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Full Length Research Paper

Performance of yam microtubers from temporary immersion system in field conditions

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The yam clones ´Pacala Duclos´ and ´Belep´ *of Dioscorea alata* were used to evaluate the performance of microtubers formed in temporary immersion systems (TIS) in field conditions. Previously sprouted microtubers with a fresh weight higher than 3.0 gFW were used while *in vitro* plants and tuber crowns from conventional propagation methods served as control. In both clones there were no significant differences in qualitative morphological characters between plants from microtubers and *in vitro* plants for all traits but both differed significantly from plants obtained from tuber crowns. The same trend was observed for number, length, diameter and fresh weight of tubers produced 36 weeks after field planting. The number of tubers formed per plant raised from microtubers doubled that raised from tuber crowns in both clones. Microtubers from temporary immersion systems can be grown on the field and used in original seed production programs.

Key words: Microtuber, yam, field, temporary immersion system.

INTRODUCTION

In vitro propagation of yam by serial culture of single node cuttings has been used in the rapid multiplication of diseases-free material in elite seed yam programs (Malaurie et al., 1995; Borges et al., 2004, Ondo et al., 2007). However, the protocols of yam by serial culture of sail-node cuttings have presented low multiplication coefficients and survival of the plants in the acclimatization phase and in field (Malaurie et al., 1995; Medero et al., 1999; Chu and Ribeiro, 2002; Borges et al., 2004). The alternative end-product in the yam micro propagation process is a small tuber (microtuber) produced when *in vitro* plantlets are placed under tuber-inducing conditions (Mantell and Hugo, 1989; Salazar and Beltran 2003; Balogun, 2009).

Microtubers have a potential to be integrated into seed yam programs (Balogun et al., 2006; Chen et al., 2007). Microtubers are particularly convenient for handling, storage and distribution. Unlike micro propagated plantlets, they do not need a time consuming hardening period in a greenhouse and may be adapted easily to large-scale mechanised planting in the field (Coleman et al., 2001; Pruski et al., 2003).

Micro propagation of *Dioscorea alata* L. in semi-solid medium and in conventional size culture flasks is characterized by low microtuber formation frequencies by *in vitro* plants and microtubers with average fresh weight lower than 0.5 gFW (Balogun et al., 2006; Ondo et al., 2007; Balogun, 2009). These factors limit its use as propagule for propagation, microtuber formation and direct planting under field conditions. So, the acceptance as seed propagule has been restricted (Salazar and Beltran, 2003; Balogun et al., 2006; Chen et al., 2007).

The temporary immersion system reduces the problems caused by static liquid media because asphyxiation does not occur due to the short periods of immersion and because of limited medium convection, shear damage is minimized. With most crops, growth is sharply enhanced

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Abbreviations:TIS; temporary immersion system, **FW;** fresh weight.

and high quality propagules are produced (Escalona, 2006). TIS has been used for microtuberization in potatoes (Teisson and Alvard, 1999; Jimenez et al., 1999). Cabrera et al. (2005) developed a method for yam microtuber formation in TIS. In this culture system, 4.5 to 4.7 microtubers per plant with a fresh weight ranging from 3.15 to 3.20 gFW were obtained in yam clones 'Pacala Duclos' and 'Belep', respectively. These results exceeded the fresh weight in microtubers obtained from protocols previously described in the literature. In spite of these findings, there is a lack of publication on the performance of yam microtubers from TIS in field conditions and the available information relates to the performance of microtubers formed in semi-solid culture media and in conventional size culture flasks (Balogun et al., 2006).

Microtuber fresh weight has significant effect on sprouting, as well as on their response in greenhouses and in field conditions (Yu et al., 2000). Thus, in yam seed production programmes, the use of microtubers depends on their yielding ability under field conditions. The main objective of this research was to evaluate the performance of microtubers from TIS in field plots.

MATERIALS AND METHODS

Researches were carried out at the tissue culture laboratory and in field plots from the Research Institute of Tropical Root and Tuber Crops (INIVIT), Santo Domingo municipality, Villa Clara province, Cuba. Yam clones `Pacala Duclos´ and `Belep´ (*D. alata* L.) were used. Nodal segments in the third subculture were taken as explants for microtuber production and *in vitro* plants from *in vitro* germplasm bank at INIVIT.

Description of microtuber production in temporary immersion system (TIS)

Glass flasks (5000 ml) with silicone caps were used. Couple flasks were connected by an autoclavable silicone tubing (ID = 6.0 mm), one was used as medium reservoir and the other one as culture flask. A sterilised filter (0.22 µm, Midisart Satorius Co.) was fitted to each flask for ventilation. The immersion frequency and duration were regulated by means of a programmable timer connected to a two-way solenoid electro valves. Three temporary immersion systems for each clone were used, as well as, an immersion time of 10 min every 6 h.

Microtubers were formed in TIS in two culture stage

Shoot growth stage

During the shoot growth stage (six weeks culture), each system containing 1500 ml liquid multiplication medium was inoculated with 50 single nodal segments and incubated at 25 ± 2°C under cool-white fluorescent tubes (135 - 150 µmol m^{-2} s^{-1}) with a 16 per 24 h photoperiod.

Tuber induction stage

In the tuber induction stage (18 weeks culture) the whole medium was changed in each system for 3000 ml liquid tuber induction medium (100 g. l^{-1} sucrose) and plants were incubated in constant dark.

In both culture stages, an immersion time of 10 min was used each 6 h, that is, four immersions per day (Cabrera et al., 2005). Microtuber harvesting was carried out after 24 weeks of culture. In order to enhance sprouting, microtubers were immersed in a solution at 28.89 µM gibberellic acid during 10 mins. Later, cultures were placed in a growth chamber (RUMED) in darkness for eight weeks at 26 ± 2.0°C and 90% relative humidity. Previously sprouted microtubers were transferred to field for planting.

Production of *in vitro* plants

Plantlets were multiplied as single node explants on 30 ml MS (Murashige and Skoog, 1962) medium in glass flasks (250 ml). Five nodal segments were placed in each vessel. Sucrose (30 gl^{-1}) was used as a carbon source and media were solidified with 6.0 gl^{-1} agar-E (BioCen). The pH of the medium was adjusted to 5.7 before autoclaving. Cultures were incubated at 25 ± 2.0°C under white fluorescent tubes (135-150 µmol$m^{-2}s^{-1}$) with a 16 per 24 h photoperiod. After 35 days, single stem plantlets were cut into single-node explants and placed on fresh MS medium in glass flasks for further multiplication. The process was repeated until the required numbers of plantlets were obtained.

In vitro plant acclimatization was carried out for 45 days in a culture house covered with a plastic mesh, which reduced the light intensity by 70%. Irrigation took place with microsprinklers through the microjet system with a frequency of 10 min and 6 irrigations per day. With this frequency, a relative humidity of 85-90% was guaranteed. Polystyrene boxes with 70 holes and an artificial substrate formed by a mixture of casting and zeolite (3:1) were used.

Evaluation of agronomic traits in field conditions

In order to evaluate the response of microtubers and *in vitro* plant in field conditions, planting took place as follow:

i) Microtubers with 3.0 gFW from TIS previously sprouted in growing chambers.
ii) *In vitro* plants previously acclimatized with 30 cm length stems and at least four leaves.
iii) Tuber crowns with 100 gFW from plants of commercial plantations according to Yam Technical Instructive (Control) (MINAG, 2004).

In field conditions, 1200 microtubers and the same quantity of *in vitro* plants and tuber crowns per clone were planted. The experimental design was a randomized block with four replications. Experimental plots consisted of 4 mound rows, with 40 plants each. Plantations took place, on brown carbonated soils on April 15, 2006 (Hernandez et al., 1999). Planting distance was 1.00 x 1.00 m. Irrigation and other cultural practices were developed following the Yam Technical Instructive (MINAG, 2004).

In order to study the stability of morphological characters, the total population was evaluated 24 and 36 weeks after culture according to yam morphological descriptors from the International Institute of Phytogenetic Resources, (IPGRI/IITA, 1997). The list from IPGRI involves 134 descriptors. 72 descriptors which included qualitative characters of the vegetative development of stems, leaves, suscep-tibility to biotic stress and characteristics related to tubers were used.

After 36 weeks of culture in field conditions, 20 plants from internal ridges per treatment were selected. During hand harvesting, the following variables were evaluated:

i) Number of tubers/ plant.

Table 1. Mean values of tuber yield parameters in different yam propagules under field conditions.

Treatments	Tuber number per plant	Tuber length (cm)	Tuber diameter (cm)	Tuber fresh weight (KgFW)
cv. ´Pacala Duclos´				
Microtubers	3.96 ± 0.21*a	18.76 ± 0.63 a	14.60 ± 0.46 a	2.92 ± 0.13 a
In vitro plantlets	3.76 ± 0.24 a	18.23 ± 0.58 a	14.93 ± 0.66 a	2.88 ± 0.16 a
Tuber crowns	1.50 ± 0.27 b	16.93 ± 0.43 b	12.90 ± 0.60 b	2.45 ± 0.14 b
cv. ´Belep'				
Microtubers	3.06 ± 0.11*a	20.83 ± 1.46 a	16.77 ± 0.35 a	3.66 ± 0.30 a
In vitro plantlets	2.97 ± 0.15 a	19.63 ± 1.66 a	16.47 ± 0.48 a	3.41 ± 0.46 a
Tuber crowns	1.43 ± 0.27 b	17.50 ± 0.89 b	13.77 ± 0.23 b	2.76 ± 0.24 b

*Means with distinct letters in the same column differ for values of P ≤ 0.05 by Dunnett´s C.
600 plants from microtubers, *in vitro* plantlets and tuber crowns were evaluated.

ii) Tuber length (cm).
iii) Tuber diameter (cm).
iv) Tuber fresh weight (kgFW).

Data statistical processing was carried out through variance analyses. For multiple mean comparisons, Dunnentt´s C tests were applied because no variance homogeneity with P < 0.05 significance level was found.

RESULTS AND DISCUSSION

No variability was observed in qualitative morphological characters for both clones among all treatments. Also, no significant differences were noticed between plants obtained in TIS and *in vitro* plants in relation to tuber number, tuber length, diameter length and fresh weight in both clones after 36 weeks of culture. However, microtubers and *in vitro* plants differed significantly from plants raised from tuber crowns. Plants from microtubers produced double the number of tubers formed by plants raised from tuber crowns in the two clones (Table 1).

Plants from microtubers formed in TIS and *in vitro* plants of yam clone ´Belep´ did not show variability in morphological characters in comparison to plants obtained from conventional propagation methods as control.

Yam plants (cv. ´Belep´) from microtubers developed in a temporary immersion system showed good performance under field conditions, without significant differences in comparison with *in vitro* plants with respect to tuber number, tuber length, tuber diameter and tuber fresh weight per evaluated plant 36 weeks after culture, but differences were noticed with plants from tuber crowns. In this yam clone, plants obtained from microtubers formed in TIS doubled tuber number per plant in relation to control plants.

In both clones, higher tuber yield obtained in microtubers formed in TIS and *in vit*ro plants in field conditions compared to plants from conventional propagation could be due to *in vitro* physiologic rejuvenation where tissues lost signs from mother plants and resulted in an increment of the physiologic vigour in certain agronomic variables (Cardone et al., 2004). According to Kawakami et al., (2005), the highest plant vigour coming from biotechno-logical methods may be due to rejuvenation, lack of antagonism with micro organisms that affected plants in their natural habitat and cleaning obtained through tissue culture, all of which were difficult to separate.

Until now, the present studies have not been developed about the stability of morphological characters of yam plants starting from the microtubers. According to Balogun (2009), evaluations of this type constitute aspects that should be investigated if it is sought to develop protocols for microtubers formation that could be used for the conservation and germoplasm exchange, as well as to establish a program of production of vegetable material of plantation in this cultivation. These results showed that the use of microtubers formed in TIS will be an important alternative for original seed production in this crop. Up to now, yam microtubers have shown an inferior response in field conditions in relation to tuber number, tuber length, tuber diameter and tuber fresh weight per plant in comparison with *in vitro* plants showing a fresh weight lower than 3.0 gFW, due to their formation in semisolid culture medium and in conven-tional size culture flasks (Chen et al., 2007).

Pruski et al. (2003) suggested for potato culture that microtubers should not be used for original seed production, because tuber number per plant was from 20 to 30% lower than in the case of *in vitro* plants. However, Balogun et al. (2004) offer greater possibilities to yam microtubers than to *in vitro* plants when a seed program is started through biotechnological methods. *In vitro* plants are too fragile and small for a direct plantation into field conditions and so, the acclimatization stage that increases material management and production cost is needed; unlike plantations carried out with microtubers where management is easier and brings the possibility to mechanise planting (Ondo et al., 2007).

Results obtained from evaluation of the agronomic response of microtubers in field conditions corroborated

with those from Jimenez (2005) in potato. Potato microtubers formed in TIS in comparison with *in vitro* plants did not show significant differences in relation to tuber number per plant. Yu et al. (2000) and Pruski et al. (2003) stated that fresh weight of potato microtubers for direct plantation showed important effect on sprouting and later crop development. The effect of TIS in yam tuber for-mation was proved in field conditions as these culture systems facilitate a higher microtuber number per plant and production of microtubers with a fresh weight higher than 3.0 gFW. Thus, microtubers have shown a good response for seed production in field conditions.

Conclusion

The plants of the microtubers of both clones of yam clone did not show differences in the qualitative morphological characters that were evaluated in field; they presented the best answers in the quantitative characters.

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