Plant Tissue Cult. **12**(2) : 147-154, 2002 (December)

PTC

Increased Growth of Micropropagated Banana (*Musa paradisiaca*) with VAM Symbiont

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Key words : Micropropagation, VAM, Banana, Increased growth

Abstract

Banana plantlets regenerated through shoot-tip culture were subjected to routine hardening procedure. One set of plantlets was supplied with VAM consortium (5 gm) in its rhizosphere and the other set was treated as control. Appropriate conditions like temperature and humidity were maintained for the plantlets subjected to hardening during the experiment. Various plant growth parameters including chlorophyll content of leaves and percentage root colonization by VAM were studied at regular intervals of 20 days. The results revealed a significant increase in the overall growth of VAM inoculated banana plantlets over the control.

Introduction

Banana is one of the important horticultural crops extensively cultivated in India. It has been conventionally propagated through suckers which is a time-consuming method with lower rates of multiplication. To overcome this, the standardized protocols for shoot-tip culture have been commercially exploited by various tissue culture industries. In fact, plant tissue culture forms an important and advantageous tool for rapid generation of elite clones. Although this technique has got several successful applications, there are still certain hitches, which limit its widespread use.

One of the major shortcomings of tissue culture raised plantlets is the lack of any indigenous symbiont. The plantlets, generated *in vitro* under aseptic conditions, eliminate all microbes including natural symbionts also. Vesicular-arbuscular mycorrhizal fungi form one such group of fungus that establishes the symbiotic relationship in the roots of higher plants (Bolan 1991; Barea 1991).

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VAM fungi avail of the nutrients from the cortical root cells to sustain themselves and in turn provide an array of benefits to the host. VAM has been reported to cause increased nutrient uptake (Pearson and Jakobsen 1993), reduced disease occurrence in host (Caron 1989), reduced transplant mortality (Biermann and Linderman 1983) and injury (Menge et al. 1978), improved water relations (Gianinazzi et al. 1990) and chlorophyll content (Jasrai and Thaker 2002) increased drought tolerance (Davies et al. 1992), increased contents of DNA and RNA (Senthilkumar et al. 2000), improved rooting and survival (Strullu 1985) and overall growth (Wang et al. 1993) of micropropagated plants.

The present study was carried out with an aim to study the effects and corresponding symbiotic association of VAM fungi on *in vitro* raised banana plantlets (*Musa paradisiaca* var. *Robusta*). The growth parameters taken into consideration comprise shoot-length and weight, root-length and weight, leaf-length, breadth and area along with its chlorophyll content.

Materials and Methods

Shoot-tip explants from the selected suckers of elite banana plants (*Musa paradisiaca* var. *Robusta*) were established on MS medium. Rapid multipli-cation was achieved following the method reported earlier (Cronauer and Krikorian 1984).

The VAM consortium for the experiment was isolated from rhizosphere of rhodes grass (*Chloris guyana*) growing in semi-arid grassland at Nadiad (22.41_N and 72.55_E), Gujarat. The isolation procedure required the use of a graded set of sieves following the wet sieving and decanting technique (Gerdemann and Nicholson 1963). The isolated consortium was subjected to bulking through pot cultures of certified seeds of *Sorghum bicolor* var. *Harasona*. Three cycles of four weeks growth were carried out while maintaining semi-arid conditions. The bulked consortium containing spores was then quantified under a binocular microscope (Fig. 3).

The micropropagated banana clones of similar weight and height were planted in polybags (1 lt capacity), containing the soil mixture comprising soil, sand and compost (2 : 1 : 1; v/v) at the rate of 1.25 kg/polybag (Fig. 1). The soil mixture was pre-sterilized in an autoclave at 121_C (30 min) to ensure elimination of any symbiont in the soil mixture. One set of plants was kept as control (non-mycorrhizal) and another set was inoculated with 5 gm of VAM consortium at the rate of 215 spores/gm in the rhizosphere. Both the sets were

maintained under appropriate conditions for hardening (Jasrai et al. 1999) in the Botanical Garden of the M.S. University of Baroda.

The growth parameters were recorded at an interval of 20 days through random selection of plants from both sets. To extract chlorophyll a known amount (25 mg) of freshly harvested leaf tissue (without mid-vein) was finely shreded into approximately 1 mm pieces and transferred immediately to the test tubes containing 5 ml dimethyl sulphoxide (DMSO). For complete extraction the test tubes were subjected to incubation (30 min) at 61_C in a water bath (Barnes and Balagner 1992). On cooling to room temperature, the optical density of the same was measured using UV-visible spectrophotometer (Shimadzu). The chlorophyll being photo-oxidative in nature the whole procedure was followed in dark. The chlorophyll content was calculated using an appropriate formula (Maclachalan and Zalik 1963).

To determine the mycorrhizal infection and colonization of roots the segmented (1 cm) fresh roots were stained in acid fuschin (0.02 %). A thorough clearing (3 cycles) of roots with 10 % KOH followed by the sequential treatments with 3 % sodium hypochlorite (60 min), ammonical hydrogen peroxide (60 min) and HCl were adapted and documented in the form of photographs (Fig. 4) using steriomicroscope (Zeiss). The percentage root colonization by the symbiont was determined using Nicholson's formula (Nicholson 1960) :

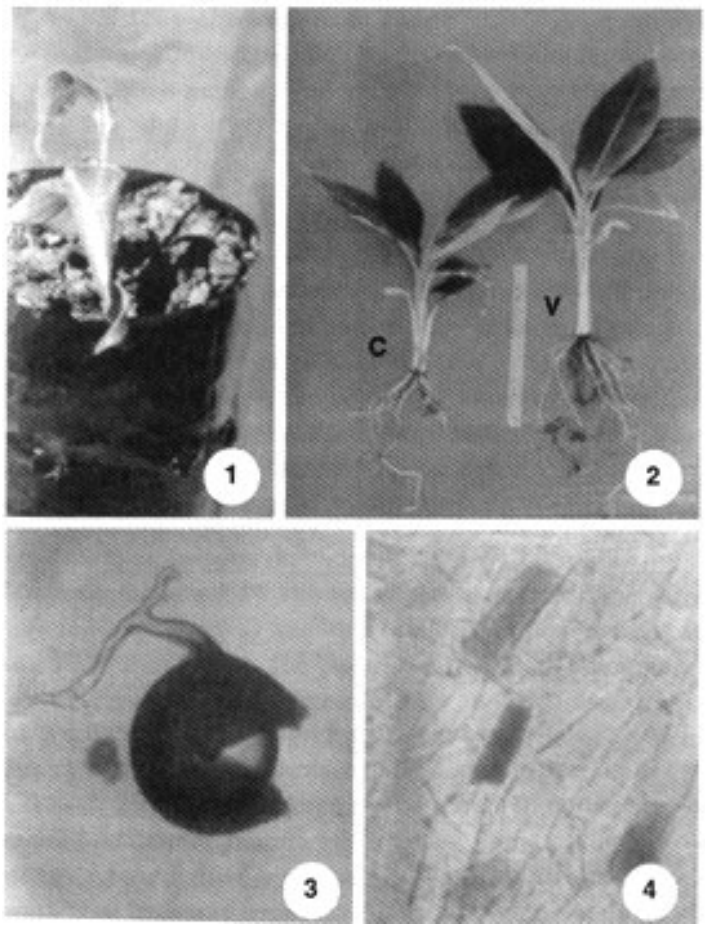
$$\text{Percentage root colonization} = \frac{\text{Number of infected segments}}{\text{Total number of segments observed}} \times 100$$

Results and Discussion

The shoot-tips inoculated on the MS supplemented with 6-benzyl adenine (4.4 µM) resulted in greening of the explants accompanied by their growth within a week of its incubation. On an average eight - ten multiple shoots developed during each subculture. For root induction further growth and the proliferated shoots were transferred to MS supplemented with IAA (0.1 µM). The well developed plantlets of appropriate size were screened and transferred to polybags as per procedure developed earlier in our laboratory (Jasrai et al. 1999).

The direct irrigation may at times lead to waterlogging which is, inhibitory to the growth and proliferation of VAM. Therefore, the perforated polybags with plantlets were maintained in moist sand beds so that the required amount of water gets supplied through capillary action.

The study of growth parameters at the end of 20 days revealed no significant difference in the mycorrhizal plants over the non-mycorrhizal counterparts (control). Similar results were reported earlier for *Gerbera* and *Nephrolepis* (Wang et al. 1993). However, the subsequent data revealed a significant increase in growth rate among all parameters of mycorrhzial plants in contrast to the control set of plants (Fig. 2).



Figs. 1 - 4 : 1. Micropropagated banana inoculated with VAM. 2. Growth of micropropagated banana after 100 days, C-control (non-mycorrhizal); V - VAM inoculated. 3. Germinating spore of VAM with hyphae. 4. Arbuscules in the cortical cells of the infected roots of banana.

The root length, unlike other parameters, showed an insignificant increase in mycorrhizal plants as against the control. However, the steep increase at the end of 40 days continued till the next interval but later on

remained stabilized, thus exhibiting a normal growth - a sigmoid curve. This could be due to the limited volume available in the polybags for root proliferation.

The significant increase in overall growth of plantlets (Table 1) could be attributed to the mycorrhizal colonization in the roots of the VAM inoculated set of plants. There was a relative increase in the growth parameters of host and percentage root colonization by VAM. The mycelium being much smaller in diameter can penetrate those soil particles, which are otherwise not accessible by root hairs of the host, thereby channeling the nutrients (Gupta and Mukerji 1999). Furthermore, the increased absorptive surface area due to VAM must have resulted in an increased nutrient uptake including Mg.

Table 1. Comparison of root and shoot parameters of the VAM inoculated banana plantlets over control at regular intervals.

Growth parameters (cm)	Treatment	Incubation period (days)				
		20	40	60	80	100
Shoot length	Control	4.40	4.90	6.62	8.20	11.5
		± 0.053	± 0.352	± 0.860	± 0.436	± 0.392
	VAM inoculated	4.40	5.98	8.33	11.35	14.00
		± 0.081	± 0.395	± 0.970	± 0.322	± 0.437
Root length	Control	4.26	5.70	9.50	18.50	23.00
		± 0.080	± 0.302	± 0.163	± 1.080	± 0.462
	VAM inoculated	4.32	7.33	13.1	18.50	25.00
		± 0.037	± 0.173	± 0.140	± 0.712	± 0.594
Shoot weight	Control	2.26	2.96	9.45	14.53	19.40
		± 0.030	± 0.137	± 0.158	± 0.665	± 0.347
	VAM inoculated	2.25	4.11	17.31	18.97	26.46
		± 0.045	± 0.141	± 0.361	± 0.719	± 0.419
Root weight	Control	0.34	0.76	2.29	3.86	6.12
		± 0.050	± 0.034	± 0.140	± 0.410	± 0.447
	VAM inoculated	0.35	0.88	3.55	6.70	10.99
		± 0.093	± 0.033	± 0.040	± 0.823	± 0.503

The progressive increase in the chlorophyll levels, can be attributed to VAM, along with the increase in leaf - length and breadth, including the total leaf area (Table 2), leading to the enhanced photosynthetic efficiency of the leaves. The high production of photosynthates could have resulted in better growth of mycorrhiza-associated plants. According to Subramanium (2000), the fungal counterpart utilizes about 10 - 15 % of the photosynthetic carbon of the

host for its proliferation. The subsequent increase in percentage root colonization from 18 % at the end of 40 days to as high as 67 % in the following 60 days could be due to increased production of photosynthates (Fig. 2). The greater spread of mycelium in soil would again benefit the host. Thus, different components benefit each other resulting finally in the better growth of each partner of the symbiotic team.

Table 2. Comparison of the leaf parameters of VAM inoculated banana plantlets over the control at regular intervals.

Growth parameters	Treatment	Incubation period (days)				
		20	40	60	80	100
Length (cm)	Control	8.89 ± 0.052	10.44 ± 0.378	12.01 ± 1.686	12.36 ± 0.698	12.30 ± 0.231
	VAM inoculated	8.95 ± 0.032	11.42 ± 0.505	15.89 ±1.770	17.82 ± 0.716	20.00 ± 0.198
Breadth (cm)	Control	3.14 ± 0.148	3.65 ± 0.172	4.25 ± 0.619	4.66 ± 0.299	7.20 ± 0.179
	VAM inoculated	3.15 ± 0.036	4.35 ± 0.271	6.07 ± 0.490	6.92 ± 0.150	8.00 ± 0.209
Area (cm2)	Control	28.4 ± 0.120	38.11 ± 0.550	49.81 ±1.157	57.59 ± 0.927	86.40 ± 0.410
	VAM inoculated	29.0 ± 0.068	49.68 ± 0.776	90.71 ± 1.33	123.3 ± 0.866	160 ± 0.407
Chlorophyll content (gm/ fresh wt)	Control	1.149 ± 0.050	1.25 ± 0.338	1.33 ± 0.43	1.408 ± 0.579	1.45 ± 0.407
	VAM inoculated	1.153 ± 0.084	1.31 ± 0.410	1.59 ± 0.378	1.845 ± 0.692	2.12 ± 0.623

The benefits of VAM fungi have also been demonstrated in various other micropropagated plants of horticultural interest like *Gerbera* and *Nephrolepia* (Wang et al. 1993), *Persea americana* (Azcon-Aguilar et al. 1992), *Rhododendron hybrida* (Lemoine et al. 1992), *Malus pumila* and *Prunus amygdalus* (Sbrana et al. 1994), *Syngonium* and *Draceana* (Adholeya and Gaur 1999).

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