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A Simple Bioreactor System for Production of Storage Organs of Chinese Yam (*Dioscorea opposita* Thumb.)

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Abstract

Storage organ of *Dioscorea opposita* was efficiently generated by using a drum type bioreactor. This reactor was not equipped with a system for mechanical aeration and explants were immersed intermittently into the medium on rotation. When explants precultured on a rotary shaker were transferred to the bioreactor, approx. 230 pieces of storable organs that could be bulbils or microtubers were generated after 5 weeks. These organs sprouted within a week and eventually grew into normal plants.

Key words: bioreactor, Chinese yam, mass propagation, storage organ

Introduction

Storage organs such as microtubers generated *in vitro* have considerable merits in distribution of disease-free seedlings, stock of germplasm and transplanting. Since a longer culture period is required to obtain the storage organs comparing with the case of propagation of shoots, in general, reduction of the culture cost is indispensable. Bioreactor culture is one of key techniques for the cost reduction in production of *in vitro* seedlings (Levin and Vasil, 1989). We have tried to develop an efficient and economical bioreactor system for mass propagation of storage organs in which the explants is cultured without mechanical aeration. The system has been applied successfully in mass propagation of taro corms (Akita and Ohta, 1996). We also reported that tubers of potato (*Solanum tuberosum* L.) were propagated using a similar but simpler bioreactor system (Akita and Ohta, 1998).

Yam is one of the important root crops in the world. Since yams are mainly propagated by the storage organs (bulbil and rhyzophore), *in vitro* technique is advantageous to provide the disease-free plants. Culture conditions for *in vitro* tuberization of yam has been studied and the storage organs are sucessfully induced (e.g. Ng, 1988; Alizadeh *et al.*, 1998; Jasik and Mantell, 2000). In addition, Matsumoto (1990) reported that a bulbil-like body was directly induced on leaves of a cultivar of *D. opposita* and suggested a new route to produce storable organs of yam. Reliability of the *in vitro* derived yam as seedlings has also been examined in field test (e.g. Watanabe *et al.*, 1993, 1994). All of these reseults indicate that *in vitro* techniques can be expected to be useful tool to provide yam plants of high quality. However, there is a limited number of the reports on the application of bioreactor techniques for the production of seedlings and/or storage organs of yam (e.g. Matsumoto and Suyama, 1997).

In this study, we successfully applied our airpump-less bioreactor with some modifications to the generation of storage organs of yams and also confirmed that the organs obtained are suitable as seed tubers of yam.

Materials and Methods

Plant materials

Dioscorea opposita cv. Hatashoyamanoimo was kindly provided by Dr. K. Watanabe, Agricultural Experiment Station of Shiga. The sprouts were sterilized using 1% sodium hypochlorite solution for 15 min and washed thoroughly with sterilized water. *In vitro* stock plants of *D. opposita* was maintained on a solidified Murashige and Skoog's medium (Murashige and Skoog, 1962) supplemented with 30 g l^{-1} sucrose and 8 g l^{-1} agar (Wako, Osaka). They were cultured at 24 ℃ under illumination (12 h/day, 140 μmol m^{-2} s^{-1} at the top

of the culture vessels.

Culture conditions

Yam explants to be applied to a bioreactor were precultured as follows. Three of single nodal segments excised from the *in vitro* stock were transferred to a 300 ml flask containing 100 ml of MS liquid medium (30 g l^{-1} of sucrose) and shaken at 85 rpm under illumination from the bottom of flasks (12 h/day, 180 μmol m^{-2} s^{-1}) at 24 ℃. After 30 days of the culture, 1 ml of ethanol solution of BA (1 g l^{-1}) was added to the flask and the culture was continued for another 3 days.

In the bioreactor experiment, a 10 liter rotary drum bioreactor (20 cm in diameter and 40 cm in length, Shibata-kagaku, Tokyo) was used. A cylindrical stainless steel mesh, 15 cm in diameter and 35 cm in length, was equipped in the center of the reactor (**Fig. 1**). The bioreactor was filled with 1 liter of a modified MS medium in which the concentrations of major inorganic salts (KNO_3, NH_4NO_3 and $CaCl_2$) were reduced by half and 60 g l^{-1} of sucrose was supplemented.

The explants harvested from 10 flasks were transferred to the inside of the mesh in the reactor and cultured for 5 weeks at 0.5 rpm rotation. Bioreactor culture was carried out under dim light (12 h/day, 7 μmol m^{-2} s^{-1}) at 24 ℃.

Sprouting

Storable organs of *D. opposita* taken out from the bioreactor were washed with tap water. They were kept in a polycarbonate container [7(W) x 7(D) x 10(H) cm, Sumitomo Chemical, Osaka] or directly transplanted into a plastic container filled with a mixture of vermiculite and peatmoss (1:1). All of the samples were kept under room conditions (ca. 25 ℃).

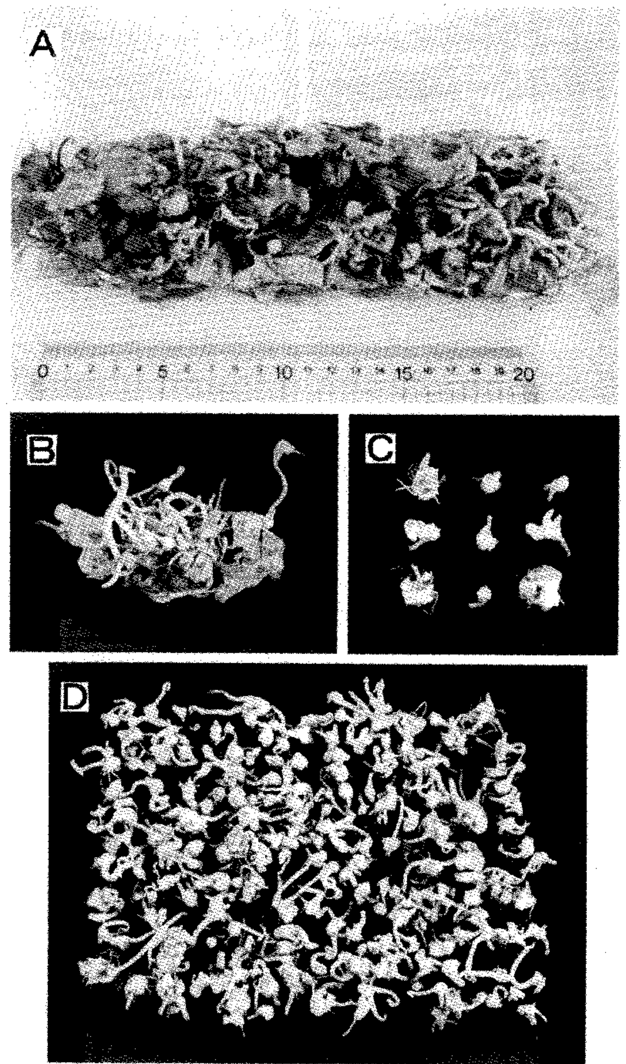

Fig. 2 *D. opposita* plants cultured in the bioreactor. (A) The appearance of shoots grown in the bioreactor. (B) White tuberous tissues formed on the axillary buds of the shoot. (C) White tuberous tissues excised from the shoot. (D) Tuberous tissues generated in a bioreactor. Numbers on scales designate length in cm.

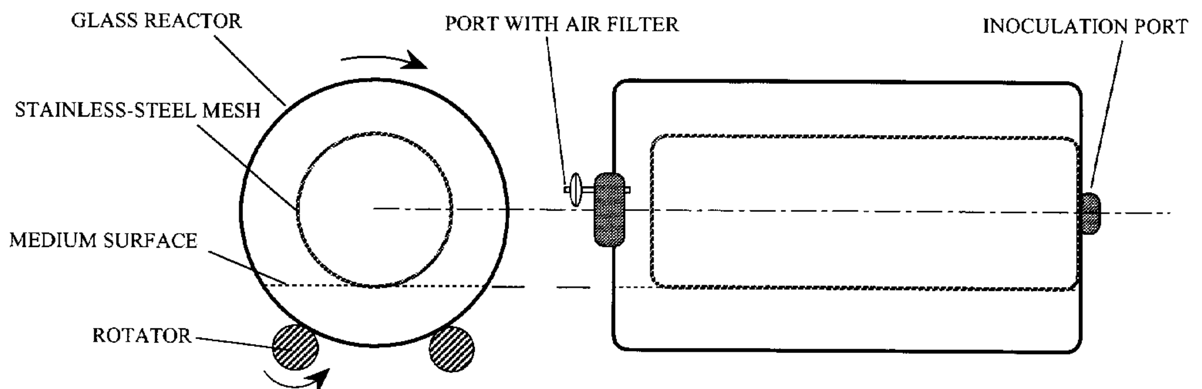

Fig. 1 Diagram of the culture system for mass propagation of storable organs of *D. opposita*. A cylindrical stainless steel mesh, 15 cm in diameter and 35 cm in length, was equipped in the center of a 10 liter rotary drum bioreactor (20 cm in diameter and 40 cm in length). Explants were transferred to the inside of the mesh through the inoculation port.

Results and Discussion

In our preliminary experiment, generation of bulbils of this cultivar was stimulated by a pulse treatment with BA at a high concentration before transferring into the medium containing increased concentration of sucrose (unpublished data). Thus, the explants of yam grown in flasks were transferred to the bioreactor after BA treatment for three days.

Whereas the medium in the bioreactor turned to brownish color during culture, the explants grew actively inside of the mesh. **Fig. 2** shows the cultured plants of *D. opposita*. The appearance of shoot-mass grown was cylindrical and white tuber-like tissues were formed on the axillary buds (**Fig. 2A** and **Fig 2B**). The surface of these tissues were soft and fragile (**Fig 2C**), but they contained a sticky sap that is typical for the storage organs (bulbils and rhizophores) of yam of this cultivar. The total weight of the plants including these tuberous tissues was 116.1 g (FW). The total number and weight of the tuberous tissues was 230 and 53.3 g (FW), respectively. Dry matter content of the shoots (stems, roots and leaves) and the tuberous tissue was 12.5% and 11.7% (w/w), respectively, meaning that tuberous tissues account for approx. 44% of the dry matter of the plants.

These tissues were storable in a plastic container under room conditions after washing with tap water. No fungal infection was observed even after several weeks. Sprouting was observed within 1 week under this condition and approx. 95% of them were sprouted in the plastic container after 4 weeks (**Fig. 3**). In addition, normal plants were grown when these storable tissues were transplanted directly on soil (**Fig. 4**). These results reasonably revealed that the tuberous tissues could be bulbils or microtubers.

Rotary bioreactor systems were employed in our previous reports on mass propagation of taro corms and potato tubers (Akita and Ohta, 1996, 1998). In this report, a cylindrical stainless steel mesh was inserted in the center of rotary vessel and this mesh might improve the efficiency of mass transfer of oxygen to the explants, i.e. the medium surface was disrupted by the mesh and the surface area increased. In addition, explants was cultured under shallower medium compared to our previous systems. Actually we also demonstrated that microtubers of potato could be efficiently produced by using this system (data was not shown).

It is well known that, in general, cultivars of yam do not generate aerial tubers (bulbils) in field conditions. However, Tatsumoto and Suyama (1998) succeeded in the induction of rhyzophores from a cultivar that rarely generates bulbils by culturing in a roller bottle. Whereas their culture system and conditions was different from ours, their and our results may suggest a possibility to produce rhyzophores or bulbils for some of yam cultivars in rotating type of bioreactor.

Dormancy was not clearly observed in our experiment. Whereas dormancy of *in vitro* derived microtubers would significantly vary in each cultivar (e.g. Matsumoto and Suyama, 1997, Alizadeh *et al.*, 1998), factors affecting on the length is unclear. In addition, field performance including the possibility of mutation should also be studied in the future.

Until now, we have proposed several types of rotating bioreactor for plant organs (Akita and Ohta, 1996, 1998). In this study, we demonstrated that our newly designed bioreactor system is applicable to yam propagation. Our simple and adjustable bioreactors including those proposed previously (Akita and Ohta, 1996, 1998) will be useful as the efficient and economical system for commercial production of high quality seedlings.

Fig. 3 Sprouts from the tuberous tissues of *D. opposita* stored in a plastic container. Numbers on scales designate length in cm.

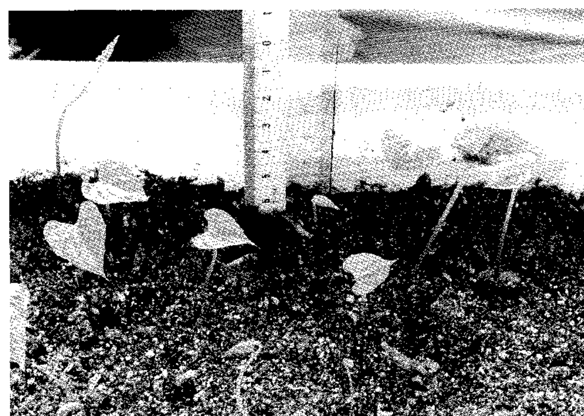

Fig. 4 *D. opposita* shoots developed from the tissues directly transplanted on soil. Numbers on scales designate length in cm.

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