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Propagation

Physiological analysis of micropropagated banana 'BRS Conquista' seedlings acclimatized under different substrates and organomineral fertilizer doses

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Abstract-In the production of micropropagated seedlings, an important step is the exposure of plants to local environmental conditions, called acclimatization. Thus, the aim of this study was to evaluate the physiological response of banana 'BRS Conquista' cultivar, under different substrates and organomineral fertilizer doses during acclimatization. The experimental design used was a 3 x 5 factorial scheme (substrates x organomineral fertilizer doses), with five replicates and five seedlings per plot. The substrates used were: coconut fiber, pine bark and 100% natural mixed commercial formula. The organomineral fertilizer used was 4-14-8 at doses of 0, 1,000, 2,000, 3,000 and 4,000 g m^{-3}. At 60 days after seedlings transplant, evaluations were performed regarding radiation incidence (Qleaf), internal CO_2 concentration (Ci), stomatal conductance (Gs), transpiration rate (E), photosynthesis (A), mesophyll conductance (Gm), chlorophyll fluorescence (F), maximum leaf fluorescence in light (Fm) and leaf area index (LAI). It was observed that the pine bark substrate provided adequate physiological levels for the development of banana seedlings during acclimatization, as well as higher leaf area values. Intermediate organomineral fertilizer doses (1,000 to 3,000 g m^{-3}) resulted in higher photosynthetic rates.

Index terms: Micropropagation, *Musa* spp., fertilization.

Análise fisiológica de mudas micropropagadas de bananeira 'BRS Conquista' aclimatadas em diferentes substratos com utilização de fertilizante organomineral

Resumo- Na produção de mudas micropropagadas, uma etapa importante é a exposição das plantas às condições ambientais locais, denominada de aclimatação. Assim, objetivou-se avaliar a resposta fisiológica da cultivar de banana BRS Conquista sob diferentes tipos de substratos e dosagens de fertilizante organomineral durante a aclimatação. O delineamento experimental utilizado foi em esquema fatorial 3 x 5 (substratos x doses de fertilizantes organomineral), com cinco repetições e cinco mudas por parcela. Os substratos foram: fibra de coco, casca de pínus e formulado comercial misto, 100% natural. O fertilizante organomineral empregado foi o 4-14-8 nas dosagens 0; 1.000; 2.000; 3.000 e 4.000 g m^{-3}. Aos 60 dias após o transplantio das mudas, foram realizadas as avaliações referentes à incidência da radiação (Qleaf), concentração interna de CO_2 (Ci), condutância estomática (Gs), taxa de transpiração (E), fotossíntese (A), condutância do mesófilo (Gm), fluorescência da clorofila (F), fluorescência máxima da folha em ambiente iluminado (Fm) e índice de área foliar (IAF). Observou-se que o substrato casca de pínus proporcionou níveis fisiológicos adequados para o desenvolvimento das mudas de bananeira durante a aclimatação assim como maiores valores para a área foliar. Doses intermediárias do fertilizante organomineral (1.000 a 3.000 g m^{-3}) resultaram em maiores taxas fotossintéticas.

Termos para indexação: Micropropagação, *Musa* spp., adubação.

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Banana (*Musa* spp.) stands out worldwide, being, in 2017, the most produced fruit, totaling 153.2 million tons (FAOSTAT, 2021). Therefore, due to its importance, studies addressing gas exchange are relevant for understanding the physiological responses to local conditions, allowing the use of information related to changes in transpiration rates, stomatal conductance, photosynthesis, fluorescence parameters and water use efficiency as physiological indicators of the presence of stress before symptoms are visible. It is also possible to extrapolate these results to establish more adequate management practices in production systems (TURNER et al., 2007; DONATO et al., 2016; ARANTES et al., 2016).

Acclimatization is an essential step in the production of micropropagated seedlings, as it involves exposing seedlings to an *ex vitro* environment, with controlled conditions close to those to which plants will be exposed during field cultivation. However, this is a transition stage that aims both at adapting seedlings to local environmental conditions and stimulating their growth and development (MARTINS et al., 2011). Among management strategies used during the acclimatization of micropropagated banana seedlings, the choice of the substrate and nutritional supplementation used is of great importance, as their physical, chemical and biological characteristics influence the physiological aspects of plants (ARANTES et al., 2016; DONATO et al., 2016).

Organomineral fertilizers have been widely used due to their higher concentration of nutrients when compared to organic fertilizers and greater amounts of organic matter when compared to mineral fertilizers (KIEHL, 2008). Studies such as that carried out by Lima et al. (2006) and Nomura et al. (2012) have been developed in order to evaluate the response of banana plants to the use of organomineral fertilizers; however, their approach is mainly phytotechnical, and it is interesting to develop studies addressing the physiological response of plants to this management.

Banana 'BRS Conquista' cultivar has stood out for being a great option for cultivation, since it has high yield potential and resistance to the main diseases that affect the crop, such as black sigatoka, panama disease and yellow sigatoka (PEREIRA; GASPAROTTO, 2008). Thus, the aim of this work was to evaluate the physiological response of banana 'BRS Conquista' cultivar under different substrates and organomineral fertilizer doses during the acclimatization phase.

The acclimatization of banana 'BRS Conquista' cultivar seedlings propagated by the vegetative micropropagation method (obtained from private companies with plant health certification) was carried out in nursery with 50% shading screen, and placed in black plastic bags with capacity of 10 dm^3, filled with 9.5 dm^3 of substrate. The experimental design used was a 3 x 5 factorial (substrates x organomineral fertilizer doses), with five replicates, with each experimental unit consisting of five plastic bags with one seedling each. The substrates used in this experiment were coconut fiber, pine bark and 100% natural commercial formula, which is a mixed substrate composed of coconut husk, forest residues and biomass residues.

Interactions between the three types of substrates were evaluated under five 4-14-8 organomineral fertilizer concentrations (0, 1,000, 2,000, 3,000 and 4,000 g m^{-3}) added to substrates in split-plots, with 50% of the recommended dose in the transplanting of seedlings to the plastic bag and the remainder divided into two applications of 25% of the recommended dose, at 20 and 40 days after transplanting.

At 60 days after transplanting, evaluations were performed regarding radiation incidence (Qleaf), internal CO_2 concentration (Ci), stomatal conductance (Gs), transpiration rate (E), photosynthesis (A), mesophyll conductance (Gm), chlorophyll fluorescence (F), maximum leaf fluorescence in light (Fm) and leaf area index (LAI). Data were collected early in the morning, always using the third or fourth leaf, counting from the apex to the base, as performed by Arantes et al. (2016).

To collect data related to gas exchange, infrared gas analyzer (IRGA) model Lcpro+® Portable Photosynthesis System (ADC BioScientific Limited, UK) was used, with ambient temperature and irradiance and air flow of 200 ml min^{-1}. Fluorescence data were obtained using fluorometer coupled to the IRGA chamber. The maximum chlorophyll fluorescence (Fm) was determined by applying a saturating light pulse of 6000 μmol m^{-2} s^{-1} for 1 second. For the leaf area index (LAI), canopy analyzer (LAI-2000, Plant Canopy Analyzer - Li-Cor, NE, USA) was used during the period of gas exchange data collection.

Variables were submitted to factorial analysis of variance (ANOVA), with 5% significance level, later, for variables significant in the F test, test for comparison of means was carried out via Tukey's test (5%) and regression study regarding doses used. All statistical analyses were performed using the Statistica (STATSOFT INC., 2004) and Sisvar Inc. software (FERREIRA, 2011).

Radiation incidence at adequate levels tends to favor plant growth, development and production. In this work, radiation incidence results obtained were very close to each other, both for the type of substrate and regarding the dose used. However, statistically, the dose of 3,000 g m^{-3} obtained lower radiation incidence value compared to the other treatments (Table 1).

Table 1. Radiation incidence (Qleaf) evaluated in micropropagated banana 'BRS Conquista' seedlings acclimatized under different substrates and organomineral fertilizer doses.

Substrates	**Doses (g m^{-3})**				
	0	1,000	2,000	3,000	4,000
	Values in µmol photons m^{-2} s^{-1}				
Pine Bark	1998.67 aA	1999.89 aA	1999.22 aA	1999,55 aA	1998.89 aA
Coconut Fiber	1998.45 aA	1999.67 aA	1999.0 aA	1999.78 aA	1999.22 aA
Formula	1999.55 aA	1998.67 aAB	1999.22 aAB	1997.78 bB	1998.11 aAB
CV %	0.04				

Equal lowercase letters in columns (substrates) and equal uppercase letters in rows (doses) do not differ from each other by the Tukey's test ($p<0.05$).

According to data presented by Marques (2017) and Robinson and Saúco (2010), radiation incidence values close to 1,998 µmol photons m^{-2} s^{-1}, such as those obtained here, are above optimum values, negatively affecting the photosynthesis rate of plants. These higher than desired values are reached when the ambient temperature is outside limits from 14 to 35°C and humidity is low, below 35% (TURNER et al., 2007; ROBINSON; SAÚCO, 2010). Thus, as a consequence, there is partial restriction of the rubisco performance (predominant enzyme in plants with C_3 photosynthetic mechanism, as is the case of banana) resulting in decreased CO_2 fixation and stomatal closure, which is not interesting for plant development (ARANTES et al., 2016).

From data obtained for photosynthesis, it appears that the formula obtained photosynthesis rates lower than the other substrates, being statistically different at doses of 1,000 and 3,000 g m^{-3}. Regarding the tested organomineral fertilizer doses for the formulated one, no significant differences were identified. For pine bark, it was observed that extremes doses (0 and 4,000 g m^{-3}) resulted in lower photosynthetic rates, with better results at dose of 1,000 g m$^{-3.}$ The photosynthetic rate of seedlings grown in coconut fiber was higher at fertilizer dose of 2,000 g m^{-3} and lower in the absence of supplementation (Table 2). Thus, for higher photosynthetic rates, intermediate organomineral fertilizer doses (1,000 to 3,000 g m^{-3}) are recommended during the acclimatization phase of banana seedlings, together with the use of pine bark and coconut fiber substrates.

Table 2. Photosynthesis (A) obtained from micropropagated banana 'BRS Conquista' seedlings acclimatized under different substrates and organomineral fertilizer doses.

Substrates	**Doses (g m^{-3})**				
	0	1,000	2,000	3,000	4,000
	Values in µmol m^{-2} s^{-1}				
Pine Bark	6.36 aB	15.54 aA	9.15 aAB	8.41 abAB	4.96 aB
Coconut Fiber	4.05 aB	8.49 abAB	14.36 aA	11.98 aAB	8.29 aAB
Formula	3.15 aA	7.12 bA	7.36 aA	2.21 bA	8.38 aA
CV %	47.64				

Equal lowercase letters in columns (substrates) and equal uppercase letters in rows (doses) do not differ from each other by the Tukey's test ($p<0.05$).

With regard to the internal CO_2 concentration, it was observed that in the absence of supplementation, the formula is inferior to the other substrates, but with the addition of fertilizer, no significant differences were observed among materials. Regarding doses, there were differences only for coconut fiber, in which the addition of fertilizer caused lower results when compared to control (Table 3).

Table 3. Internal CO_2 concentration (Ci) determined in micropropagated banana 'BRS Conquista' seedlings acclimatized under different substrates and organomineral fertilizer doses.

Substrates	Doses (g m^{-3})				
	0	1,000	2,000	3,000	4,000
	Values in mol m^{-2} s^{-1}				
Pine Bark	357.00 abA	319.89 aA	345.00 aA	308.89 aA	312.78 aA
Coconut Fiber	419.45 aA	331.67 aB	334.89 aB	342.78 aAB	333.44 aB
Formula	296.15 bA	319.45 aA	333.22 aA	371.22 aA	322.00 aA
CV %	47.64				

Equal lowercase letters in columns (substrates) and equal uppercase letters in rows (doses) do not differ from each other by the Tukey's test ($p<0.05$).

The addition of organomineral fertilizer increases the organic matter content of the substrate and decreases its porosity, reducing the water availability, which is essential for CO_2 absorption, because according to Taiz and Zeiger (2009), plants use large amounts of water as a direct consequence of CO_2 absorption to carry out photosynthesis. Most of the water absorbed by roots, about 97%, is transported and evaporated on the leaf surface by transpiration, the remainder (3%) is used to meet growth demands, photosynthesis and other metabolic processes. The reduction in soil moisture content can interfere with CO_2 levels and photosynthetic rate.

Pine bark and the formula showed interesting transpiration rate for plant metabolism (Table 4). Similar results were obtained by Marques (2017), in which the transpiration rate of 'Prata-Anã' and 'BRS Platina' banana leaves measured in the morning was 5.96 mmol H_2O m^{-2} s^{-1}.

Table 4. Mean test (Tukey, 5%) for variables transpiration rate (E), stomatal conductance (Gs), mesophyll conductance (Gm), chlorophyll *a* fluorescence (F), maximum leaf fluorescence (Fm) and leaf area as a function of the type of substrate used.

Substrates	E	Gs	Gm	F	Fm	Leaf area
Pine Bark	5.57 a	0.49 a	0.027 a	429.07 a	448.73 a	838.46 a
Coconut Fiber	3.38 b	0.37 a	0.029 a	424.36 a	440.55 a	669.93 b
Formula	5.55 a	0.34 a	0.041 a	208.51 b	217.26 b	854.20 a
CV %	43.33	111.78	154.62	15.06	14.64	43.33

Equal lowercase letters do not differ by the Tukey's test ($p<0.05$).

Fluorescence is an optical phenomenon resulting from the process of dissipating the energy absorbed by plants. The measurement of fluorescence in the chlorophyll molecule can be associated with photosynthetic efficiency, indicating the reflexes of environmental conditions and the physiological state of plants. The wavelength, when associated with fluorophores, such as hydroxycinnamic acids, flavonols, isoflavones, flavanones and phenolic acids, must be between 400 and 600 nm. When greater than 600 nm, it is associated with chlorophyll *a* present in photosystem II (BAKER, 2008; CEROVIK et al., 1999; FALCO, 2012).

Only pine bark and coconut fiber showed fluorescence levels within the expected range. The low fluorescence and photosynthesis levels obtained for the formula indicate the presence of some adversity in this cultivation system. One possibility is the impairment of metabolic functions due to the difficulty of absorption of some nutrients due to the characteristics of the mixture used in the formulated substrate, as previously mentioned and highlighted by Epstein and Bloom (2004).

Finally, leaf area evaluation shows smaller size for eedlings grown with coconut fiber compared to seedlings grown on other substrates. However, this smaller area did not compromise fluorescence levels and photosynthetic rates, which indicates good physiological performance of plants submitted to this treatment. It is noteworthy that greater leaf area and, at the same time, higher biomass contents, are related to higher setting rates and better initial seedling development (LIMA et al., 2006). For the organomineral fertilizer dose as a function of leaf area, quadratic behavior was observed, explaining 98% of data, in which higher doses provided larger sizes in terms of leaf area (Figure 1).

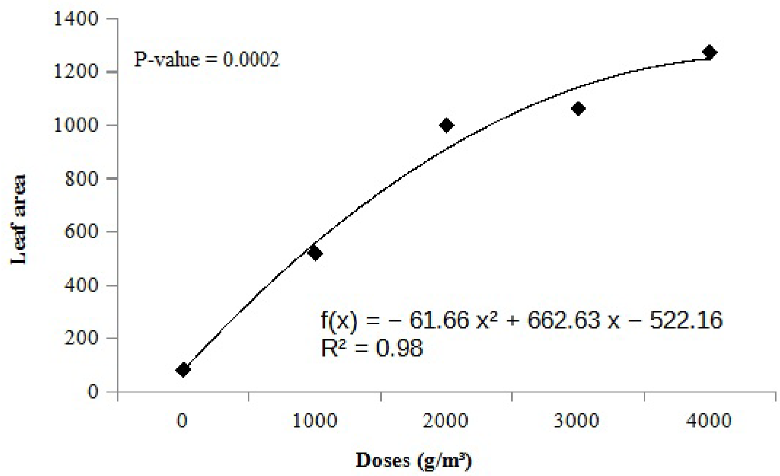

Figure 1. Regression analysis for leaf area as a function of different organomineral fertilizer doses used in micropropagated banana 'BRS Conquista' seedlings.

In view of the results obtained, it was possible to conclude that the pine bark substrate provides adequate physiological levels for the development of banana 'BRS Conquista' seedlings during acclimatization as well as higher leaf area values. Furthermore, intermediate organomineral fertilizer doses (1,000 to 3,000 g m^{-3}) resulted in higher photosynthetic rates and good results for the other variables under study.

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