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Research article

Effect of vermicompost on vegetative growth and nutrient status of acclimatized Grand Naine banana plants☆

S.A.M. Hassan [a,b], Rania A. Taha [a,b,*], Nagwa S.M. Zaied [a,b], Entsar M. Essa [c], Abd El-Rheem Kh. M [d]

[a] Pomology Department, Agriculture and Biological Research Institute, National Research Centre (NRC), 33 El Buhouth St., Dokki, Giza, 12622, Egypt
[b] Tissue Culture Technique Lab, Central Laboratories Network, NRC, Egypt
[c] Soils and Water Use Department, NRC, Egypt
[d] Plant Nutrition Department, NRC, Egypt

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ABSTRACT

Novel approach for introducing vermicompost as an acclimatization soil for banana plants was conducted. Different potting mixture of vermicompost, vermiculite, peatmoss and sand at different ratios were used to study the effect of different agricultural media on vegetative growth and leaf mineral content of banana tissue culture plantlets, after acclimatization stage. Two different trial periods were studied; 12 and 24 weeks in these agricultural media for two seasons. Results indicated that using vermicompost combined with vermiculite and sand (33.3% for each) recorded the best results for the most vegetative growth studied parameters (plant height, plants and roots length, stem diameter, leaf width and shoot and root fresh and dry weight). Moreover, vermicompost at 50% + peat moss at 50% significantly increased total chlorophyll content in banana plants. Furthermore, N, P and K plant analysis has shown that vermicompost at 75% with peat moss gave the highest mineral content values at the two periods. Addition of vermicompost to the culture media improved in vitro-produced banana plants in greenhouse.

1. Introduction

Many scientific researches were conducted to improve the survival rate and growth parameters of the tissue cultured plants during acclimatization. Culture medium is an important factor to get these enhancements. Sand, perlite, vermiculite and peat moss were the most investigated substances as Ali et al. (2011) concluded that application of potting mixture containing soil: sand: farm yard manure (2:1:1 v/v/v) was superior treatment for increasing vegetative growth and survival of banana plantlets. Furthermore, survival rate (98%) of Atlanta cultivar was reported by Han and Goo (2003) using a growth substrate of vermiculite and perlite (1:1 v/v). In addition, plum plantlets were successfully acclimatized in combination of peat moss and sand at the rate of 1:1 (Hassan et al., 2021).

Vermicompost represents a very suitable medium for plant growth showing improved growth for many plant species (Ebrahimi et al., 2021; Serri et al., 2021), that can also be used as a growth substrate in different stages of micropropagation. It is rich in macro and micronutrients, vitamins, growth hormones, enzymes such as proteases, amylases, lipase, cellulase, and chitinase, and immobilised microflora created by earthworm activity. Even after the earthworms have ejected the enzymes, they continue to degrade organic matter (Barik et al., 2011). However, vermicompost as a culture medium for acclimatization are rarely investigated. Martin et al. (2003) assured that *Anthurium andreanum* was planted in vermicompost and sand (1:3), kept in the greenhouse for acclimatization, revived growth within two weeks then transferred to a net house. In contrast, Parkhe et al. (2018) claimed that from different potting mixtures; garden soil, cocopeat, farm yard manure (FYM), vermicompost and sand with different combinations, 100 % hardening success was conducted to banana plantlets of cv. Grand Naine when garden soil and FYM (3:1) were used. This combination gave maximum height and survival of plantlets and shows outstanding performances in field condition. The positive effects of vermicompost are also highlighted in other studies (Naiji and Souri, 2018; Souri and Hatamian, 2019; Najarian and Souri, 2020).

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* Corresponding author.
E-mail address: rania_abdelghaffar@yahoo.com (R.A. Taha).

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Banana (Musa spp.) is considered as one of the most important tropical fruit crops grown in the world. This crop plays an important role in the economy of tropical and subtropical countries. Micropropagation is a unique process for banana large numbers production. Acclimatization is the most crucial process during banana micropropagation (Ozdemir et al., 2020). This is for that the in vitro raised plantlets are not readily acclimatized for ex-vitro conditions due to the non functional stomata, weak root system and poorly developed cuticle (Vasane and Kothari, 2006). Moreover, an effective acclimatization technique saved time, labour, and money, including the culture medium used, is really needed (Gantait et al., 2009).

The present investigation was carried out to study the effect of vermicompost, as a novel culture medium, combined with other agricultural media as sand, vermiculite and peat moss on vegetative growth and leaf mineral contents of ex-vitro Grand Naine banana plants acclimatization.

2. Materials and methods

This study was carried out during September to February for two seasons in the greenhouse of Pomology Dept., National Research Centre, Dokki, Giza Governorate, Egypt. Mineral leaf contents were analyzed at Soils and Water Use Department and Plant Nutrition Department, National Research Centre, Dokki, Giza, Egypt.

2.1. Acclimatization process

Grand Naine banana (*Musa spp.*) plantlets derived from in vitro cultures of Tissue Culture Technique Lab., Central Laboratories Network, National Research Centre, Dokki, Giza Governorate, Egypt as described by Taha et al. (2020) were taken away from the jars and the basal portion of these plantlets were subjected to continuous flow of tap water to get rid of medium residues then rinsed carefully in sterile distilled water. The basal part of plantlets was dipped in antifungal solution (Benlate) at the rate of 0.1% for 10 min. Then, they were planted in suitable black pots filled with wetted peat moss: sand (1:1) without any water surplus to avoid decay of plantlets roots. The planted pots were covered with transparent polyethylene bags (0.2 mm thick) after spraying water inside the bag to raise up the relative humidity (RH) to 80–90% around plantlets leaves. Small pores in bags were made after emerging the first new leaf (indicator of successful gradual acclimatization), then increased longitudinally by increasing their lengths and numbers until removing the whole bag.

2.2. Vermicompost description

The vermicompost used in this experiment was made of animal wastes (cow and horse manure), which were digested by species of earthworm such as *Eisenia andrei* and *Eisenia fetida*. Some chemical properties of vermicompost were determined in Table 1. Micronutrient content (Fe, Mn, Zn) were determined using atomic absorption spectrophotometer as described by Cottenie et al. (1982). Other chemical properties of vermicompost were measured according to the standard methods described by Cotteine (1980).

2.3. Effect of various culture media on banana vegetative growth

The gradual acclimatized Grand Naine banana plants were planted in bigger pots (20 × 20) filled with sand, peat moss, vermiculite and vermicompost in their combination as follows:

T 1: Peat moss (50%) + sand (50%)
T 2: Vermicompost (50%) + sand (50%)
T 3: Peat moss (50%) + vermicompost (50%)
T 4: Peat moss (75%) + vermicompost (25%)
T 5: Peat moss (25%) + vermicompost (75%)
T 6: Vermiculite (50%) + sand (50%)
T 7: Vermiculite (50%) + vermicompost (50%)
T 8: Vermiculite (33.3%) + vermicompost (33.3%) + sand (33.3%)
T 9: Vermiculite (25%) + vermicompost (50%) + sand (25%)
T 10: Vermiculite (25%) + vermicompost (25%) + sand (25%) + peat moss (25%)

2.4. Fertilization

Crystalon (2.0 g/l) as a source of NPK (20% N, 20% P, 20% K) was used to fertile the plants. It was applied to soil during growing season to different tested treatments, including control.

2.5. Data collection

Morphological parameters determination and chemical analysis were done for plants at two times; 12 and 24 weeks after transplanting for two seasons. Samples (three plants/treatment/time) were collected to take morphological data. Moreover, leaves were cut and dried for chemical analysis. The following morphological parameters were measured: Plant length (cm), number of leaves, root length (cm), leaf length (cm), stem diameter (cm), leaf width (cm), root and shoot fresh weight (g) were determined at two times; after 12 weeks and 24 weeks from transplanting.

2.6. Chemical analysis

Total nitrogen content was estimated by micro-Kjeldahl (Gerhardt, EV16) (Motsara and Roy, 2008). The percentages of phosphorus and potassium in the acid digested samples of banana dry leaves were determined. Phosphorus was determined calorimetrically with NH4-Metavanidate method by spectrophotometer (Unico, 2000UV). Potassium was flame-photometrical estimated in leaves samples (England, Jenway, 7PFP). Total chlorophyll content was determined in first leaves by Minolta meter (SPAD-502, Japan) and the data were expressed as SPAD units (Markwell et al., 1995).

3. Statistical analysis

Data for the two seasons were collected and average were counted. All data were subjected to statistical analysis using MStat software. The

Table 1. Some chemical properties of vermicompost used in this investigation.

pH	EC (dS m^{-1})	Moisture content	Organic matter	Organic carbon	C/N ratio	N	P	K	Fe	Zn	Mn
						%			Ppm		
7.2	2.4	25%	55.2 %	32.1 %	29.1	1.80	0.55	1.2	1320	40	110
Plant growth regulators (ppm)											
Indol Acetic Acid			Abscisic Acid			Cytokinin				Gibrilic Acid	
50.82			78.70			40.79				75.80	

comparison among means of the different treatments was determined, as illustrated by Duncan (1955).

4. Results

4.1. Vegetative growth characteristics

Data in Table 2 which collected after 12 weeks from transplanting, clearly showed that T8 (Vermiculite 33.3% + vermicompost 33.3% + sand 33.3%) surpassed other treatments in enhancing plant length, root length, stem diameter, leaf width, shoot dry weight and gave satisfactory root dry weight. Meanwhile, T3 (peat moss 50% + vermicompost 50%) gave the highest leaves number, stem diameter and leaf length. Furthermore, T9 (Vermiculite 25% + vermicompost 50% + sand 25%) gave the highest leaf width, shoot and root fresh weight (Figure 1).

Similarly, data in Table 3 which collected after 24 weeks from transplanting, clearly showed that T8 surpassed other treatments in enhancing plant length, root length, stem diameter, leaf width, shoot fresh weight and shoot and root dry weight. It gave satisfied leaves number, leaf length and root fresh weight.

Figure 1. Effect of vermicompost combined with different agricultural media on growth parameters of grand Naine banana after 12 weeks.

4.2. Leaf chlorophyll and leaf mineral contents

Results in Table 4 show the effect of vermicompost combined with different agricultural media on total chlorophyll and Leaf mineral content in the leaves after 12 and 24 weeks from transplanting. The highest total chlorophyll was obtained from T3 (peat moss 50% + vermicompost 50%) after the 12 weeks from transplanting. Furthermore, after 24 weeks, the positive effect of planting in vermicompost combinations clearly appeared as most vermicompost treatments surpassed others and T10 (Vermiculite 25% + vermicompost 25% + sand 25% + peat moss 25%) gave the highest value.

Likewise, results in Table 4 indicated that leaf mineral content was affected significantly by different treatments under this investigation. The highest N%, P% and K% in leaves were recorded by plants grown in T5 (Peat moss 25% + vermicompost 75%) at 12 weeks as well as 24 weeks after transplanting.

5. Discussion

Many researchers indicated that culture medium affects significantly primary and secondary acclimatization success of banana plantlets (Ali et al., 2011; Vasana and Kothari, 2006) but few of them investigated vermicompost on banana plants. Our results indicated that vermicompost combined with other culture materials; specially vermiculite and sand recorded the best results in terms of most studied morphological measurements during greenhouse stage of in vitro-produced banana plants. Similarly, Kashyap et al. (2017) investigated the effect of vermicompost with different methods of extraction; vermicompost, eluant or vermicompost extract for *B. monnieri* plants. Results showed that 100% survival for the three methods. Moreover, vermicompost extract medium gave the higher root numbers and plantlets weight while, vermicompost alone gave the higher number of shoots. It might be due to the high quantities of nutrients, humic acids, and humates in vermicompost that cause the plant growth response to resemble hormone-induced activity (Atiyeh et al., 2000; Edwards and Burrows, 1988). Soil amendment with vermicompost and pistachio biochar increased plant growth and performance of eggplant as well as yield and water use efficiency (Ebrahimi et al., 2021). Otherwise, sugarcane bagasse compost was used at various concentrations (20%, 30%, 40%, 80% and 100%) into soil bedding medium mixture for Pelargonium × hortorum. Generally, application of sugarcane compost stimulated plant growth. In addition, low concentrations especially 20% resulted in the highest morphological parameters including leaves number, stems number, shoots originate flowers, flowers number, inflorescence diameter, main shoot diameter, shoot and root biomass (Najarian and Souri, 2020).

Generally, our results assured that addition of vermicompost to growth environments improved banana leaves content of chlorophyll, nitrogen, phosphorus and potassium. Similarly, chlorophyll a increased in plants grown in sugarcane compost at all concentrations used compared to control plants (Najarian and Souri, 2020).

It is clear that T5 had the highest rate of vermicompost used so that, it gave superior nutrient contents. It is evident that vermicompost

Table 2. Effect of vermicompost combined with different agricultural media on growth parameters of Grand Naine banana after 12 weeks.

Treatments	Leaves no.	Plant length	Root length	Stem diameter	Leaf length	Leaf width	Shoot F.W	Shoot D.W.	Root F.W.	Root D.W.
		cm					G			
T_1	4.333	47.0	29.67	16.33	23.67	10.97	44.0	3.643	17.4	2.193
T_2	4.667	49.33	22.33	17.33	24.63	11.40	39.97	4.390	22.93	2.930
T_3	5.333	58.33	30.67	22.33	28.67	12.93	77.0	6.510	32.03	3.387
T_4	5.00	47.0	25.33	17.0	20.60	10.33	45.23	3.833	19.3	1.947
T_5	3.667	48.33	18.0	14.0	20.93	10.27	29.53	3.377	19.43	2.277
T_6	4.333	59.0	28.0	19.0	24.83	11.50	70.17	7.667	32.90	4.933
T_7	3.667	49.67	25.67	16.67	23.5	11.87	46.43	7.583	33.60	2.550
T_8	4.333	59.67	36.67	21.33	23.0	13.30	70.20	8.787	35.30	5.097
T_9	4.00	54.67	18.0	20.0	27.17	13.57	82.63	7.987	41.33	3.723
T_{10}	5.00	57.33	27.0	20.67	27.9	12.37	64.63	7.847	38.30	5.213
$LSD_{0.05}$	0.91	0.67	4.70	2.33	4.04	0.64	5.63	0.80	3.03	0.116

Table 3. Effect of vermicompost combined with different agricultural media on growth parameters of Grand Naine banana after 24 weeks.

Treatments	Leaves no.	Plant length	Root length	Stem diameter	Leaf length	Leaf width	Shoot F.W	Shoot D.W.	Root F.W.	Root D.W.
		cm					G			
T_1	6.50	51.33	29.33	6.30	33.28	17.67	56.41	4.513	17.36	2.21
T_2	6.50	49.50	21.00	6.90	37.17	17.23	46.81	3.745	21.49	2.96
T_3	5.00	59.00	29.67	4.33	38.00	13.17	85.09	6.807	31.02	3.33
T_4	7.00	50.17	24.67	6.50	37.58	18.72	45.22	3.618	19.67	1.96
T_5	6.00	45.66	18.67	6.00	42.00	19.83	32.87	2.630	19.78	2.20
T_6	8.00	56.20	27.67	6.25	31.00	14.73	63.77	5.102	32.67	4.67
T_7	8.00	54.10	25.33	6.50	37.92	19.72	46.42	3.714	33.76	2.36
T_8	9.00	63.80	37.00	8.75	39.17	20.33	90.15	7.212	35.33	5.16
T_9	10.0	57.83	18.00	7.50	34.75	17.40	70.14	5.611	41.67	3.67
T_{10}	9.00	62.17	26.67	8.00	38.41	19.17	84.84	6.787	39.00	5.23
$LSD_{0.05}$	0.90	1.63	4.80	0.74	0.75	0.49	5.04	4.00	3.05	0.210

Table 4. Effect of vermicompost combined with different agricultural media on total chlorophyll and mineral content% of Grand Naine banana.

Treatments	Total chlorophyll	Total Chlorophyll	N %	P%	K%	N%	P%	K%
	12 weeks	24 weeks	12 weeks			24 weeks		
T_1	31.33	21.0	2.25	0.21	3.15	2.24	0.22	3.14
T_2	23.27	23.0	2.25	0.20	3.34	2.24	0.21	3.33
T_3	35.90	27.0	2.56	0.23	3.45	2.55	0.24	3.44
T_4	30.77	26.95	2.30	0.24	3.23	2.30	0.25	3.24
T_5	27.83	22.60	2.63	0.25	3.55	2.62	0.26	3.54
T_6	33.33	24.85	2.31	0.12	3.27	2.32	0.13	3.28
T_7	26.23	33.05	2.52	0.17	3.41	2.53	0.17	3.42
T_8	30.57	33.10	2.48	0.14	3.38	2.49	0.14	3.39
T_9	25.17	31.70	2.51	0.21	3.41	2.52	0.21	3.42
T_{10}	30.47	39.45	2.61	0.22	3.50	2.62	0.23	3.51
$LSD_{0.05}$	2.57	6.33	0.01	0.008	0.04	0.01	0.008	0.04

enhances uptake of nutrients by plants and this may be by providing all nutrients in readily available form. As, Mahmud et al. (2020) assured that the application of vermicompost reduced soil acidity and produced macro and micronutrients content (N, P, K, Mg, Ca, S, Fe, Zn, B and Al) in the soil and plants. In fact, the technology of vermicomposting was found to provide better phosphorus nutrition from different organic wastes (Ghosh et al., 1999). In that manner, highest phosphorus concentration content was occurred at 20% sugarcane bagasse compost substitution. Moreover, a large amount of nitrogen and potassium were observed at 40% sugarcane bagasse compost (Najarian and Souri, 2020).

Vermicompost proved to be a very suitable organic fertilizer or substrate with high porosity, aeration, drainage and good water holding capacity (Edwards and Arancon, 2005). It has also significant levels of growth promoting substances including minerals, enzymes, and phytohormones (Naiji and Souri, 2018; Souri and Hatamian, 2019; Najarian and Souri, 2020). It was found to include growth-promoting auxins, cytokinins, and gibberellins (Suhane, 2007).

6. Conclusion

Survival rate and growth enhancement are crucial for acclimatization stage of tissue culture derived plants. Addition of vermicompost to the culture media improved in vitro-produced banana plants in greenhouse. As for, using vermicompost combined with vermiculite and sand (33.3% for each), recorded the best results for the most vegetative growth studied parameters. Vermicompost at 75% with peat moss at 25% gave the highest mineral content values at the two periods of the study. Vermicompost proved to be a novel culture medium for enhancing plant growth in the greenhouse.

Declarations

Author contribution statement

S.A.M. Hassan: Performed the experiments; Analyzed and interpreted the data.

Rania A. Taha, Nagwa S.M. Zaied: Conceived and designed the experiments; Wrote the paper.

Entsar M. Essa, Abd ElRheem Kh. M: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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