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Improvement of in vitro method to screen for dry spell lenient banana assortments by sorbitol incited osmotic pressure

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Abstract

The identification of drought tolerant banana varieties under natural environment is complicated by difficulties in field management, variation in phenotype and unexpected rainfall events. A study to develop an alternative and rapid technique to screen for drought tolerant banana varieties by an *in vitro* technique was carried out. Effects of 0.09 M sucrose, 0.09 M sorbitol and 0.0 M sugar on growth of banana plantlets were compared under *in vitro* conditions. Results from this experiment proved that sorbitol is not a source of energy for *in vitro* banana plantlets and it could be used as a neutral osmotic inducer. Exploration of different levels of osmotic stress induced by 0.1 to 0.5 M sorbitol in the media and their effects on the growth of banana plantlets proved that the concentration of 0.2 M sorbitol is the highest concentration to reveal different growth parameters. The application of this concentration on banana varieties of Williams, Popoulou, Obino l'Ewai, Lep Chang Kut, Mbwazirume (negative control: drought sensitive), and Cachaco (positive control: drought tolerant) showed that all varieties were affected by sorbitol osmotic stress but the degree of sensitivity is different. Significant differences in reduction of gain of fresh and dry weight, new roots and leaves, and leaf area were observed between Cachaco and Mbwazirume. For most growth parameters, Cachaco showed the lowest reduction and Mbwazirume presented the highest reduction due to osmotic stress. The varieties Williams and Lep Chang Kut showed a level of drought tolerance after Cachaco. Lep Chang Kut had the lowest reduction of gain of new root and fresh weight, and water content; whereas, Williams occupied the second position of low reduction of gain of leaf area, number of leaves, and the third position in low reduction of gain of new root and dry weight. After, Obino l'Ewai occupied the fourth position and Popoulou came as the fifth position. Mbwazirume was the last in the tolerance of sorbitol induced osmotic stress with high reduction in many growth parameters evaluated. From this study, an *in vitro* technique to screen drought tolerant banana varieties was developed, and the drought tolerance of Cachaco and Lep Chang Kut and drought sensitivity of Mbwazirume were proved. The total gain of fresh and dry weight, number of new leaves and leaf area were identified to be appropriate growth parameters for identifying drought tolerant banana varieties under *in vitro* condition.

Keywords: Banana, drought tolerance, *in vitro* screening, osmotic stress, sorbitol.

INTRODUCTION

Banana is a worldwide important crop. Its annual production was estimated at 102 Mt and the area occupied by banana plantations was estimated at 4.8 × 10^{-6} ha (FAOSTAT, 2010). Banana is the fifteenth of the world's imported commodities and the fourth most important food crop after rice, wheat and maize in many developing countries. It contributes as a source of food, employment and incomes in its major production areas

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(Heslop-Harrison and Schwarzacher, 2007; FAOSTAT, 2010). Banana is the source of beverages, feeds and materials for handicraft, medicine and ceremonies (Rossel, 1999). Alcohol, vinegar and wine are usually produced from fruits of banana. After harvesting, stems and leaves of the banana plants are commonly used as feeds (Nelson et al., 2006). It has been observed that fibres of banana leaves exhibit the highest ash, carbon and cellulose content, hardness and tensile strength (Jústiz-Smith et al., 2008). These fibres serve in various handicraft to fabricate baskets, mats, table mats, photo frames, ear rings, souvenir boxes, bags, wall hangings, trays, hats, hand bags, season cards, folders, key holders, neckties, bow ties, waist coats, necklaces and other useful materials (Handcrafts, 2006). Potash diuretic and disinfectant effects and sap strong astringent effects of banana lead to its use in traditional medicine (Nelson et al., 2006). Moreover, in many African countries, banana plants are used in different ceremonies related to birth, funeral rites and religious (Rossel, 1999). Despite its importance, its production is challenged by biotic and abiotic constraints.

Banana plants are susceptible to a wide range of diseases and pests. Some of these pests and diseases are highly aggressive, easily spread and very difficult to eradicate (Nelson et al., 2006; Robinson and Saúco, 2010). These biotic constraints affect banana production and cause a significant impact on food availability and economy in many developing countries (Nelson et al., 2006; Teycheney et al., 2007). Abiotic stresses such as fluctuation of photoperiod, quality of light, winds, soil moisture and water supply influence the growth and production of banana (Lassoudière, 2007). Banana plant is very sensitive to water deficiency. When there is a severe water deficiency, all the leaves fall prematurely and the banana pseudo-stem collapses (Stover and Simmonds, 1987; Lassoudière, 2007). Under a prolonged drought period, Mahouachi, (2009) observed that the gas exchange and growth parameters were reduced drastically. Emerging leaves and growing fruits are extremely affected by drought stress (Turner et al., 2007). It was revealed that the decline of 100 mm of rainfall caused maximum bunch weight losses of 1.5 to 3.1 kg and 8 to 10% of the total production. van Asten et al. (2011) found that the water shortage is the most important yield reducing constraint in the rain fed East African highland banana production. Farmers reported that the drought stress is the second most important constraint of banana production in Rwanda, Burundi and Eastern Democratic Republic of Congo after soil fertility (Murekezi and van Asten, 2008; Bouwmeester et al., 2009). Therefore, water availability is critical for banana production.

Drought is a major environmental factor that determines the growth, productivity and distribution of plants. It is the most serious and worldwide yield reducing stress in agriculture (Ober, 2008). Drought affects more than 10% of arable soil (Bray et al., 2000; Zidenga, 2006) and this drought condition is continually distressed by the explosive increase of world population, continuous deterioration of arable land, shortage of fresh water, and the current climate change. The increase in drought stress threatens the global agriculture production and food availability. It has been estimated that two thirds of the yield potential of major crops are routinely lost due to drought stress (Bray et al., 2000; Lafitte et al., 2004; Zidenga, 2006; Magombeyi and Taigbenu, 2008). Therefore, the sustainability of production will depend on the identification and development of new drought tolerant varieties (Cochard et al., 2008).

Studies on tolerance to drought stresses have been done on various crops such as rice, maize, potato, cassava, bean and others (Bartels and Sunkar, 2005; Hirasawa et al., 2006), but studies on banana are few. The causes that limit these studies could be the fact that banana is perennial crop which has a long generation time. Moreover, the screening for resistance to drought stresses is complicated by difficulties of field management, phenotypic variations and unexpected events (Lafitte et al., 2004). Indeed, new techniques to identify banana varieties tolerant to drought stresses are needed. The objective of this study was to develop an *in vitro* technique to screen for drought tolerant banana varieties.

MATERIALS AND METHODS

Plant materials were obtained from the Bioversity International Transit Centre (ITC) of *Musa* Germplasm Collection, based in the Laboratory of Tropical Crop Improvement of K. U. Leuven, Belgium, in which this study was carried out. The description of banana varieties used in the study is presented in Table 1.

Preparation of culture media

The culture media used in the study were the proliferation culture medium (PCM), regeneration culture medium (RCM) as described by Strosse (2003) and modified RCM. The semi solid PCM (4.4 g × l^{-1} Murashige and Skoog (1962) (MS) salts with vitamins, 0.09 M sucrose, 10 µM benzyl amino purine (BAP), 1 µM indole acetic acid (IAA) and 2.5g × l^{-1} gelrite) was used to multiply *in vitro* plantlets and to maintain the stock of plant materials. The liquid RCM (only differing from PCM in its BAP concentration: 1 µM BAP) was used to prepare plantlets and as a control in the experiments. The modified RCM (liquid RCM without sucrose, and liquid RCM supplemented with 0.09 M sucorse and 0.1 to 0.5 M sorbitol) were used as treatments in the experiments and to mimic drought stress conditions. All reagents of culture media, except gelrite acquired from Belgolabo, Belgium, were purchased from Duchefa, the Netherlands. Precise quantity of reagents were weighed with an analytical balance and dissolved in the distilled water. The pH of the solution was adjusted at 6.12 with a pH meter and 1 N potassium hydroxide or 1 N hydrochloridric acid to increase or to decrease respectively the pH. 5 ml of RCM and modified RCM and 20 ml of PCM were distributed in tubes of diameter 2 cm and height 15 cm.

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Table 1. Description of banana varieties used in the study.

Variety	Genome	ITC No.	Country donor	Collection date	Use
Banksii	AA	466	Honduras	1988	Wild species
Laterita	-	627	Colombia	1989	Wild species
Cachaco	ABB	643	Colombia	1986	Cooking
Lep Chang Kut	BBB	647	Thailand	1989	Cooking
Mbwazirume	AAA	84	Burundi	1986	Cooking
Obino l'Ewai	AAB-P	109	Nigeria	1986	Cooking
Popoulou	AAB	335	Nigeria	1987	Cooking
Williams	AAA	365	Australia	1988	Dessert

Source: mgis.inibap.org/.

The tubes containing the culture media were covered with caps and the sterilization was carried out in the autoclave at 121°C for 20 min. The culture media were removed from the autoclave and kept until they cooled prior to starting the inoculation. The water potential (Ψ_w) of sterilised liquid culture media was determined with a dewpoint potentiometer WP4.

Inoculation procedures and growth conditions

Clusters of 2 or 3 plantlets were inoculated in each tube containing the PCM. Inoculated tubes were kept in a growth room (at 25 ± 2°C, 45 to 52% humidity, long day periods of 16 and 8 h under continuous light of 50 $\mu Em^{-2}S^{-2}$). After four weeks, well developed plantlets were selected and transferred individually on liquid RCM and remaining plantlets were sub-cultured to a new PCM. After four weeks, plantlets on the RMC were removed from the tube and explants of 3cm of length with three roots of 1 cm were excised. Weight of each explant was determined with an analytical balance prior inoculation. For each variety, 7 to 10 explants were inoculated on the control medium (RCM) and on the stressing media (modified RCM). Inoculated tubes were covered with their caps and wrapped with parafilm, and kept in growth room for six weeks with a refreshing of culture medium every two weeks.

Identification of osmotic inducer of banana

Explants of Banksii variety were grown on RCM (control), RCM without sucrose, and on RCM where sucrose was replaced by 0.09 M sorbitol (stress condition). To identify a suitable concentration of osmotic inducer, explants of two wild banana varieties of Banksii and Laterita were grown for five weeks on RCM (control) and RCM supplemented with 0.1 to 0.5 M sorbitol (stress conditions). Prior inoculation, fresh weight of explants was determined.

Effects of sorbitol induced osmotic stress on growth of different banana varieties

Six banana varieties (Williams, Mbwazirume, Cachaco, Obino l'Ewai, Lep Chang Kut and Popoulou) were tested. Explants of these varieties were grown on RCM (control) and RCM supplemented with 0.2 M sorbitol (stress) for six weeks.

Data collection and analysis

At the end of each experiment, number of new roots (GNR), number of new leaves (GNL), gain of leaf area (GLA), gain of fresh weight (GFW), gain of dry weight (GDW) and water content (WC) were determined. Plantlets were removed from the tubes and fresh weight (FW) was immediately determined with the analytical balance. Number of leaves and roots were counted and leaf area was determined by excising each leaf and drawing its surface on millimetre paper. To determine the dry weight (DW), plantlets were dried in the oven to constant weight at 70°C for 72 h. The WC and GDW were determined according to the following formula: WC (%) = $[(FW-DW) \times FW^{-1}] \times 100$, GDW = FDW - IDW, IDW= $[IFW \times (100 - WCc)] \times 100^{-1}$, WCc = FWc - DWc, where FDW: final dry weight, IFW: initial fresh weight, WCc: water content of control (%), IDW: initiation dry weigh, FWc: fresh weight on control, DWc: dry weight on control. Data collected were analysed using GenStat 14th edition. This analysis consists of ANOVA and comparison of means with Turkey test.

RESULTS

Osmotic inducer of banana plantlets

The dissolution of sucrose and sorbitol in a solution of culture medium causes a decrease of Ψ_w of the culture medium (Table 2). The culture medium with 0.09 M sucrose presented a low Ψ_w (-0.37 MPa) compared to the Ψ_w of the culture medium in which sucrose was replaced by 0.09 M sorbitol (-0.31 MPa). The results showed also that the Ψ_w of the culture medium decreased as the concentration of sorbitol increased.

Capacity of sorbitol to induce an osmotic stress for banana plantlets

Three different culture media; RCM (control), RCM omitted sucrose and RCM where sucrose was replaced by sorbitol were compared to verify if sorbitol could be metabolised by *in vitro* banana plantlets. This experiment showed that plantlets grown on the RCM with sucrose present a high growth rate than the plantlets grown on the other culture media (Figure 1).

The comparison of growth parameters of gain of fresh and dry weights (GFW and GDW), gain of new roots and

Table 2. The Ψ_w of different culture media used in the study.

Composition of culture medium	Ψ_w (Mpa)
RCM without sugar	-0.09
RCM with 0.09 M sorbitol	-0.31
RCM with 0.09 M sucrose	-0.37
RCM with 0.09 M sucrose and 0.1 M sorbitol	-0.63
RCM with 0.09 M sucrose and 0.2 M sorbitol	-0.85
RCM with 0.09 M sucrose and 0.3 M sorbitol	-1.17
RCM with 0.09 M sucrose and 0.4 M sorbitol	-1.32
RCM with 0.09 M sucrose and 0.5 M sorbitol	-1.72

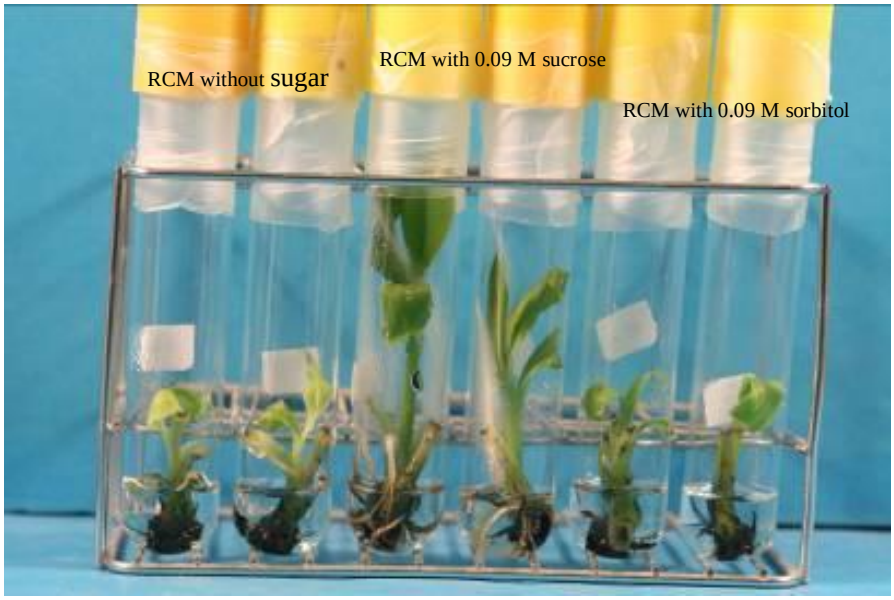

Figure 1. Growth of banana plantlets (variety of Banksii) on culture media with different sugars. Photo of plantlets at five weeks old.

leaves (GNR and GNL), and gain of leaf area (GLA) (Table 3) revealed significant differences between plantlets grown on the culture medium with sucrose and culture medium without sugar and with sorbitol. However, there was no significant difference between growth effects of culture media without sugar and with sorbitol. Plantlets grown on culture medium with sucrose presented a high GFW, GDW, GNR, GNL and GLA than plantlets grown on the culture media with sorbitol and without sugar. It was also observed that plantlets grown on the three different media had significant variations of WC (Table 3).

Suitable concentration of sorbitol to induce osmotic stress for *in vitro* plantlets of banana

Effects of osmotic stress induced by different concentrations of sorbitol on the growth of *in vitro* plantlets of banana varieties Laterita and Banksii were investigated. The aim of this experiment was to identify the concentration of sorbitol that could be used to mimic the drought stress condition in the screening process. The RCM with 0.1, 0.2, 0.3, 0.4 and 0.5M sorbitol and RCM without sorbitol (control) were tested. The Ψ_w of medium decreased from -0.37 to -1.72 Mpa, when the culture medium was supplemented with 0.5 M sorbitol (Table 2). In terms of growth, the two banana varieties did not show any significant difference for all evaluated growth parameters on the control medium. However, it was observed that as the concentration of sorbitol increased, the plantlet growth decreased (Tables 3 and 4). The effects of control and stressing media on all investigated growth parameters were significantly different. Plantlets grown on the control medium showed the highest averages of GFW, GDW, GNR, GNL, GLA and WC. Plantlets grown on culture media with high concentrations of sorbitol (0.4 and 0.5 M) did not show

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Table 3. Effects of culture media with different sugars on growth of *in vitro* plantlets of banana (variety of Banksii).

Treatment	GDW	GFW	GLA	GNL	GNR	WC
RCM without sugar (control)	0.009^{a}	0.183^{a}	86.700^{a}	0.500^{a}	0.333^{a}	91.480^{a}
RCM with 0.09 M sorbitol	0.025^{a}	0.237^{a}	90.300^{a}	0.667^{a}	0.500^{a}	93.330^{b}
RCM with 0.09 M sucrose	0.090^{b}	0.874^{b}	366.800^{b}	2.833^{b}	5.333^{b}	94.990^{c}

Values are means of six plantlets of five weeks old. Numbers followed by the same letter are not significantly different at P - value < 0.01, and multiple mean comparisons with Tukey test.

Table 4. Growth of plantlets of banana varieties Laterita and Banksii on control (RCM) and stressing culture media (RCM with 0.1 to 0. 5 M sorbitol).

Treatment	GNL		GNR		GLA		GFW		GDW		WC	
(M)	Laterita	Banksii	Laterita	Banksii	Laterita	Banksii	Laterita	Banksii	Laterita	Banksii	Laterita	Banksii
Control	2.50^{c}	2.75^{c}	3.50^{c}	5.25^{c}	492.00^{c}	658.75^{c}	1.00^{d}	1.59^{c}	0.09^{b}	0.11^{c}	90.90^{d}	93.17^{d}
0.1	1.50^{b}	1.25^{b}	2.00^{b}	3.50^{b}	185.00^{b}	259.50^{b}	0.55^{c}	0.69^{b}	0.07^{ab}	0.08^{bc}	89.04^{cd}	90.67^{cd}
0.2	1.25^{b}	0.50^{ab}	1.00^{ab}	1.50^{ab}	148.00^{b}	31.00^{ab}	0.26^{b}	0.28^{ab}	0.06^{ab}	0.05^{ab}	87.11^{bcd}	89.64^{bcd}
0.3	0.00^{a}	0.00^{a}	0.25^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.05^{ab}	0.02^{a}	0.04^{a}	0.04^{ab}	86.00^{abc}	85.55^{abc}
0.4	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	-0.06^{a}	-0.01^{a}	0.04^{a}	0.03^{a}	82.79^{ab}	87.19^{a}
0.5	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	-0.06^{a}	0.00^{a}	0.04^{a}	0.03^{a}	83.72^{a}	84.22^{ab}

The values are means of five plantlets of five weeks old. Numbers followed by the same letter are not significantly different at P-value <0.01, and multiple mean comparisons with Tukey test.

any growth. In terms of GFW, we observed the negative values, indicating that plantlets lost some weight. It was also observed that as the sorbitol concentration increased, the WC content of plantlet decreased (Tables 3 and 4).

The plant grown on RCM supplemented with 0.3, 0.4 and 0.5 M sorbitol did not develop any new leaves and roots for both banana varieties (Tables 4 and 5). This observation was highlighted by the reduced GFW and GDW (Tables 3 and 4). Indeed, in the screening process, it is necessary to quantify the growth parameters. Therefore, the concentration of 0.2 M sorbitol should be the suitable concentration that banana plantlets can tolerate and show growth characteristics under *in vitro* conditions.

Effects of sorbitol induced osmotic stress on plantlets growth of different banana varieties

Banana varieties: Cachaco, Mbwazirume, Williams, Obino l'Ewai, Lep Chang Kut and Popoulou were used in this experiment. According to previous studies, it is known that Cachaco is drought tolerant and Mbwazirume is a drought sensitive (Thomas and Turner, 2001). These varieties were used as negative and positive control to identify the drought tolerance of other four banana varieties. The results of this experiment showed a drastic decrease of growth under stress medium (Figure 2).

The evaluation of plantlets in terms of GFW, GDW, GNR, GNL, GLA and WC revealed that varieties grow differently on the control medium (Table 6). Therefore, brut data of growth on the stressing culture medium are not comparable. These data have been transformed in terms of reduction due to osmotic stress following this formula: $Y_i = [(\mu - y_i) / \mu] \times 100$, where Y_i: reduction due to osmotic stress for each sampled plantlet, μ: mean of a growth parameter for plantlets grown on the control medium, and y_i: quantified value of growth parameter for each plantlet grown on the stressing medium.

Table 5. Growth parameter of plantlets on control (RCM) and stressing culture media (RCM with 0.1 to 0.5 M sorbitol) without considering variety effects.

Treatment (M)	GLA	GNL	GNR	GFW	GDW	WC
Control	575.400^{c}	2.625^{c}	4.375^{c}	1.291^{c}	0.099^{c}	92.04^{d}
0.1	222.200^{b}	1.375^{b}	2.750^{b}	0.620^{b}	0.076^{bc}	89.85^{cd}
0.2	79.400^{a}	0.875^{b}	1.250^{a}	0.268^{a}	0.054^{ab}	88.37^{bc}
0.3	0.000^{a}	0.000^{a}	0.125^{a}	0.035^{a}	0.043^{a}	85.77^{ab}
0.4	0.000^{a}	0.000^{a}	0.000^{a}	-0.032^{a}	0.034^{a}	83.97^{a}
0.5	0.000^{a}	0.000^{a}	0.000^{a}	-0.030^{a}	0.034^{a}	84.99^{a}

The values are means of five plantlets of five weeks old. Numbers followed by the same letter are not significantly different at P-value <0.01, and multiple mean comparisons with Tukey test.

Figure 2. Growth of banana varieties on the control culture medium (RCM) and stressing culture medium (RCM with 0.2 M sorbitol). 1: Mbwazirume, 2: Williams , 3: Popoulou, 4: Cachaco, 5: Obino l'Ewai, 6: Lep Chang Kut. Photos of plantlets at six weeks old.

Table 6. Variation in growth rate between banana varieties on the control culture medium (RCM).

Variety	GFW	GDW	GNR	GNL	LA	WC
Cachaco	2.422^{ab}	0.178^{c}	4.900^{ab}	3.600^{b}	2374.000^{cd}	92.560^{b}
Lep Chang kut	0.602^{a}	0.065^{a}	3.700^{a}	2.600^{a}	497.000^{a}	89.080^{a}
Mbwazirume	2.161^{bc}	0.134^{b}	4.100^{a}	3.000^{ab}	1461.000^{b}	93.690^{bc}
Obino l'Ewai	1.963^{b}	0.118^{b}	8.600^{c}	2.600^{a}	2058.000^{bc}	93.950^{c}
Popoulou	2.013^{b}	0.120^{b}	6.200^{b}	3.700^{b}	1626.000^{bc}	94.000^{c}
William	2.660^{c}	0.170^{c}	6.300^{b}	3.700^{b}	2861.000^{d}	93.600^{bc}

Values are means of five plantlets of six weeks old, numbers followed vertically by the same letter are not significantly dif ferent at P-value <0.01, and multiple mean comparisons with Tukey test.

Table 7. Growth reduction (%) of plantlets of different banana varieties due to sorbitol induced osmotic stress.

Variety	DFW	GDW	GNR	GNL	GLA	WC
Cachaco	68.95^{a}	8.78^{a}	44.90^{a}	33.33^{a}	72.63^{a}	5.794^{b}
Lep Chang kut	67.50^{a}	13.17^{ab}	27.03^{a}	65.38^{b}	100.00^{c}	2.623a
Mbwazirume	86.44^{b}	32.09^{b}	29.27^{a}	73.33^{b}	100.00^{c}	8.300^{b}
Obino l'Ewai	74.44^{a}	34.32^{b}	45.35^{a}	34.62^{a}	81.54^{ab}	6.819^{b}
Popoulou	76.51^{a}	31.19^{ab}	40.32^{a}	37.84^{a}	88.38^{b}	7.622^{b}
William	75.14^{a}	27.71^{ab}	41.27^{a}	32.43^{a}	76.27^{a}	7.335^{b}

Values are means of five plantlets of six weeks old, numbers followed vertically by the same letter are not significantly different at P-value <0.01, and multiple mean comparisons with Tukey test.

The standardized results of growth parameters indicated that all investigated banana varieties were affected by a sorbitol induced osmotic stress; but, the degree of sensitivity is different. Significant differences in reduction of GFW, GDW, GNR, GNL and GLA were observed between banana varieties Cachaco and Mbwazirume. For most growth parameter, Cachaco showed the lowest reduction and Mbwazirume presented the highest reduction due to osmotic stress. However, there was no significant difference in the WC reduction (Table 7). The varieties Williams and Lep Chang Kut showed a level of drought tolerance after Cachaco. Lep Chang Kut had the lowest reduction of GNR, GFW and WC, whereas, Williams occupied the second position of low reduction of GLA, and GNL, and the third position in low reduction of GNR and GDW (Table 7). Obino l'Ewai and Popoulou ranked fourth and fifth respectively. The last in the tolerance to sorbitol induced osmotic stress was Mbwazirume with significant reduction in many growth parameters evaluated.

DISCUSSION

The addition of medium components, especially macronutrients and carbon sources in distilled water causes a considerable decrease of osmotic potential (ψ_s) of the solution (George, 1993). The increase of solute concentration in the solution results in more negative Ψ_s. Consequently, the decrease of solution Ψ_s affects strongly the Ψ_w (Taiz and Zeiger, 2006). This is the case of the results of this study in which the increase of sorbitol concentration caused the decrease of Ψ_w of the culture medium. It was also revealed that the MS salts in the culture medium with 87.6 mM sucrose contributed approximately 50% of the overall medium Ψ_w. The results of this study did not fit in this range, because the contribution of the MS salt with vitamins (culture medium without sugar) to Ψ_w of the culture medium was estimated at 24.32%.

Similarly, George (1993), and de Paiva and Otoni (2003) observed that the sterilization of culture medium causes a degradation di- and tri-saccharides incorporated in the culture medium and this degradation causes a decrease of Ψ_w of the culture medium. They also reported that the sterilization of culture medium containing sucrose as the only carbon source resulted in 10 to 15% carbohydrate hydrolysis which was pH dependent. Therefore, the hydrolysis of sucrose into its components causes an increase of osmotic tress of the culture medium. This finding was reapproved by the results of this study where the culture media with the same concentration of different sugars presented different Ψ_w. The Ψ_w of the RCM with 0.09 M sucrose and 0.09 M sobitol were -0.37 and -0.31 Mpa, respectively. The Ψ_w difference of these culture media could be due to the heat of sterilisation which might have caused the hydrolysis of sucrose into its components, glucose and

fructose.

The growth of *in vitro* plantlets depends on nutritional and controlled environmental factors (Zryd, 1988). Optimization of compositions of culture medium is an important approach to fasten the micropropagation process and improve the quality of plantlets (Liu et al., 2006). Under *in vitro* conditions, explants require carbohydrate as a source of energy (de Paiva Neto and Otoni, 2003) and different sugars such as sucrose, glucose, maltose, fructose, sorbitol and others were proven to have effects on a growth of *in vitro* plantlets (Liu et al., 2006). However, most studies have concluded that sucrose supports the highest growth rate (Petersen et al., 1999; Fuentes et al., 2000; Mello et al., 2001). These observations were reconfirmed in this study where plantlets grown on culture medium with sucrose showed the highest growth, GFW, GDW, GNR, GNL and GLA (Figure 1, Table 3).

The osmotic stress due to 0.09 M sucrose was higher than the osmotic stress due to 0.09 M sorbitol (Table 2). However, plantlets grown on the RCM with sorbitol showed a poor growth (Figure 1, Table 3). This observation reveals that the observed high growth rate is due to a source of energy. This means that sorbitol is not a source of energy for banana plantlets. Similar observation was reported by Melo et al. (2001) in the cell culture of bean (*Phaseolus vulgaris*). It was suggested that the negative effects of sorbitol on the plant growth is due to its inability to be metabolized by plant cells. This inability could be associated with its reduced uptake, the absence or insufficient sorbitol dehydrogenase in these plant species (Jain et al., 1997).

The ability to metabolize different types of carbohydrates differs within plant species and responses of explant cultures to different treatments of carbohydrates are genotype dependent (Cuenca and Vieitez, 2000). Therefore, the choice of the osmotic inducer should be based on the target species because the solute may be absorbed and metabolized by plantlets (Karhu, 1997; de Paiva Neto and Otoni, 2003). Sorbitol is known as an osmotic regulator like mannitol, polyethylene glycol (Mello et al., 2001; Gopal and Iwama, 2007; Al-Khateeb, 2008) and sodium chloride (Legocka and Kluk, 2005; Hirasawa et al., 2006). However, it was revealed that sorbitol can be used as a source of energy (Wang et al., 1999; Al-Khayri and Al-Bahrany, 2002). The results of this study showed that sorbitol is not a source of energy for banana plantlets. Therefore, it can be used as an osmotic inducer in banana studies.

The osmotic stress affected the plant growth, stem height, foliage weight, root number and root dry weight (Wang et al., 1999; Deblonde and Ledent, 2001; Tourneux et al., 2003; Lahlou and Ledent, 2005; Jager et al., 2008; Szira et al., 2008). A decrease of total fresh and dry matter, emergence of new leaves, leaf area, number of living leaves, root volume, shoot and root growth were reported on various crops suffering from drought stresses (Kallarackal et al., 1990; Firth et al., 2003; Gopal and Iwama, 2007; Hamidou et al., 2007; Jager et al., 2008; Hund et al., 2009). Drought condition decreases the plant metabolic pathways. Consequently, there is a reduction in the amount of metabolites and plant biomass (Kulkarni and Phalke, 2009). Gopal and Iwama (2007) observed also a severe reduction in the foliage growth of potato plantlets on a culture media with 0.3 and 0.4 M sorbitol. It is possible that sorbitol at high concentrations induces a very strong osmotic stress which exceeds the plantlet capacity of osmotic adjustment. Consequently, plantlets grown on these culture media suffer from a severe water deficit due to high osmotic stress. These could be the causes of reduced plantlet growth observed in the stressing medium of this study.

The results from the experiment investigating the effects of sorbitol induced osmotic stress on plantlets growth of different banana varieties highlighted the complexity of drought tolerance of banana varieties. Under field conditions, it has been suggested that root system is an indicator of drought tolerance. A variety with a strong and well developed root system is considered as drought tolerant (Kulkarni and Phalke, 2009). It has been also reported that the development of a strong root system is an adaptive response to water deficit. This is because many and thick roots could have some advantages for water uptaking and transporting, and maintaining a plant water status under a drought condition (Azhiri-Sigari et al., 2000; Kato et al., 2006; Xiong et al., 2006; Kulkarni and Phalke, 2009; Markesteijn and Poorter, 2009). However, the hypothesis that a drought tolerant banana variety could develop a strong root system does not hold under *in vitro* condition. Cachaco which is a drought tolerant banana variety did not show a well developed root system (Figure 2, Table 7). This behavior of Cachaco could be due to its high endogenous cytokinine / auxine ratio which inhibits the emission of roots (unpublished laboratory results).

A variety that presents a smallest reduction of growth rate under a stress condition is suggested to be drought resistant or tolerant. This was re-confirmed by our results where Cachaco, a presumed drought tolerant banana variety, showed the lowest growth reduction and Mbwazirume, a drought sensitive cultivar, presented the highest reduction of growth rate. Exciting results were observed on the variety Lep Chang Kut which its growth reduction was closer to that of Chacaco. This observation reveals that Lep Chang Kut could be drought tolerant but its general growth rate under *in vitro* condition was very low compared to other varieties in this study (Figure 2, Table 6). Consequently, it was difficult to detect its drought tolerance ability because the applied *in vitro* protocol seems to be not optimized for cultivar Lep Chang Kut.

Cachaco and Lep Chang Kut had the lowest reduction of WC due to water osmotic stress. Two phenomena are the main causes of reduction of plant WC. Firstly, a plant can lose its water if the ψ_w of the soil is more negative than the ψ_w of the plant. Secondly, a plant can decrease its WC by accumulation of compatible solutes (Taiz and Zeiger, 2006). Indeed, considering the high growth rate of Cachaco on the stress medium (Figure 2) and the small growth reduction due to water stress of Cachaco and Lep Chang Kut (Table 7), it could be that the reduction of WC of these cultivars resulted from the accumulation of compatible solutes. The results of this study revealed that the growth parameters related to leaves (NL, LA), GFW and GDW are relevant for identifying drought tolerant banana varieties under *in vitro* condition.

Conclusion

This study identified that sorbitol is a neutral osmotic inducer to study the drought tolerance of banana varieties and showed that the concentration of 0.2 M sorbitol is a suitable concentration to screen for drought tolerant banana varieties under *in vitro* condition. Moreover, the growth parameters of GLA, GFW and GDW of leaves, total GFW and total GDW were proved to be relevant in identifying drought tolerant banana varieties under *in vitro* condition.

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