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Minimal cost production of banana (*Musa* spp.) using modified MS medium *in vitro* and acclimatization in hydroponics system *ex vitro*

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In vitro production of banana at low cost and acclimatization of plantlets using hydroponics system in the greenhouse were studied. Hydroponics system allows for higher accuracy in terms of controlling the surrounding environmental conditions, which then allows for a higher possibility of increasing productivity and enhancing the quality of plants. Increased number of vigorous shoots produced in modified MS could be mainly due to the synergistic effect of enhancing the elements on improving the metabolic activities in shoot initiation and development. Cytokinin helps in shoot proliferation, and increasing of minerals which plays a significant role in shoot growth and development. Of the different MS medium, the best response in terms of roots number attained with M5 and M6. The relative humidity in the hydroponic system provided a consistent decrease, allowing the gradual acclimatization of the regenerated plants to the unique environment compared to greenhouse potted plants. Hydroponic system which turned to be the best and lowest cost of acclimated of banana during current study. Plant growth parameters in terms of maximum root number, leaves number, plant length, stem diameter and increased weight occurred when plants were adapted and kept in hydroponics. Analysis of the results has shown that the survival rate and growth vigor was higher in plants grown hydroponically as opposed to their traditionally grown ones. Response to the nutrient medium used was also encouraging in the hydroponically grown plantlets.

Keywords: Acclimatization, banana, greenhouse, hydroponics, *in vitro*, rizogenesis

INTRODUCTION

Banana (*Musa* spp.), which is a member of the family Musaceae, are one of the world's most important subsistence crops. It is grown in large numbers in the tropics and subtropics in all types of agricultural system, ranging from small, mixed, subsistence gardens, to large commercial monocultures. In many developing countries, this crop acts as a staple food or the cornerstone of the country's economy. Banana is heavy in potassium, calcium, phosphorus, minerals, carbohydrates, and vitamin-C as well as being famous for its availability throughout the entire year, sufficient production as well as high acceptability to the consumers. Moreover, it is of foremost importance for tannin, latex and fiber production (Al-Amin et al. 2009). Many biotic and abiotic factors are efficient for small yield and production of banana. The virus is one of the major problems. There is a growing demand for disease free and healthy planting materials of banana, yet the conventional clonal propagation method appears to be unable to supply that demand. The richness of vegetative propagated banana and plantain remained significantly limited by virus disease (Lepoivre, 2000). Micro-propagated banana plants have two significant advantages in their size, and septic quality which makes it easier to add new varieties to production areas also their nematode-free status makes it possible to improve soil parasite control and lowered the use of nematicides (Navarro et al. 1994). Hydroponics is developing into a highly functional as well as an effective substitute in comparison to traditional farming cultivation on the soil. It can come into use in areas where arable land is very scarce as well as in areas where the ground was excessively used, which causes a disruption of chemical and biological properties, more often problems occurring in protected cultivation, and high infestation of plant pathogens. Therefore, hydroponics as a method has been preferably used even in tropical countries with abundant land. Hydroponics offers high-quality of products and limits the use of pesticides in comparison to traditional cultivation of land, as well as the wide benefits in great capacity of production, and independent of climate and soil conditions that have been previously mentioned (Castellane and Araújo, 1995). Maximum systems are dynamic, and there is forced circulation of water or air to oxygenate the solution. It is seen that there is a trend to use the NFT (nutrient film technique) system. However, there is still a lack of published data on the adaptation of banana plants produced through hydroponic systems on the development and survival Menezes (2010). Hydroponic systems that constitute a solid substrate for the simulation of the machining impedance of original soil is significant alterations of root and whole plant growth compared to traditional hydroponics (Slota et al,2016). Thus, the object of the study was to investigate different modified MS medium designed to produce *in vitro* Banana at acceptable quality and plantlets acclimatization. Moreover, the use of soilless cultures, such as hydroponics and greenhouses were evaluated with the view of improving the quality and growth of the banana plants in soilless culture.

MATERIALS AND METHODS

The experiments were carried during the period from 2014 to 2016, in the gene transfer and germplasm conservation Laboratory. Explants were collected from field grown banana plants, Department of Plant Biotechnology, Genetic Engineering and Biotechnology Research Institute (GIBRI), University of Sadat City.

Investigate different modified MS (Murashige and Skoog, 1962) medium aiming to produce *in vitro* banana at acceptable quality:

Preparation of materials explants

The shoot explant from suckers was collected by carefully removing the leaves to obtain the apical bud w few sheaths. Then washed with sterile water and sterilized as described (Ahmed et al. 2014). The isolated and surface sterilized explants were prepared as explants in initiation and multiplication shoots. The individual meristems were directly inoculated in 350ml culture jar each containing 50ml of MS medium supplemented with 2mg/l BA, 30g/l sucrose and 6g/l agar (Dharaneeswara Reddy et al. 2014).

Induction of shoots

Effect of modified MS medium on shoot growth and development

The shoot was taken from initiation stage were transferred to MS medium (control treatment) and amended of MS medium which consisted of three different concentrations of nutrient (KH_2PO_4, $CaCl_2$ and $MgSO_4$). Thus, a total of eight different medium treatments were applied (Table 1).

All various media was supplied with 3mg/l BA, 30g/l sucrose, and 6g/l agar. Three shoots (Cluster) were provided in each 350ml glass jar which contained 50ml one of the treatments (medium). Each jar was viewed as a replicate. The study included ten replicates for each treatment. The experiment was replicated twice. The data on shoot proliferation were recorded after six weeks as shoots number/jar, leaves number/jar, shoot length (cm) and weight/ jar. Based on the best results obtained for shoot growth and development, only four treatments (media) were selected to continue for root development evaluation.

Induction of rooting

Effect of modified MS basal nutrient medium on rooting

The effects of modified media included different strengths of M3, M4, M5 and M6 media supplemented with 0.5mg/l IBA, 0.5mg/l charcoal, 30g/l sucrose and 6g/l agar. Shoots were generated from *in vitro* multiplication stage (3–4cm long) were individually separated and five shoots were cultured on modified MS medium. The following parameters were estimated after one month: number of roots per plantlet, root length (cm), the number of leaves and plant length (cm).

Table:1 Different medium treatments applied in the study.

Modified medium	MS salt strength	Elements level (g/l)		
		KH_2Po_4	$CaCl_2$	$MgCl_2$
M1	Full	0.085	0.220	0.185
M2	Full	0.170	0.440	0.370
M3	¾	0.085	0.220	0.185
M4	¾	0.170	0.440	0.370
M5	½	0.085	0.220	0.185
M6	½	0.170	0.440	0.370
M7	¼	0.085	0.220	0.185
M8	¼	0.170	0.440	0.370

Acclimatization of plantlets

Effect of modified MS *in vitro* and two methods of in-greenhouse hardening on a growth of banana plantlets *ex vitro*

In vitro produced plants from M3, M4, M5 and M6 of modified MS rooting experiment were subjected to two methods of acclimatization using:

(1): Hydroponic system:

The plantlets were acclimatized and developed in the hydroponic system NFT (Fig.1). The system consisted of a small plastic tray (50 cm width x 100 cm length x 10 cm depth). The base of the tray was supplied, with small clay beads 0.5 in diameter. The banana plantlets were transplanted, and the roots were covered, with the clay beads. The nutrient solution was pumped from a small feeding tank to a polyethylene distribution tube. This system has the advantage of allowing the roots to be in direct contact with the nutritive solution (NPK), allowing an appropriate intake of nutrients. The plastic tries covered with a transparent plastic lid (50 cm width x 100 cm length x 15 cm height), creating a microenvironment with a high relative humidity, through which light can pass while protecting the plantlets against a possible mechanical damage or the attack of insects. The nutrient solution was implemented to the plants' roots through Hydroponic system forms a layer of cultivation along the canals where plantlets, keeps its roots. The technique allows the wide range of acclimatization and can be prepared in rigid or flexible tubes with different sections, diameters and lengths.

(2): Plants transferred to pots (6 cm) which included (peat: perlite 3:1v/v) in the nursery and covered with transparent plastic packets for producing highest grower and development. Maximum leaves number, plant length (cm), root number, root length (cm), leaves the area, thickness of stem and determination of chlorophyll (a and b) and total chlorophyll. For the plants adapted in greenhouse pots, as the plastic bag was gradually removed the relative humidity decreased, allowing the plants to adapt to the new environment slowly**.**

Determination of biochemical parameters by extraction of chlorophyll (Arnon, 1949)

One gram of fresh leaves was taken and ground with 10ml of 80% acetone. Then centrifuged at 5000 –10000rpm for 5min. The supernatant was shifted, and the procedure was repeated, till the residue becomes colorless. The absorbance of the solution was read at 645nm and 663nm against the solvent (acetone) blank. The quantity of chlorophyll a, b and total chlorophyll were calculated using the following equation: Chlorophyll a: 12.7 (A663) – 2.69 (A645), Chlorophyll b: 22.9 (A645) – 4.68 (A663) and Total Chlorophyll: 20.2 (A645) + 8.02 (A663).

Statistical analyses:

The randomized factorial design was applied, and data were the examination of variation. Separation of means between treatments was determined using LSD test at 5% (Steel and Torrie, 1980).

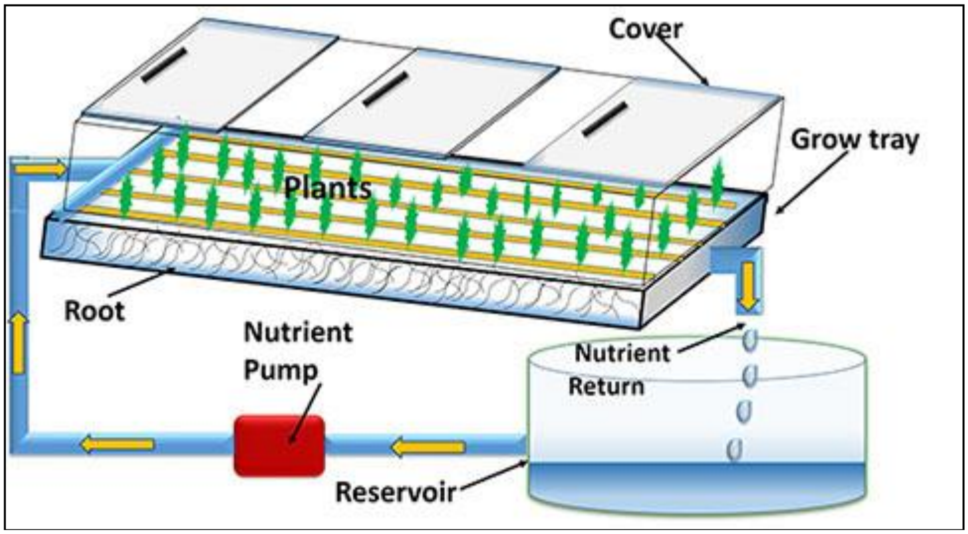

Figure 1. Sequence assembly of hydroponics system to the adaptation of banana.

Table 2: Effect of modified MS medium on growth and development of *banana* grown after two months of culture.

Modified of media	Shoots number	Shoot length (cm)	Leaves no.	Weight (g)	Growth vigor
MS (control)	11 .25 c	5.25 bcd	28.50 d	13.00 d	++
M1	9.25 d	3.25 e	13.00 f	11.00 ef	+
M2	7.50 ef	3.62 e	23.00e	11.75 e	+
M3	15 .50 b	5.00 cd	36.50 c	15.75 c	++
M4	17.25 a	6.50 ab	39.75 b	17.25 b	+++
M5	15.50 b	7.13 a	37.50 c	16.50 bc	++++
M6	16.50 ab	6.25 abc	48.50 a	18.50 a	++++
M7	8.50 de	4.00 de	10.00 g	10.25 f	-
M8	6.50 f	3.5 e	10.50 g	10.50 f	-
L.S.D at 5%	1.47	1.26	1.76	1.17	

RESULTS

Induction of shoots:

One of the difficulties that restrict obtaining the sufficient number of planting material of a chosen clone is that during its growth period, the rate of multiplication in banana is limited to 5-20 suckers per plant. Micropropagation techniques facilitate production of a great number of plantlets/unit in time, thus helping in rapid introduction and dissemination of new varieties.

Effect of modified MS medium on shoots growth and development:

From the results obtained, the statistical analysis emerges that the growth and development of shoots were efficient with modified MS basal nutrient medium. The shoots obtained from the modified M4 basal medium gave the maximum regeneration, number of shoots and gave moderated growth value than other treatments as shown in (Table 2) and (Fig. 2). The tallest shoots were seen in the modified M5 medium. Maximum leaves number, fresh weight, and growth value were obtained on modified M6 medium than other treatments. Hence, the increased number of produced healthy plants might be attributed to the synergistic effect of modified elements on enhancing the metabolic activities in shoot initiation and development

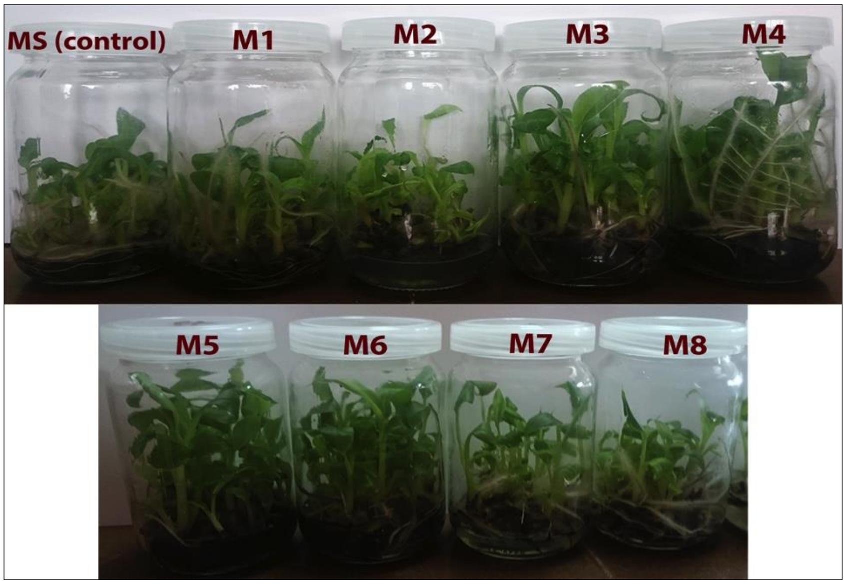

Figure 2: Development of banana shoots on modified MS medium after two months of culture.

Table 3: Effect of modified MS medium supplemented with 0.5mg/l IBA on induction and development of roots from *in vitro* cultured after six weeks.

Modified MS media	Root number	Root length (cm)	Leaves no.	Plant Length (cm)	Growth vigor
M3	4.80	6.20	5	7.50	++
M4	5.40	5.60	6	7.90	+++
M5	6.80	6.90	5	8.50	++++
M6	6.60	8.60	5	7.40	++
L.S.D at 5%	**1.09**	**0.93**	**NS**	**NS**	

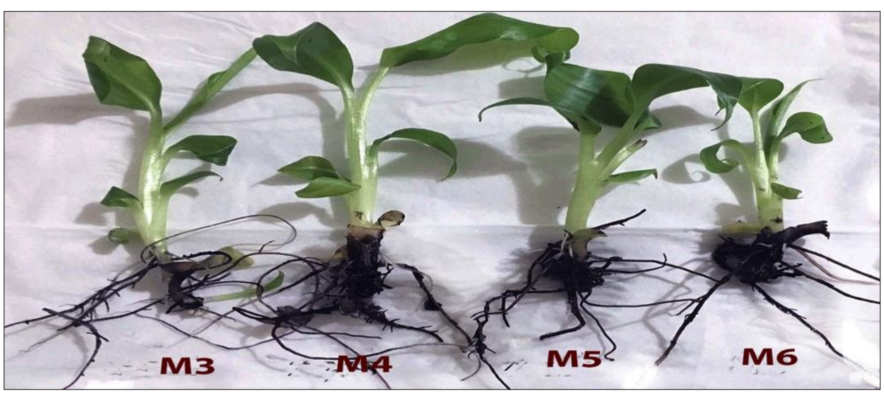

Figure 3: Development and initiation of roots on different modified MS basal medium after six weeks.

Induction of rooting

Effect of modified MS medium on rooting*:*

Root growth and development varied with different MS strengths. Vigorous roots of *in vitro* grown plantlet were given by M5 and M6 media (Table 3). The highest root length was increased under M6 medium. The root number and root length after 30 days were remarkably the highest indicating that M5 and M6 were essential for successful root induction of banana. The use of M5 for rooting produced with highest and tallest plants which grew vigorously than other treatments, so the same can be used further as a recommendation for salt strength leading to minimizing the cost of media. Of the different MS concentrations tested, the best response to roots number was obtained with M5 and M6 (Fig. 3). Small mineral salt concentration in the *in vitro* rooting medium can be adopted as an effective low-cost *in vitro* rooting procedure for a banana.

Acclimatization of plants

Effect of *in vitro* modified MS medium and different acclimatization in greenhouse methods:

Plantlets were adapted via hydroponic installation had a better development than those adapted in the greenhouse pots. The results corresponding to the increase in root number, leaves number, plant length, and leaves the area as well as stem thickness during the adaptation in hydroponics from *in vitro* M4 modified medium as shown in (Fig. 5). It was noted that the tallest roots formed at M4 changed medium and adapted in the potted greenhouse than those adapted in the hydroponic system as shown in (Table 4). The highest content of chlorophyll a, b and total chlorophyll was obtained from M3 modified medium than another treatment (Table 5). In general, it was found that both; increased number of leaves and roots length observed when plants were adapted to the hydroponics system, doubling the original number of new structures after two months. On the other hand, the adaptation of plants in greenhouse pots increased root length. The relative humidity in the hydroponics system presented a consistent decrease, allowing the gradual acclimatization of the regenerated plants to the new environment. Hydroponic systems, have advantages such as being able to save water and nutrients due to the closed system in order to recycle and attempt to reduce possible environmental contamination of nutrients and pesticides (Fig 6). Moreover, they have the lowest cost of installation, as well as easiness of operation and equipment sterilization between cultivation seasons

Table 4: Effect of *in vitro* modified MS medium on root number, root length, leaves number and plant length at different methods of acclimatization in the greenhouse after six weeks.

Acclimatization methods	Modified MS Medium	Root number	Root length(cm)	Leaves no.	Plant Length (cm)
Method 1 (Pots cover)	M3	5.80CD	7.20D	3.40D	7.70C
	M4	7.80B	21.60A	5.00B	9.30CD
	M5	6.40E	14.40B	4.00CD	7.80DE
	M6	5.20DE	6.30D	4.20B	7.50E
Method4 (Hydroponics system)	M3	6.60C	7.00D	5.00B	8.70C
	M4	9.20A	11.00C	7.00A	13.50A
	M5	8.50AB	10.40D	6.40AB	11.00B
	M6	5.80F	5.00E	4.20C	9.50C
L.S.D at 5%		1.03	2.24	0.73	1.52

Table 5: Effect of *in vitro* modified MS medium on leaves area, steam thickness, accumulation of chlorophyll at different acclimatization methods in the greenhouse after six weeks.

Adaptation Methods	Modified MS media	Leaves area(cm)	Stem thickness(cm)	Chl.a	Chl. b	Total chl.
Method1 (Pots cover)	M3	2.55 d	0.52D	17.22	7.54	24.75
	M4	3.07 c	0.76B	18.31	9.11	27.22
	M5	2.47 d	0.56CD	14.18	5.94	19.90
	M6	1.83 e	0.44D	13.85	5.75	19.54
Method4 (Hydroponics system)	M3	1.65 f	0.54D	23.15	16.35	39.26
	M4	4.50 a	1.00A	22.84	14.92	37.16
	M5	3.25 b	0.70BC	21.35	11.98	33.27
	M6	1.32 g	0.44D	7.56	6.07	13.51
L.S.D at 5%		1.69	0.14			

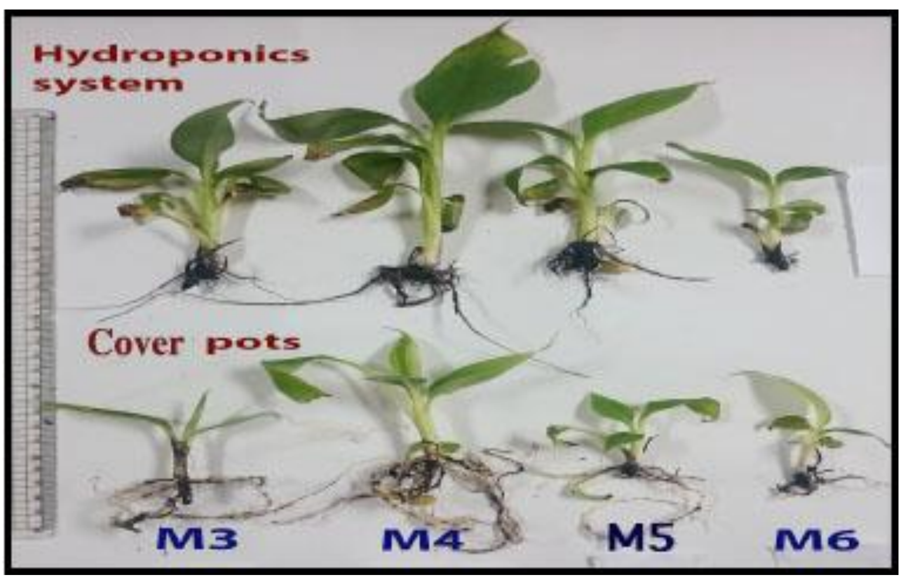

Figure 5: Effect of *in vitro* modified MS media on growth and development at acclimatization of plants using hydroponics system and greenhouse.

Figure 6: Development and acclimatization of banana plants using hydroponics system *Ex Vitro.*

DISCUSSION

Induction of shoots

Effect of modified MS medium on shoots growth and development

The modified elements improved *in vitro* growth and development of banana. Nitrogen (N) is a fundamental element for plant cell and tissue cultures, being essential for the synthesis of DNA, RNA, and proteins (Oksman-Caldentey 1994). Inorganic macronutrient and micronutrient levels used in most plant tissue culture media are based on levels established in the plant tissue culture medium developed by (Murashige, and Skoog, 1962) for tobacco tissue culture "MS medium" (Nassar, 2004). The active factor in the medium is the ions of different types rather than the compounds. One type of ion can be contributed by more than one compound, example NO_3^- ions may be contributed by NH_4NO_3 as well as KNO_3 (Bhojwani and Razdan, 1983). Wu et al. (2005) who reported that Ca Cl_2 was one of the factors that affected the pH value of MS during the process of autoclaving. On the other hand, supplying $Ca(NO_3)2.4H_2O$ instead of $CaCl_2.2\ H_2O$ decreased the concentration of K indirectly. Sindhu et al. (2012) reported that little mineral salt strength at various stages of plantlet development had a positive effect. Maximum, number of shoots and roots of banana varieties (*Poovan and Monthan*) in media containing low-cost trio particles compared to media with standard KH_2PO_4 (Dhanalakshmi and Stephan 2014).

Induction of rooting

Effect of modified MS medium on rooting

An efficient *in vitro* rooting of banana obtained with the establishment of optimum concentration of MS in a tissue culture medium for banana is necessary. Small mineral salt concentration in the *in vitro* rooting medium can be adopted as an effective low-cost *in vitro* rooting procedure for a banana. The present results are similar to the findings of Sindhu et al. (2012) after hardening, plantlets developed on ½ or ¾ strengths MS medium could be acclimatized and grew well *ex vitro*. Maximum rooting of banana was obtained on ½MS supplemented with IBA 1mg/l Ahmed et al. (2014). Root initiation in modified MS media containing MS nutrients formed sub-roots, which grew vigorously (Dhanalakshmi and Stephan 2014). The maximum root length (6.46 cm) was obtained in half MS media supplemented with IBA 1.5 mg/l followed by IAA 1.5 mg/l treatment (4.32 cm) respectively (Kumar, 2016).

Acclimatization of plants

Effect of *in vitro* modified MS medium and different acclimatization in greenhouse methods

Hydroponics is a plant growth system that provides a more an efficient control of nutrient supplemented. Plantlets were adapted via hydroponic system had a better development than those adapted in the greenhouse pots. The relative humidity in the hydroponics system presented a consistent decrease, allowing the gradual acclimatization of the regenerated plants to the new environment. Hydroponic systems, have advantages, lowest cost of installation, easiness of operation and equipment sterilization between cultivation seasons, saving water and nutrients due to the closed system for recycling and possible reduced environmental contamination with nutrients and pesticides. Macedo *et al*. (2003) succeeded in achieved pineapple plants derived from *in vitro* propagation were more developing *ex vitro*, using the floating hydroponics. The hydroponic cultivation has as main advantages compared to the rational use of water and nutrients supplied to plants so that plants may present a further development in short-time intervals. Nutrient solutions require accurate and special control in order for the growth of plants in hydroponic gardens with an inert substrate like rockwool (Espacios y Paisajes system) or without substrates (NGS system) (Guzman, 2004). Adaptation of three types of *in vitro* plants like, herb (Strawberry - *Fragaria* × *ananassa* Duch.), shrub (Rose - *Rosa indica* L.) and tree (Date palm - *Phoenix dactylifera* L.) is what the hydroponics techniques are tested for. Results have shown that hydroponically grown plantlets have significantly higher survival rate and growth vigor when compared to their conventionally grown controls (Al-khalifah 2010). After hardening, plantlets developed on ½ or ¾strength MS medium could be acclimatized and grew well *ex vitro* (Sindhu et al. 2012 and Al-Moshileh et al. 2017). Both hydroponic systems have become hydroponic vertical gardens able to reuse water continuously. The application of fertilizer is executed by compound solid fertilizer mixtures manufactured for use in fertigation, with different ratios between the three major elements and microelements in the form of chelates. The nutrient solution depends on the type of plants (Salasa et al. 2012).

CONCLUSION

Banana is a necessary food, and considered as one of the most extensively shipped fruits in the world. The demand for planting material of banana is very high in the tropical and sub - tropical regions. Plantlets were acclimated via hydroponic installation had a better development and a lower cost than those adapted in the greenhouse pots. Our hydroponic system also proved useful for fast adaptation, natural growth, and allowed for rapid harvest. Moreover, the hydroponic system also allowed for water re-use, hence we anticipate savings in utilized water quantities.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

AB, AN and AE designed and performed the experiments and also wrote the manuscript. AN designed the hydroponic system for plant acclimatization. AB and AN performed data analysis. AB, AN and AE designed experiments and reviewed the manuscript. All authors read and approved the final version.

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