Notas Científicas

Simulation of in vitro water deficit for selecting drought-tolerant banana genotypes

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Abstract – The objective of this work was to determine a method to simulate water stress in vitro to select drought-tolerant banana (*Musa* sp.) genotypes. The BRS Tropical and Prata Anã cultivars were grown in vitro in an MS liquid medium containing sucrose and benzylaminopurine (BAP), supplemented with different concentrations of polyethylene glycol (PEG) or sorbitol. The yield stability index of dry matter was evaluated. Cultivation for 30 days in a medium containing 15 g L^{-1} PEG or 36.4 g L^{-1} sorbitol is appropriate to simulate water stress in vitro.

Index terms: *Musa*, PEG, sorbitol, tissue culture.

Simulação de deficit hídrico in vitro para seleção de bananeiras tolerantes à seca

Resumo – O objetivo deste trabalho foi determinar uma metodologia para simulação de estresse hídrico in vitro, para seleção de genótipos de bananeira (*Musa* sp.) tolerantes à seca. As cultivares BRS Tropical e Prata Anã foram cultivadas in vitro em meio MS líquido, contendo sacarose e benzilaminopurina (BAP), e suplementado com diferentes concentrações de polietileno glicol ou de sorbitol. Avaliou-se o índice de estabilidade de rendimento de matéria seca. O cultivo por 30 dias em meio contendo 15 g L^{-1} de PEG ou 36,4 g L^{-1} de sorbitol é apropriado para simular a condição de estresse hídrico in vitro.

Termos para indexação: *Musa*, PEG, sorbitol, cultura de tecido.

Soil water stress is among the limiting factors when cultivating banana plants (Placide et al., 2012; Vanhove et al., 2012). In the context of climate change and of the conservation of water resources, and considering agricultural activity is responsible for 72% of the water consumed in Brazil (ANA, 2013), it is clearly necessary to look for alternative strategies to cope with water shortages. The search by genetic improvement programs for genotypes that use water more efficiently, with a greater capacity of osmotic adjustment and stomatal control, is considered the most efficient and economical strategy to address the problem of drought. Banana cultivars that contain the B genome appear to be more drought-tolerant that those with only the A genome (Robinson & Galán Saúco, 2010).

Field studies to select drought-tolerant banana genotypes are difficult because of the long cultivation cycle and the need for large areas due to the size of the plants. Thus, in vitro selection is advantageous because it is faster and less plant material is required, allowing for the rapid multiplication of selected genotypes (Rai et al., 2011), control of conditions, and the detection of differences in reduced growth (Vanhove et al., 2012).

There are reports of using polyethylene glycol (PEG) or sorbitol as water stress inducers of various plant species, including banana (Rai et al., 2011; Bidabadi et al., 2012; Placide et al., 2012; Vanhove et al., 2012). Sorbitol is a solute that is normally not metabolized by plants, and PEG is an impermeable, long-chain polymer. These compounds induce water stress by both reducing the osmotic potential of the culture medium and not being metabolized by the plants. However, it is necessary to consider the ideal concentrations of the inducers to discriminate among

tolerant and sensitive genotypes, based on growth parameters, without lethally damaging the plants. The ideal concentration is one where none of the cultivars completely stops growing, but growth is reduced compared with a control with no inducers (Placide et al., 2012).

Growth reduction is an important parameter to evaluate the tolerance of a plant to water stress. This is because growth is directly related to mass, and the possible loss of mass when a plant is under stress is a fundamental parameter when discriminating tolerant genotypes (Vanhove et al., 2012).

Despite the existence of reports of in vitro selection of drought-tolerant banana plants (Bidabadi et al., 2012; Placide et al., 2012; Vanhove et al., 2012; Moreno-Bermudéz et al., 2015), there are still no results for the Prata Anã and BRS Tropical cultivars. Prata Anã is among the most cultivated and commercialized cultivar in Brazil, and the BRS Tropical cultivar tends to be drought tolerant (Silva et al., 2009). For this reason, it is necessary to establish an adequate method to study the simulation of water stress in vitro for these cultivars.

The objective of this work was to determine a method to simulate water stress in vitro to select drought tolerant banana genotypes.

The Prata Anã (AAB genome) and BRS Tropical (AAAB genome) banana cultivars were assessed. The BRS Tropical cultivar is a tetraploid hybrid produced by crossing Yangambi N.2 and the diploid M53, and was obtained from Embrapa's banana breeding program. Plants of the two genotypes with the most vigorous and uniform growth, maintained in culture medium (Murashige & Skoog, 1962) for 50 days to root, were selected and used as explants. These were cut to a height of 3.0 cm by removing the leaves and maintaining three roots (1 cm long each).

The explants were transferred to a liquid medium composed of MS salts, 30 g L^{-1} sucrose and 4 mg L^{-1} benzylaminopurine (BAP), supplemented with different concentrations of PEG or sorbitol, with the purpose of simulating water stress in vitro. The final volume of the medium was adjusted with sterilized distilled water. For both the inducers, four different concentrations were used: 15, 30, 45, and 60 g L^{-1} PEG_{6000} (Bidabadi et al., 2012); or 18.2, 36.4, 54.6, and 72.8 g L^{-1} sorbitol (Placide et al., 2012; Vanhove et al., 2012). For the control treatment, plants were maintained in a MS medium that lacked an inducer. The osmotic potentials of the culture mediums containing PEG or sorbitol were determined according to Villela et al. (1991) and Placide et al. (2012), respectively.

The plants were maintained in a growth room with light intensity of 36 μmol m^{-2} s^{-1}, photophase of 16 hours, and temperature of 27±2°C. For the explants, the culture medium was replenished and fresh material was collected every 15 days.

The duration of the experiment was 60 days. At the end, the dry matter was quantified for both the aerial part and roots after drying the material in a dryer with forced ventilation at 70±2°C for 72 hours.

Discriminating among the tolerant and sensitive material was done by calculating the yield stability index of dry matter (YSIdm) (Bouslama & Schapaugh, 1984), with the formula YSIdm = Ye/Yi, where Ye and Yi are, respectively, the dry matter of each genotype cultivated under stress and no stress. Thus, the higher the YSIdm, the greater the tolerance to drought. The index of fresh matter (YSIfm) was calculated in a similar way, but with fresh matter of the cultivated genotype under stress and no stress.

An experiment was performed for each inducer. Each experiment consisted of five treatments (four concentrations of PEG or four of sorbitol and one control) and two genotypes (Prata Anã and BRS Tropical), with 36 replicates; each replicate was one plant. The data were subjected to the F-test of the analysis of variance, at 5% probability. The averages of the treatments were compared using Tukey's test, at 5% probability. In relation to the YSIdm, a completely randomized design was used in a 5x2 factorial arrangement.

In this work, the biomass was the parameter used to discriminate the tolerance of the genotypes (Vanhove et al., 2012). A decrease in biomass is a consequence of a reduced photosynthetic rate and the deviation of energy destined for growth to activate and maintain metabolic activities associated with adapting to drought (Munns et al., 2002). A lower biomass in plants subjected to the condition of water stress has been reported in studies about drought tolerance, including bananas (Van Asten et al., 2011; Bidabadi et al., 2012; Placide et al., 2012; Vanhove et al., 2012).

In relation to the PEG inducer, after 60 days of cultivation with the 15 g L^{-1} concentration, both genotypes differed from the control, tolerated water deficit, and showed characteristics of in vitro growth

(Figure 1 A). The results confirm the hypothesis that the adequate concentration to simulate water deficit in vitro is the one where none of the cultivars tested completely stop growing, but exhibit reduced growth compared with the control (Placide et al., 2012).

Bidabadi et al. (2012) found that 30 g L^1 PEG was an adequate concentration to select and characterize in vitro drought-tolerant banana cultivars. Moreno-Bermúdez et al. (2015) also successfully simulated water stress in vitro using 30 g L^{-1} PEG. However, in the present study, concentrations above 30 g L^{-1} did not differ significantly among the treatments and caused a more drastic reduction in plant growth, which means higher concentrations of PEG are not recommended to select the genotypes under in vitro drought conditions (Figure 1 A).

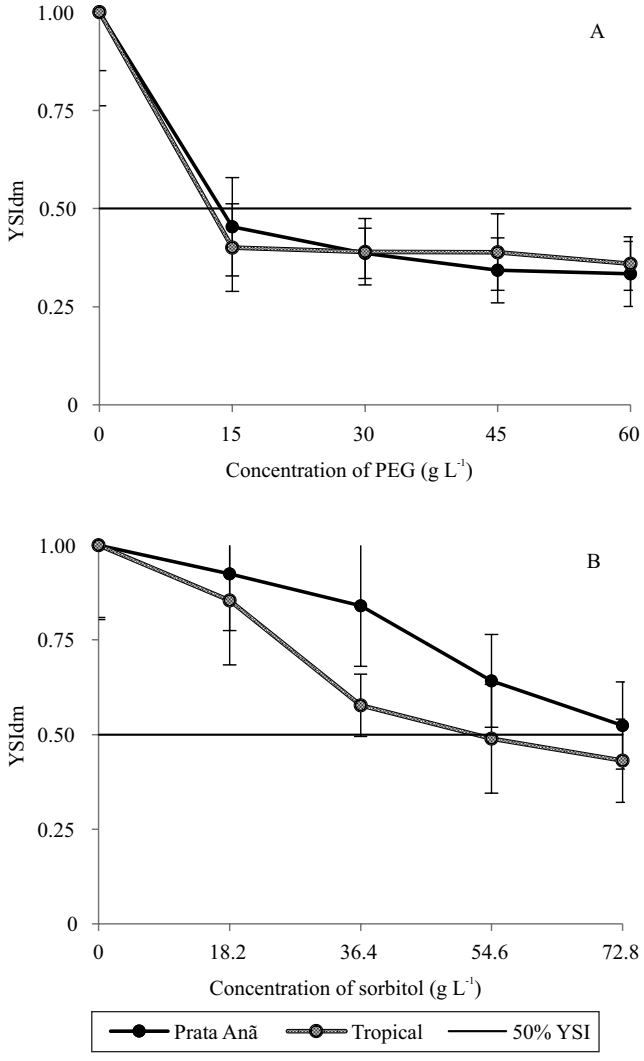

Figure 1. Yield stability index of dry matter (YSIdm) for the BRS Tropical and Prata Anã banana (*Musa* sp.) cultivars after 60 days of cultivation in vitro in MS medium with different concentrations of polyethylene glycol (PEG) (A) and sorbitol (B).

For the sorbitol inducer, both cultivars evaluated also developed less, with lower dry matter (YSIdm) for the treatments compared with the control after 60 days of cultivation; however, the proportion was lower than that to using PEG (Figure 1 B). For the treatments with 36.4 g L^{-1} sorbitol, both genotypes differed from the control, tolerated the water deficit, and showed characteristics of growth in vitro (Figure 1 B). Therefore, this is considered an adequate concentration for this inducer. Other studies that simulated water stress in vitro for bananas, using the Williams, Mbwazirume, Cachaco, Obino l'Ewai, Lep Chang Kut and Popoulou cultivars, indicated a 36.4 g L^{-1} concentration of sorbitol as adequate for selecting drought tolerance. In addition, and in agreement with the results obtained here, these studies suggest that sorbitol concentrations over 36.4 g L^{-1} cause excessive osmotic stress in the medium, drastically affecting the capacity of the plants to absorb water (Placide et al., 2012; Vanhove et al., 2012).

The YSIfm analysis indicated that the action of the water stress on the plants was already visible in the fourth week of cultivation and, therefore, this time is sufficient to discriminate between the genotypes that are tolerant and sensitive to the drought conditions simulated and tested in vitro (Table 1). These results

Table 1. Yield stability index of fresh matter (YSIfm) for the BRS Tropical and Prata Anã banana (*Musa* sp.) cultivars at 15, 30, 45, and 60 days of cultivation in vitro in MS medium, with the addition of polyethylene glycol (PEG) or sorbitol to determine the best concentration to simulate water deficit[(1)].

Concentration	Prata Anã			
	15 days	30 days	45 days	60 days
15 g L^{-1} PEG	0.57A	0.43B	0.36C	0.29D
36.4 g L^{-1}Sorbitol	0.44AB	0.47A	0.42B	0.37C
	BRS Tropical			
15 g L^{-1} PEG	0.41 A	0.37B	0.34BC	0.30C
36.4 g L^{-1}Sorbitol	0.40A	0.33B	0.33B	0.30B

[(1)]Means followed by equal letters, in the lines, do not differ by Tukey's test, at 5% probability.

corroborate those obtained by other authors (Van Asten et al., 2011; Bidabadi et al., 2012; Placide et al., 2012).

Thus, it is concluded that the Prata Anã and BRS Tropical cultivars, grown under water stress induced by PEG, present a greater reduction in growth than those grown in sorbitol. Furthermore, the best concentrations for simulating water stress in vitro are 15 g L^{-1} PEG and 36.4 g L^{-1} sorbitol when growing these cultivars in MS medium. A period of 30 days of cultivation, for both inducers, is the most appropriate amount of time to simulate the condition of water stress and to select tolerant genotypes.

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References

ANA. Agência Nacional de Águas. **Conjuntura dos recursos hídricos no Brasil**: relatório do ano de 2012. Brasília, 2013. 432p. Available at: <http://arquivos.ana.gov.br/institucional/spr/conjuntura/PDFs%20agregados/ANA_Conjuntura_Recursos_Hidricos_Brasil_capitulos_.pdf>. Accessed on: Mar. 16 2017.

BIDABADI, S.S.; MEON, S.; WAHAB, Z.; SUBRAMANIAM, S.; MAHMOOD, M. In vitro selection and characterization of water stress tolerant lines among ethyl methanesulphonate (EMS) induced variants of banana (*Musa* spp., with AAA genome). **Australian Journal of Crop Science**, v.6, p.567-575, 2012.

BOUSLAMA, M.; SCHAPAUGH, W.T. Stress tolerance in soybeans. I. Evaluation of three screening techniques for heat and drought tolerance. **Crop Science**, v.24, p.933-937, 1984. DOI: 10.2135/cropsci1984.0011183X002400050026x.

MORENO-BERMÚDEZ, L.J.; REYES, M.; GÓMEZ-KOSKY, R.; URQUIZA, M.R.; CHONG-PÉREZ, B. Efecto del estrés hídrico inducido con PEG 6000 sobre el contenido de agua de plantas in vitro de *Musa* spp. Grande naine (AAA) y Pelipita (ABB). **Biotecnología Vegetal**, v.15, p.251-254, 2015.

MUNNS, R.; HUSAIN, S.; RIVELLI, A.R.; HARE, R.A. **Progress in plant nutrition**. Dordrecht: Kluwer Academic, 2002. 188p.

MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. **Physiologia Plantarum**, v.15, p.473-497, 1962. DOI: 10.1111/j.1399-3054.1962.tb08052.x.

PLACIDE, R; CHRISTIAN, C.S.; RONY, R. Development of in vitro technique to screen for drought tolerant banana varieties by sorbitol induced osmotic stress. **African Journal of Plant Science**, v.6, p.416-425, 2012. DOI: 10.5897/AJPS12.101.

RAI, M.K.; KALIA, R.K.; SINGH, R.; GANGOLA, M.P.; DHAWAN, A.K. Developing stress tolerant plants through *in vitro* selection - An overview of the recent progress. **Environmental and Experimental Botany**, v.71, p.89-98, 2011. DOI: 10.1016/j.envexpbot.2010.10.021.

ROBINSON, J.C., GALÁN SAÚCO, V. (Ed.). **Bananas and plantains**. 2nd ed. Oxford: CAB International, 2010. 311p. (Crop production science in horticulture series, 19). DOI: 10.1079/9781845936587.0000.

SILVA, A.J.P. da; COELHO, E.F.; MIRANDA, J.H. de; WORKMAN, S.R. Estimating water application efficiency for drip irrigation emitter patterns on banana. **Pesquisa Agropecuária Brasileira**, v.44, p.730-737, 2009. DOI: 10.1590/S0100-204X2009000700013.

VAN ASTEN, P.J.A.; FERMONT, A.M.; TAULYA, G. Drought is a major yield loss factor for rainfed East African highland banana. **Agricultural Water Management**, v.98, p.541-552, 2011. DOI: 10.1016/j.agwat.2010.10.005.

VANHOVE, A.C.; VERMAELEN, W.; PANIS, B.; SWENNEN, R.; CARPENTIER, S.C. Screening the banana biodiversity for drought tolerance: can an in vitro growth model and proteomics be used as a tool to discover tolerant varieties and understand homeostasis. **Frontiers in Plant Science**, v.3, p.1-10, 2012. DOI: 10.3389/fpls.2012.00176.

VILLELA, F.A; DONI, L; SEQUERA, E.L. Tabela de potencial osmótico em função da concentração de polietileno glicol 6.000 e da temperatura. **Pesquisa Agropecuária Brasileira**, v.26, p.1957-1968, 1991.

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