Global Journal of Crop, Soil Science and Plant Breeding

ISSN: xxxx-xxxx Vol. 1 (1), pp. 014-019, November, 2013. © Global Science Research Journals

Full Length Research Paper

Acclimatization of micropropagated *Musa cavendischii* cultivar roatan plants submitted to doses of fertigation and auxin

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Accepted 30 October, 2013

Micropropagation of banana is suggested to produce great number of healthy plants but the success depends on the ability to transfer plants from *in vitro* conditions to greenhouse or field environment, at low cost and with high survival rates. In this study, the effects of the fertilization and indolebutyric acid (IBA) applied in the nutritive solution on growth of *Musa cavendischii* plantlets were evaluated during acclimatization process in greenhouse. First, *in vitro* plantlets of *M. cavendischii* were transplanted to pots of 150 cm^3 containing perlite and transferred to greenhouse for their acclimatization during 11 weeks. The experiment was established according to a completely randomized design with 5 treatments and 20 replications per treatment. The experimental unit was one plant transplanted in each pot, resulting from the combinations of daily applications of 10 ml of the Steiner's nutritive solution at 10, 25, 50, 75 and 100%, respectively (Factor A), without and with 1 mgL^{-1} of the auxin IBA (Factor B). After 11 weeks of acclimatization, the results shows that, the higher plants with respect to plant fresh, dry weight and height, and leaf width corresponded to the treatments from 75 to 100% of the Steiner's solution. The IBA application had no significant effects on the growth of the *M. cavendischii* plants. There was no significant interaction between fertigation and IBA applications. The plants fertigated at 100% of nutriments concentration had 27.7 cm of height, 259.9 cm^2 of foliar area, and 1.01 mg of chlorophyll g^{-1} of foliar fresh weight.

Key words: Acclimatization, fertilizer, micropropagation, indolebutyric acid, plant nutrition.

INTRODUCTION

In diverse tropical regions banana plays an important role on basic family diet and constitutes a source of carbohydrates. The average world production is around 67,139,570 tons (Venkatachalam et al., 2007). Propagation of banana plants is done asexually by means of shoots which develop from underground corms in the base of plants having more than one year old in field. In case of infected vegetative material, pathogens disseminate disease, affecting performance and quality of crops, making replacement of infected plants necessary. According to Pierick (1990), the micropropagation offers an efficient method to produce clonal populations of diverse species in a short time and for banana disease free mass propagation is feasible (Khan et al., 2001;

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Rai et al., 2012).

When the plants are propagated through tissue culture, chemical (culture medium) and physical (incubation) conditions determine the explant response whether occurring in unorganized cell proliferation or morphogenesis. Venkatachalam et al. (2007) described a standardized protocol adapted to the rapid clonal multiplication at commercial scale, of one cultivar of banana, in which they considered the costs of production, but in addition demonstrated by means of DNA analysis of a great number of micropropagated plants, that these did not display cases of somaclonal variation and the plants had high degree of genetic fidelity with regard to the stock plants selected to be propagated. According to Robinson et al. (1993), the micropropagated plants adequately acclimatized begin rapid growth when they are established in field, and they display superior growth and yield than the plants obtained by conventional propagation.

Inoculation of arbuscular mycorrhizal fungi (AMF) to the roots of micropropagated plantlets plays a beneficial role on their hardening or *ex vitro* acclimatization (Kapoor et al., 2008). Some reports indicate that, AMF confers tolerance to banana plantlets in saline stress (Yano-Meloet al., 2003). However, detailed analyses of the growth in response to mineral nutrition by drip irrigation system (fertigation) in *Musa* sp. have not been elucidated. Enhanced mineral nutrition of micropropagated plants is key to their survival and growth under *ex vitro* conditions.

Micropropagated plants supplied with suitable levels of nutriments during acclimatization in greenhouse conditions and later in nursery have shown greater vigor and capacity to grow, as well as higher yield in the later definitive plantation (Enríquez et al., 2000). Auxin indolebutyric acid (IBA) is used in nurseries as a promoter of rooting cuttings of many species and for rooting shoots in *in vitro* conditions and also promotes growth of aerial part of plant. In the present work, fertigation and IBA doses incorporated in a nutritive solution were evaluated considering its effect on the vegetative development of micropropagated *Musa cavendischii* plants during their acclimatization in greenhouse conditions.

MATERIALS AND METHODS

The investigation was carry out in the laboratory of plant tissue cultures and the greenhouse of acclimatization, at Technological Institute of the Valley of Oaxaca, Xoxocotlán, Oaxaca, Mexico. Two hundred *M. cavendischii* cultivar Roatan plants were obtained through the *in vitro* culture of vegetative buds excised from corms of stock plants selected in Pluma Hidalgo, Pochutla, Oaxaca, and following the methodology described by Luna-Ramírez et al. (2010). The micropropagated plants which had in average 12 cm height and 4 leaves were transferred to pots of 150 cm^3 containing perlite and were established in greenhouse for their acclimatization where they were kept for 11 weeks, during which these plants were watered by nebulization daily during 10 sec every 12 min, from 11 to 14 h. The total of plants were separated in 5 groups of 40 plants, which after the nebulization they were fertigated at substratum level with 10 ml of mineral salts (10, 25, 50, 75, and 100%) of the Steiner´s formulation (1984).The Steiner´s formulation at 100% contains in mgL^{-1}: 166.42 N, 30.68 P, 276.44 K, 182.34 Ca, 49.09 Mg, 111.01 S, 1.33 Fe, 0.62 Mn, 0.11 Zn, 0.44 B, 0.020 Cu, 0.048 Mo. The plants submitted to each aliquot were separated in two sub groups, to apply them the respective nutritive solution with two variants:

(I) Nutrient solution without IBA

(II) The nutrient solution with 1 mgL^{-1} IBA

So, 10 treatments were generated. Each plant was fertigated daily with 10 ml of the corresponding treatment. The experiment was established in accordance to a completely randomized design with factorial arrangement 2 × 5. The experimental unit was a plant and each treatment had 20 replicates.

At the end of the acclimatization period, 10 plants of each treatment were harvested at random to evaluate their height, length, and diameter of the stem, root volume, number of leaves, width of the larger leave, foliar area, chlorophyll content in the leaves, total fresh weight, foliar fresh weight, stem fresh weight, weight of aerial part (stem plus leaves), root fresh weight. In order to measure the accumulated dry matter in leaves, root, stem, the plant was separated in these organs which were put into paper bags and then into a dryer oven for 72 h, afterward the dry organs were weighed using an analytic balance. The total biomass (dry matter of leaves, stem, and root) as well as the relationship aerial part-root (leaves and stem/root) were obtained. Data were submitted to analysis of variance and the Tukey´s tests ($\alpha = 0.05$).

RESULTS AND DISCUSSION

The analysis of variance (Tables 1 and 2) determined that, the fertigation dose induced differences of response in the vegetative characters, fresh and dry weight of plant structures, and the root characteristics, after 11 weeks of acclimatization, except in fresh and dry weight of stems. In contrast, the addition of auxins partially inhibited growth responses in plant height, number of leaves, leaf area, fresh and dry weight of root, stem and total, and root volume. The interaction between fertilizer and auxin presented a significant effect on foliar dry weight. The addition at the same time of IBA and nutritious solution did not promote additional plant growth.

When the micropropagated plants were in the acclimatization stage for 11 weeks, it was determined that, those plants fertirrigated in doses from 10 to 75% of the Steiner´s formulation (1984) a large proportion (75 to 100% of the total) of plants was adapted. In groups of vitro plants fertigated with aliquot 100% with or without the auxin AIB, only the 60 and 75% of those plants were adapted observing that the death of plants occurred during the first 20 days of acclimatization, which was attributed to the nutrients supply in high doses. Therefore, in the firsts weeks the plantlets suffered stress by low humidity, high light intensity and switch from heterotrophic to autotrophic, and this determine the survival rate (Chandra et al., 2010; Kumar and Rao, 2012). The foregoing suggests that, during the first 20

Table 1. Means square and significance of analysis of variance of the responses of micropropagated *Musa* plants after 11 weeks of acclimation during which received nutritious solution at different doses with or without AIB.

Sources of variation	DF	Plant height	No. leaves	Leaf width	Stem diameter	Chlorophyll content	Stem length
Fert.	4	462.75**	3.42**	23.89 **	0.60**	0.878**	2.03*
Aux.	1	27.61*	1.51^{ns}	4.46**	0.04^{ns}	0.032^{ns}	1.17^{ns}
Fert-Aux	4	3.15^{ns}	1.51^{ns}	0.26^{ns}	0.07^{ns}	0.023^{ns}	1.06*
Error	70	6.44	0.658	0.379	0.04	0.013	0.46

Sources of variation	DF	Foliar area	Fresh weight				
			Total	Aerial part	Leaves	Stem	Root
Fert.	4	819.0**	225.12**	171.9**	165.02**	0.34^{ns}	12.13*
Aux.	1	170.0**	77.07 **	22.75**	17.77*	1.036*	15.81*
Fert-Aux	4	233.0^{ns}	25.41*	5.72^{ns}	4.16^{ns}	0.40*	7.87*
Error	70	2015.8	8.30	2.51	3.14	0.18	2.11

Fert = fertilization, aux = dose of auxins, fert-aux = interaction fertigation-auxins; df = degrees of freedom; * = value F, significant (Pr < 0.05), ** = value F, highly significant (Pr ≤ 0.01), ^{ns}= value F, not significant (Pr > 0.05).

Table 2. Means square and significance of analysis of variance of the responses of micropropagated *Musa* plants after 11 weeks of acclimation during which received nutritious solution at different doses with or without AIB.

Sources of variation	DF	Root volume	Relationship aerial part-root	Dry weight			
				Total	Leaves	Stem	Root
Fert.	4	17.37**	4.42*	1969223**	1185477**	1042.2^{ns}	147788*
Aux.	1	28.80**	3.84*	726567*	88644^{ns}	4500.0*	197309*
Fert-Aux	4	8.58^{ns}	2.92*	111112^{ns}	61964^{ns}	1006.0^{ns}	69868*
Error	70	3.78	0.92	82550	43307	559.7	29666

Fer = fertigation, aux = dose of auxins, fert-aux = interaction fertiligation-auxins, df= degrees of freedom;* = value F, significant (01 < Pr < 0.05); ** = value F, highly significant (Pr ≤ 0.01), ^{ns} = value F, not significant (Pr > 0.05).

days of acclimatization, the nutrients supply to these micropropagated plants should be in low doses (50% of nutrients concentration) and the subsequent days when the 77 days of acclimatization stage had passed it was observed that the plants reached different size in positive relationship with the level of nutrients supply.

Smaller plants were those fertigated with the lowest nutrient dose and as plants were fertirgated with increasing doses up to 100% of nutrients, they reached larger sizes (Tables 3 and 4). In the first 10 days of acclimatization, micropropagated *M. cavendishii* plants displayed slow growth and the lower leaves formed during the *in vitro* culture, died. However, in the following days, plants displayed active growth and development of new leaves, although with some differences arising from the aliquots (10, 25, 50, 75 and 100%) of nutrient solution applied to them. Such morpho-physiological changes in plantlets are due to effects of *ex vitro* conditions as suggested by other authors. For example, at *in vitro* conditions, the plantlets have less developed cuticle, epicuticular waxes and functional stomatal apparatus, causing high stomatal and cuticular transpiration or a transplantation shock during the first step of the acclimatization process (Pospišilova et al., 1999; Hazarika, 2006; Chandra et al., 2010; Kumar and Rao, 2012).

When 77 days of acclimation had passed, it was determined that, the plants fertigated at 100% of nutrient concentration reached greater diameter and length of stem. This same pattern was found in fresh weight and root volume but at doses from 25 to 100% (Table 3). As the plants were fertigated with increasing doses of nutrients, they developed bigger leaf area but also with the larger concentration of chlorophyll in their leaves. And so, those plants fertigated with nutrient solution at 10% of nutrients concentration and the plants fertigated at 100% of nutrients concentration of the Steiner´s formulation had respectively 77.1 and 259.9 cm^2 of leaf area as well as 0.44 and 1.01 mg of total chlorophyll g^{-1} foliar fresh weight, magnitudes significantly different (Tukey,test p < 0.05) (Table 4).

It is possible that, the plants with greater leaf area and more chlorophyll per unit of foliar fresh weight would perform more net photosynthesis, because also, those plants accumulated more dry matter (Table 4). According to Marschner (1995), the cells of green leaves, had more

Table 3. Characteristics of micropropagated *Musa* plants fertigated with different doses of nutrients, and with or without auxin during eleven weeks of acclimatization.

Fertigation (%)	Auxin (mg L^{-1})	Plants adapted[1] (%)	Plant height (cm)	Stem length (cm)	Stem diameter (cm)	Root volume (cm^3)	Number of leaves	Leaf width (cm)
10	1	90	13.63^{f}	0.85^{a}	0.79^{bc}	6.68^{c}	6.25^{bc}	2.90^{e}
10	0	100	14.11^{ef}	0.87^{a}	0.82^{c}	6.93^{bc}	5.75^{c}	3.32^{de}
25	1	90	17.08^{def}	1.09^{a}	1.04^{ab}	8.56^{ab}c	6.75^{ab}c	3.70^{d}e
25	0	85	18.12^{de}	1.13^{a}	1.17^{a}	9.31^{abc}	6.75^{abc}	4.17^{cd}
50	1	90	20.72^{cd}	1.11^{a}	1.06^{ab}	8.87^{abc}	6.75^{abc}	4.32^{cd}
50	0	90	22.93^{bc}	1.01^{a}	1.24^{a}	10.12^{ab}	7.37^{ab}	5.11^{bc}
75	1	90	25.35^{ab}	1.02^{a}	1.35^{a}	8.31^{abc}	7.62a	5.63^{ab}
75	0	90	25.53^{ab}	1.01^{a}	1.18^{a}	8.37^{ab}c	6.62^{ab}c	5.72^{ab}
100	1	75	25.77^{a}	1.18^{a}	1.25^{a}	7.18^{bc}	7.25^{ab}	5.78^{ab}
100	0	60	27.72^{a}	1.08^{a}	1.33^{a}	10.87^{a}	6.75^{ab}c	6.37^{a}

Fertigation (%)	Auxin (mg L^{-1})	Fresh weight (g)				
		Total	Aerial part	Leaves	Stem	Root
10	1	5.10	2.51^{f}	2.24^{c}	0.27^{a}	3.28^{b}
10	0	5.57^{de}	2.80^{f}	2.49^{bc}	0.31^{a}	2.77^{b}
25	1	9.16^{cde}	4.68^{f}	4.4 ^{bc}	0.22^{a}	4.48^{ab}
25	0	9.69^{bcd}	5.20^{e}	4.75^{bc}	0.45^{a}	4.49^{ab}
50	1	10.64^{bc}	6.01^{d}	5.79^{abc}	0.22^{a}	4.63^{ab}
50	0	13.96^{ab}	8.62^{c}	8.17^{ab}	0.45^{a}	5.34^{ab}
75	1	13.22^{bc}	9.27^{b}	8.90^{ab}	0.37^{a}	3.95^{ab}
75	0	12.99^{bc}	9.20^{b}	8.80^{ab}	0.40^{a}	3.79^{ab}
100	1	13.01^{bc}	9.83^{ab}	9.30^{a}	0.53^{a}	3.18^{b}
100	0	18.44^{a}	11.92a	11.47^{a}	0.45^{a}	6.52^{a}

In column, means with same letter does not differ significantly (Tukey's test, $p < 0.05$); [1]Define in terms of the total of transplanted plantlets

Table 4. Characteristics of micropropagated *Musa* plants fertigated with different doses of nutrients and with or without auxin, after 11 weeks of acclimatization.

Fertigation (%)	Auxin (mg L^{-1})	Foliar area (cm^2)	Chlorophyll (mg g^{-1})	Dry weight (mg)				
				Total	Leaves	Root	Stem	RPA/R
10	1	72.42^{d}	0.42^{e}	625.0^{c}	270.9^{d}	320^{c}	26.13^{ab}	0.97^{b}
10	0	81.80^{cd}	0.33^{e}	664.3^{c}	302.4^{d}	330^{c}	27.75^{ab}	1.19^{b}
25	1	134.42^{cd}	0.62^{d}	1004.9^{bc}	468.5^{cd}	500^{abc}	28.25^{ab}	1.14^{b}
25	0	153.04^{bc}	0.61^{d}	1173.8^{b}	560.0^{bcd}	550^{abc}	54.63^{a}	1.12^{b}
50	1	153.83^{bc}	0.72^{d}	1061.6^{bc}	529.3^{bcd}	510^{abc}	16.13^{b}	1.79^{b}
50	0	213.85^{ab}	0.64^{d}	1475.4^{ab}	793.4^{abc}	620^{ab}	53.25^{a}	1.35^{ab}
75	1	222.74^{ab}	0.87^{bc}	1313.4^{ab}	801.6^{ab}	490^{abc}	18.88^{b}	1.81^{ab}
75	0	230.79^{a}	0.75^{c}	1343.8^{ab}	816.5^{ab}	490^{abc}	22.50^{ab}	1.76^{ab}
100	1	234.90^{a}	0.97^{ab}	1438.8^{ab}	1031.8^{a}	380^{bc}	22.25^{ab}	3.32^{ab}
100	0	285.01^{a}	1.04^{a}	1740.0^{a}	962.6^{a}	700^{a}	25.50^{ab}	1.41^{a}

In column, means with same letter does not differ significantly (Tukey's test, $p < 0.05$); RPA/R = relationship aerial part/root;

than 75% of the total organic Nitrogen in the chloroplasts, mainly into enzymes; in addition, in groups of plants that receive nutriments from sub optimal until optimal supply, there are high positive correlations between the content of mineral nutrient in the leaves and the rate of net photosynthesis. According to Jones (1983), the accumulation of dry matter, particularly during the phase of vegetative growth, is in linear function of the amount of

intercepted solar radiation and the CO_2 fixation and so those factors such as nutrition and water condition of the plant have effect on the plant performance by altering the leaf area index, the interception of light and net photosynthesis.

The plants fertigated with doses from 75 to 100% of the Steiner´s universal solution (1984) displayed a significant growth in root, stem and total fresh and dry weight (Tables 3 and 4). This fact indicates that fertilization during the acclimation of micropropagated plantlets of *Musa*, improves the further development of leaf area, stems and roots, in view of the greater accumulation of dry matter. Plants control their development through endogenous synthesis of growth regulators which acting firstly at the level of receptors in the cell membranes and later at the level of gene expression. Both the amount of growth promoters synthesized in various organs of the plant, such as the amount of receptors at level of membrane, are in direct relation and are components of the physiological condition of the plant; thus also the magnitude of growth is an expression of the physiological condition of the plant. In reference to the effect of the addition of auxins, the results show differential effects between the evaluated variables. For example, the addition of 1 mg L^{-1} of IBA inhibited the growth of leaves, stem and root; as well as the accumulation of fresh and dry weight. These results suggest that the IBA was applied in higher dose than the optimum and induced lower plant growth and development.

The major response in plant growth and chlorophyll content at high doses of mineral nutrition indicated that *M. cavendishii* plantlets absorb nutrients once they developed roots during first seven weeks of acclimatization. This fact also was observed in micropropagated plants of *Fragaria* x *ananassa* cv. Elvira and the mineral nutrition conferred similar effects in shoot fresh and dry weights than nine species of arbuscular mycorrhizal fungi such as *Glomus* sp., *Gigaspora* sp. and *Scutellospora* sp. (Taylor and Harrier, 2001).

The fertigation with the Steiner´s universal solution at 75 to 100% of nutrient concentration without addition of IBA, favored a stronger plant growth and development in plant height, leaf area, number and size of leaves, stem and root growth, fresh and dry weight of leaves, stem, root and total (Tables 3 and 4).The plants fertigated at 10% of nutrients concentration with the presence or absence of auxin, between 47 and 50% of their total biomass was concentrated in the root and their relationship aerial part-root was 0.97 to 1.19, while the plants fertigated at 100, 26.5 to 40.2% of the total biomass was at the root and their relationship aerial part-root was from 1.41 to 3.32. The plants fertigated at 10% of nutrients concentration and those plants fertigated at 100% of nutrients concentration, with or without IBA had 5.33 and 14.97 g of total fresh weight, 13.63 and 27.7 cm in height, the stem diameter of 0.76 and 1.29 cm, the roots volume of 6.75 and 9.02 cm^3, 5.9 and 7 leaves having 3 and 6 cm wide; the leaf area of 77.1 and 259.95 cm^2. The fresh weights were stem 0.29 and 0.30 g, foliar 2.36 and 10.38 g and root 2.68 and 4.8 g. The accumulated dry matter was 285 and 996.5 mg foliar, 325 and 540 mg the root, in all cases magnitudes significantly (Tukey, $p < 0.05$) different.

The acclimatization process evaluated *M. cavendischii* plantlets which was supported with fertigation and IBA added to nutritive solution and this showed that, the first 2 or 3 weeks are crucial to the plantlets survival and particularly the fertigation in high doses enhanced the growth *M. cavendischii* plantlets under *ex vitro* conditions. In this work, the fertigation had significant effects on plantlets survival and growth, and this practice can complement the proposals of other authors which suggest the utilization rhizosphere bacteria and arbuscular mycorrhizal fungi which induce metabolic changes and enhance the tolerance to abiotic and biotic stresses (Yano-Melo et al., 2003; Kumar and Rao, 2012) and in other cases, the preconditioning under shadow is recommended (Scaranari et al., 2009)

Conclusion

The acclimatization of micropropagated *M. cavendischii* c.v. Roatan plants was realized in a greenhouse for 11 weeks, and those plants fertigated with the Steiner's nutritive solution whether at 75 or 100% of nutriment, reached the major growth in term of plant height, length of the stem, number of leaves, leaf width, foliar area, fresh and dry weight, and content of chlorophyll. The critical periods of plant adaptation to the *ex-vitro* conditions were the first 20 days. The use of 1 mg L^{-1} of the IBA in the nutritive solution did not have effect in inducing additional growth of the plants.

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