 2.5 mM solution of colchicine for a maximum of 96 h did not affect the survival of the plants until seven months after treatment.

Latado et al. (2007) have found that colchicine was toxic to explants of orange 'Pera-de-abril' and tangor 'Murcott'. They observed symptoms of toxicity and mortality, including

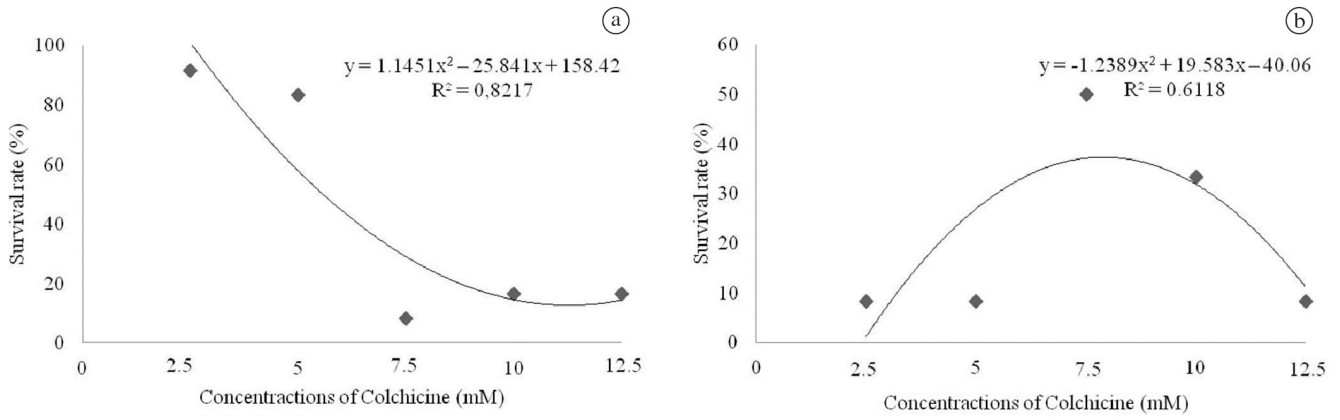

Figure 2. Survival rate of diploid banana explants subjected to various concentrations of colchicine for 24 h (a) and 48 h (b).

browning and decay of the tips of the segments. Wakana et al. (2005) have investigated the development of branches and grafting success in three species of *Citrus* whose cuttings were treated with a solution containing 20 mM colchicine.

Wu and Mooney (2002) observed a colchicine-induced decrease in the capacity for regeneration and an associated increase in mortality in several species of *Citrus*. The study investigated *Citrus* callus and immature seeds. In addition to the method of applying the antimitotic, the use of high concentrations of colchicine or inappropriate exposure times for particular tissues may cause the death of cells and plants because colchicine tolerance varies according to the species (SCHIFINO-WITTMANN, 2000).

4 Conclusions

The exposure of diploid banana 1318-01 to colchicine at 2.5 mM for a 24-h period yields relatively high survival in explants.

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