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Short Communication

In vitro basal and nodal microtuberization in yam shoot cultures (*Discorea rotundata* poir, cv. Obiaoturugo) under nutritional stress conditions

Mbanaso, E.N.A.*, Chukwu, L.I. and Opara, M.U.A.

National Root Crops Research Institute, Umudike, P.M.B. 7006, Umuahia, Abia State, Nigeria.

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Single nodal explants excised from vines of greenhouse-grown white Guinea yam (*Dioscorea rotundata* poir cv Obiaoturugo) were initiated *in vitro* on a medium consisting of Murashige and Skoog's (MS) basal salt supplemented with Gamborg's B5 vitamins, 0.5 µM benzylamino purine (BAP), 0.1 µM naphthalene acetic acid (NAA), 0.2 µM gibberellic acid (GA_3), 20 mgl^{-1} L-cysteine, 30 gl^{-1} sucrose and solidified in 8 gl^{-1} agar. They were incubated for 14 months with regular monthly subculture under continuous illumination at 24 ± 1°C and 1000 lux light intensity. Subsequently, single and double leafed segments from these were transferred to a simpler liquid medium made up of MS basal salts and vitamins, reduced sucrose level (20 gl^{-1}) and without growth regulators. The cultures were kept stationary, and without subculture for 6 months at 27 ± 1°C, 16 h illumination and at 2000 – 2500 lux light intensity. The shoot cultures began to produce excessive roots at the nodes apart from the shoot tip. Subsequently microtubers developed at the position of the axiliary buds subtended by the leaf petiole as well as at the base of some shoots. On transfer whole or segmented into fresh medium, the microtubers sprouted and produced plantlets.

Key words: *Dioscorea rotundata*, nodal, microtuberization, nutritional stress.

INTRODUCTION

Food yams (*Dioscorea* spp.) are widely grown in many tropical regions of the world as an important staple. In West Africa for instance, *Dioscorea rotundata* is the premier cultivated species. Over the years, farmers have been able to select several cultivars/landraces of this species which they grow according to local preferences and adaptation. Nigeria is currently the world's leading producer of yams (FAOSTAT, 2006). Edible tubers are usually produced subterranean unlike in other species such as *Dioscorea bulbifera* where aerial tubers are found. Routine propagation is carried out vegetatively via the use of tuber pieces known as setts. This method is slow and encourages the build-up and transfer of viral diseases as well as soil-borne pathogens from generation to generation.

Practical approaches to clone yams rapidly *in vitro* under controlled conditions have been developed and successfully applied (Mantell et al., 1980). The application of meristem tip culture (Mantell et al., 1980) and somatic embryogenesis (Ammirato, 1984) can result in mass multiplication of yam plantlets free from viruses. However, large scale establishment of *in vitro*-derived plantlets in the field, particularly where specialized post-flask handling is lacking, can be a problem (Mantell and Hugo, 1989).

Yam microtubers are hardier propagules than plantlets. Induction of viable microtubers *in vitro* by the manipulation of media components have also been demonstrated in *Dioscorea* species (Mantell and Hugo, 1989; Ng, 1988). The use of microtubers for propagation and international germplasm exchange is well described by Ng (1988). For *D. rotundata* species, induced microtubers are known to develop mainly at the base of *in vitro* shoots or occasionally from nodes just above the surface of the culture media (Ng, 1988). This paper describes nutritional stress as an inducer of nodal microtubers in cultures of *D. rotundata* cultivar 'Obiaoturugo'.

*Corresponding author. E -mail: embanaso@yahoo.com. Tel: +234-8035827231.

Figure 1. Stressed yam shoots cultures producing roots excessively from the nodes.

MATERIAL AND METHODS

Source of ex-plants

Mother plants were established from healthy 'Obiaoturugo' seed yams (weighing 200 ± 5 g) which had broken dormancy, and were planted singly in 40 cm black polybags filled with sterilized top soil and kept in a greenhouse. Light watering with tap water was carried out twice daily until sprouts were produced. Young healthy sprouts were removed from mother plants after two months of growth, de-leafed, and cut into smaller pieces. These were washed first, under running tap water to remove dirt and then rinsed in 75% ethanol for 3 min. Explants were transferred to 2% sodium hypochlorite solution in which a few drops of tween 20 were added, and shaken continuously for 20 min. Explants were then rinsed once in sterile distilled water and transferred to the same concentration of sodium hypochlorite solution for another 10 min. After three rinses in sterile distilled water, explants were transferred to covered sterile Petri dishes containing a film of sterile distilled water.

Initiation and culture of ex-plants in culture media

Explants were dissected into single nodal segments and seeded two per culture vessel (4 oz baby food jar) containing 20 mls of medium each. The culture medium was made up of Murashige and Skoog's (MS) (1962) basal salts, supplemented with Gamborg's B5 vitamins (Gamborg et al., 1968); myo-inositol (100 mgl^{-1}); benzyl amino purine (0.5 µm); naphthalene acetic acid (0.1 µm); Gibberellic acid (0.2 µm) and L-cysteine (20 mgl^{-1}). Sucrose content was 30 gl^{-1} and the medium was solidified in 8 gl^{-1} agar (Difco Bacto Agar). The pH was adjusted to 5.8 before autoclaving at 120°C for 15 min at 15 psi. Cultures were incubated at 24 ± 1°C under 1000 lux continuous illumination. Plantlets from these explants were multiplied by single nodal culture under the above conditions for a period of 14 months with regular monthly passages before transfer to stationary liquid medium consisting of MS basal salts and vitamins (Murashige and Skoog, 1962), 100 mgl^{-1} myo-inositol and 20 gl^{-1} sucrose and incubated at 27 ± 1°C and 2500 lux under 16 hours illumination cycle.

Microtuberization under stress

For this, de-leafed single or double nodal segments were seeded two per 16 oz baby food jar containing 10 ml of medium. No further subculturing was carried out and cultures were left stationary and undisturbed for 6 months on culture shelves without replenishing the medium.

RESULTS AND DISCUSSION

Eight days after the initial introduction *in vitro*, axillary buds burst from the explants producing shoots which developed an average of two to three nodes per month, with large well formed leaves. On transfer to the simpler medium which had no plant growth regulators, reduced sucrose level and in bigger culture vessel, shoot proliferation improved resulting in the development of multiple shoots each producing three to five nodes per month.

Following a period of undisturbed growth in stationary liquid medium, the plantlets started producing roots excessively from the nodes (Figure 1). These grew downwards towards the medium, from virtually all the aerial nodes apart from the shoot tip. From these nodes, aerial microtubers began to form at the position of the auxiliary buds subtended by the leaf petioles (Figure 2). The microtubers were very hairy and grew slowly, some reaching up to 100 mg after 4 months. Microtubers were formed also at the base of some of the shoots as had previously been described (Ng, 1988) (Figure 3). By this time the culture medium was almost exhausted. On subculture of plantlets these features continued as soon as the cultures were established. The aerial microtubers sprouted immediately on transfer to fresh culture medium producing shoots only from the point of attachment to the main shoot. On dissection longitudinally into smaller pieces and transfer to fresh medium, the dissected pieces produced new sprouts which grew into plantlets also from the section of point of attachment to the mother shoots.

D. rotundata cv. 'Obiaoturugo' is one of the elite cultivars which are well-adapted to some parts of the South-eastern regions of Nigeria. It flowers profusely producing mainly pistilate florets. The ovary later develops into 3-lobed capsules. Nine to ten months after planting, large cylindrical tubers with rounded bottom are produced. Naturally, tubers are formed underground and no aerial tubers have been previously described either *in vitro* or

Figure 2. Stressed yam shoots cultures producing microtubers from the nodes.

Figure 3. Stressed yam shoots cultures producing microtubers at the base of the shoots

in vivo. The ability of yams (*Dioscorea* spp.) to form tubers *in vitro* is an alternative physiological development towards a successful micropropagation programme targeted at overcoming the problems of the high mortality rate experienced during the post-flask management of the fragile *in vitro*-derived plantlets. *In vitro* microtuberization in *D. rotundata* species is induced by the manipulation of sucrose levels in the medium and day length during culture (Ng, 1988). Increase in the frequency of tuberization occurs at 5% sucrose level under short-day (10 h light and 14 h darkness) Microtubers were formed mainly at the base of the shoot or just above the surface of the culture medium. In this work *D. rotundata* cv. 'Obiaoturugo') is reported to produce both basal and aerial microtubers *in vitro*. The phenomenon was observed in cultures under nutritional stress cultures and was carried over to subsequent cultures as if the plantlets were primed. It is being suggested that the excessive production of roots and microtubers at the nodes of these plantlets was an attempt to perenate in response to stress resulting from spent culture medium during a period of undisturbed vegetative growth. It is possible that a condition of sugar famine occasioned by the exhaustion of media components triggered cultures to begin to relocate food reserves to perenating bodies rather than vegetative shoots. It can be inferred that the cultivar 'Obiaoturugo' of the *D. rotundata* may have an inherent capability of producing both basal and aerial microtubers, under nutritional stress, a feature previously observed in some *Dioscorea cayenensis*-*D. rotundata* complex under slow-growth *in vitro* germplasm conditions (Malaurie et al., 1993). This finding opens an otherwise inadequately explored area for maximizing low-cost *in vitro* microtuber production in edible *D. rotundata* species that needs to be investigated further and fully exploited.

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