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Production of yam microtubers using a temporary immersion system

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Abstract

Yam clones 'Pacala Duclos' and 'Belep' in temporary immersion system culture showed favourable results on shoot growth stage and in the development of microtubers in comparison with solid culture media. Cultures in temporary immersion systems in both clones obtained a higher microtuber number per plant, with greater fresh weight and diameter in comparison with solid culture media. Besides, 45 and 47% of microtubers greater than 3.0 gFW for 'Belep' and 'Pacala Duclos' clones respectively, were obtained. Those tubers may be planted without acclimatization and may be stored for a prolonged period of time.

Introduction

Yam (*Dioscorea* spp.) is an economically important food crop in many tropical countries especially in West Africa, South Asia and the Caribbean.

Yam microtubers offer several advantages over *in vitro* plants, since they can be stored and transplanted directly into field conditions without an acclimatization stage. Also, handling and shipping are easier, thus facilitating commercialisation and international exchange of germplasm. The main problems associated in yam and potato with microtuber production in conventional flasks are low tuber yield (1–1.5 tubers/plants) and the small tuber size that limits direct transplanting to field conditions (Jiménez et al., 1999; Salazar and Beltran, 2002).

Several approaches have been made in order to improve tuber quality and the number of tubers per plant by changing the culture medium components, e.g., high sucrose content (60 g l^{-1}), addition of plant growth regulators, manipulation of culture conditions, such as, temperature, light and photoperiod (Mantell and Hugo, 1989; Ng, 1992; Salazar and Beltran, 2002; Bazabanaka et al., 2003).

Temporary immersion systems (TIS) open possibilities to automate some *in vitro* culture stages (Alvard et al., 1993), more scaling up facilities are available, an increment in the biological efficiency and productivity of the propagated material (Lorenzo et al., 1998; Escalona et al., 1999; Martre et al., 2001). Plant morphology and physiological performance of crops in TIS are very similar to plants in *ex vitro* condition providing a higher surviving rate (Teisson et al., 1996; Etienne and Berthouly, 2002).

A TIS for potato microtuber production was designed using 4 l flasks. This culture technique showed several advantages to solid culture: i.e., a three-fold increase in shoot length, more internodes per plant and improved vigour. In the tuber induction stage, microtubers can be induced from all plant nodes, indicating that tuberization is not restricted to specific regions (Jiménez et al., 1999).

The aim of this study was to establish a semi-automated system for microtuber production and to investigate the effect of temporary immersion of explants on plant quality during shoot growing stage and in the development of microtubers during induction tuber stage.

Materials and methods

Plant material and culture media

Nodal segments of 'Pacala Duclos' and 'Belep' (*Dioscorea alata* L.) clones were obtained from previously cultivated plants in glass flasks (250 ml) containing 30 ml solid multiplication culture medium (Murashige and Skoog, 1962), supplemented with 0.5 mg l^{-1} Kinetine, 30 g l^{-1} sucrose and 7.0 g l^{-1} agar-E (Biocen). Subcultures were carried out every 35 days.

For induction and development of microtubers, shoots in the third subculture and a culture medium similar to the one previously analysed were used, but supplemented with 100 g l^{-1} of sucrose. The culture medium was autoclaved for 20 min at 121 °C and pH was adjusted to 5.7 before autoclaving.

Temporary immersion system (TIS) description

Glass flasks (5000 ml) with silicone caps were used. Couple flasks were connected by an autoclavable silicone tubing (ID = 6 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 TIS for each clone were used and an immersion time 10 min every 6 h.

Shoot growth stage

During the shoot growth stage (5 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-h per 24-h photoperiod.

Tuber induction stage

In the tuber induction stage (16 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.

Culture in solid culture medium

Thirty glass flasks were inoculated with five nodal segments per flask (250 ml) containing 30 ml solid multiplication culture medium was used and cultures were incubated like in TIS. After culturing for 5 weeks, plants were changed to glass flask (250 ml) containing 50 ml solid culture medium for microtuber induction and development, and incubated in constant dark.

Parameters evaluated and data analysis

Quality of the shoot and tubers was determined by measuring the length of the plantlets (cm) and the number of internodes produced during the shoot growth stage (5 weeks culture) and at harvesting (16 weeks culture) microtubers numbers, fresh weight (gFW) and diameter (cm) per plant were recorded. Microtubers were classified into three categories according to weight (less than 1.0 gFW from 1.0 to 3.0 gFW and more than 3.0 gFW) and it permitted to establish the microtuber percentage per catergories.

Experiments were repeated three times and reliability of data was obtained.

Data were analysed using a simple ANOVA and mean separation was done by a Duncan's multiple range test.

Results and discussion

Shoot growing stage

A TIS culture showed a favourable and positive effect during shoot growth stage (5 weeks culture) in both yam clones, see Table 1. Plants cultivated in TIS showed higher shooting length, as well as, higher internodes number per plant in comparison with plants cultivated in solid culture medium.

Table 1. Effect of temporary immersion system (TIS) and solid medium on shoot growth stage from two yam clones with 5 weeks of culture

Treatments	'Pacala Duclos'		'Belep'	
	Shoot length (cm)	Internodes number	Shoot length (cm)	Internodes number
TIS	9.6 ± 0.15* a	4.1 ± 0.18 a	9.1 ± 0.24 a	3.9 ± 0.17 a
Solid medium	4.7 ± 0.21 b	2.2 ± 0.10 b	4.5 ± 0.35 b	1.9 ± 0.11 b

*Standard error.
Different letters in one column within a clone represent significant differences by Duncań s multiple range test, $p \leq 0.05$.

Results corroborated those obtained by Jiménez et al. (1999) in relation to the use of TIS on potato culture where plants presented three times higher stem length and internodes number than plants cultivated in solid culture medium. Basail et al. (2003) in cassava obtained a higher stem length and internodes number and the multiplication coefficient using TIS was increased tremendously.

Besides, these systems provide explants with the best culture conditions for growing because explants growth is favoured because contact with media has a short duration with time to renew atmosphere in flask. So, disadvantages of solid culture media are decreased and a high photosynthetic activity is obtained (Akita and Takayama, 1994; Teisson et al., 1999; Etienne and Berthouly, 2002).

Tuber induction stage

Microtuber formation was achieved in both clones in this study culture conditions. The greatest microtuber number per plant, fresh weight and diameter were obtained in TIS in these two clones in comparison with solid culture medium Table 2.

Temporary immersion system favoured tuber formation in relation to tuber length and shoot base in comparison with solid media limited to specific regions. The biggest area of the shoot in contact with the culture medium in TIS contributed to microtuber formation. These results coincide with those reported by Jiménez et al. (1999) on potato crop. In the microtuber formation induction, the temporary immersion of explants stimulated the increment of tuber formation regions (buds), and resulted in higher microtuber production in both clones. As all buds are in contact with culture medium during short period, the inducting tuberization sign reaches all buds at the same time. This effect is not given by static culture systems. A similar observation on potato crop was previously carried out by Akita and Takayama (1994), where tuber formation regions were increased by an intermittent culture system for a complete explant immersion in culture medium each 6 h.

The classification analysis of microtubers according to their fresh weights is shown in Table 3. The use of TIS in both clones resulted in the highest microtuber percentage with weights more than 3.0 gFW in comparison with results obtained with solid media. Tubers of this weight

Table 2. Effect of temporary immersion system (TIS) in the microtuber formation in two yam clones after 16 weeks of culture

Treatments	'Pacala Duclos'			'Belep'		
	Microtubers number/plant	Fresh weight (gFW)	Diameter	Microtuber number/plant	Fresh weight (gFW)	Diameter
TIS	4.7 ± 0.30* a	3.20 ± 0.05 a	1.13 ± 0.01 a	4.5 ± 0.22 a	3.15 ± 0.04 a	1.10 ± 0.02 a
Solid medium	1.7 ± 0.24 b	0.33 ± 0.11 b	0.35 ± 0.08 b	1.4 ± 0.35 b	0.33 ± 0.14 b	0.30 ± 0.04 b

*Standard error.
Different letters in one column within a clone represent significant differences by Duncan's multiple range test, $p \leq 0.05$.

Table 3. Percentages of microtubers classified according to fresh weights (gFW) obtained in temporary immersion and solid culture media in two yam clones

Categories	'Pacala Duclos'		'Belep'	
	TIS (%)	Solid medium (%)	TIS (%)	Solid medium (%)
<1.0 g	18	57	19	60
1.0–3.0 g	37	35	36	36
>3.0 g	47	6	45	4

are the most suitable for commercial production because they may be directly planted into the field without acclimatization stage and storage for a long period of time with no detriment in weight to affect next generation. But, differences were noticed in solid culture media where the higher percentage corresponded to microtubers with weights less than 1.0 gFW.

Conclusions

Temporary immersion is a valuable option for yam microtuber production. The technique induces more tubers per plant and increases the size and weight of tubers. This allows new opportunities for commercial laboratories dealing with yam seed production, because these tubers can be stored and directly transplanted without an acclimatization stage. The TIS can also be used for shoot multiplication during the planting season, when *in vitro* plants can be immediately acclimatized and transplanted. Thus, several strategies are accessible by combining the induction and storage of microtubers with *in vitro* plant production, according to the seasonal patterns of yam farming. Further improvements should be made to increase tuber size and number of tubers per plant by manipulating the immersion programmes, cultures conditions and medium exchanges frequency. A highly valuable alternative for the commercial production of microtuber is offered by temporal immersion system.

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