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Somatic embryogenesis and plant regeneration in purple food yam (*Dioscorea alata* L.)

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ABSTRACT

A study was conducted to establish a reliable procedure for somatic embryogenesis and plant regeneration from callus cultures of purple food yam (*Dioscorea alata* L.). The procedure involved three steps; (1) culture of nodal stem segments from greenhouse-grown plants to generate *in vitro* plantlets; (2) induction of callus from the leaf, petiole and nodal stem tissues; and (3) initiation of somatic embryo from callus. Results showed that the agar-solidified Murashige and Skoog (MS) medium containing 30 gl^{-1} sugar, 0.1 gl^{-1} α-cysteine , 10 mgl^{-1} calcium pantothenic acid, 2.0 mgl^{-1} asparagine, 2.0 mgl^{-1} arginine, 80.0 mgl^{-1} adenine sulfate ($AdSO_4$) and 0.1 mgl^{-1} naphthalene acetic acid (NAA) effectively broke dormancy of lateral buds of nodal stem cultures from both 'VU-2' and *'Kinampay'* varieties. Production of multiple adventitious shoots occurred after transfer of in vitro nodal pieces to the same medium added with 1.0 mgl^{-1} benzylamino purine (BAP) or, MSA medium. Callus was effectively induced from the vegetative tissues in MS medium added with 1.0 mgl^{-1} 2,4-Dichlorophenoxy acetic acid (2,4-D) or, with picloram. Among the three types of explants, the nodal stem was the most suitable which produced purplish nodular embryogenic callus. A higher percentage of nodal stem-derived calli produced globular embryos in MS medium containing 1.0 mgl^{-1} 2,4-D and 0.5 mgl^{-1} BAP, or in 1.0 mgl^{-1} picloram and 0.5 mgl^{-1} BAP than, in the plant growth regulator-free medium (control). The maturation of embryos was facilitated by one-month culture in MS medium containing 0.1 mgl^{-1} ABA and 100 mgl^{-1} glutamine. This step improved the germination of somatic embryos in one-half strength PGR-free MS medium containing 100 mgl^{-1} glutamine (regeneration medium). All somatic embryo-derived plantlets were morphologically normal and established well in soil.

Keywords: callus, multiple adventitious shoots, nodal stem explant, plantlet, somatic embryo

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INTRODUCTION

Aromatic purple varieties of greater yam (*Dioscorea alata* L.) are among the important food yams that are highly sought because of their culinary value. Despite their economic importance, however, production is still low due to limited supply of planting materials and lack of resistance to viral and fungal diseases (Saleil *et al.* 1990). Although in vitro multiplication of *Dioscorea* species has been performed using explants since 1980s (cited by Shu *et al*. 2005), this technique has not been routinely applied in purple yams due to tissue oxidation and necrosis.

So far, reports of de novo regeneration in *D. alata* were limited to callus derived from petiole explants (Fautret *et al.* 1985) and somatic embryos derived from root cells (Twyford and Mantell 1996) of white yams. Likewise, somatic embryogenesis has been reported in several *Dioscorea* species (Osifo, 1988; Twyford and Mantell 1996; Shu *et al*. 2005) but these did not include the purple yams. To date, the technical know how on the production of unlimited number of somatic embryos, which are uniform in size and shape and retain the capacity to give rise to normal plants, is not available for any species (Petrivica, 2004).

In this paper, we report the production of plants from purple varieties of *D. alata* through the induction of callus and somatic embryos using excised nodal stems of in vitro plants. The effects of plant growth regulators on adventitious shoot formation, callus induction, somatic embryo formation and plant regeneration are described.

MATERIALS AND METHODS

Preparation of explants

Greenhouse-grown plants (2 month-old) from two purple varieties of *D. alata*, `VU-2` and *`Kinampay'* (Type D, LA-609) were used as source of nodal stem explants for initial in vitro culture. Nodal explants were prepared from vines that were thoroughly washed with tap water and then soaked in 0.1% (v/v) of fungicide solution (Benlate; Dupont,

Wilmington, Delaware, USA) for 10 min followed by rinsing in sterile distilled water four times. Finally, the explants were surface sterilized in a commercial chlorine bleach solution (containing 4.5% chlorine) for 20 minutes and then, rinsed four times in a sterile distilled water prior to cutting into single node stem pieces about 10-15 mm long.

Culture medium and incubation condition

The MS basal medium (Murashige and Skoog 1960) containing 30 gl^{-1} sugar (commercial white table sugar), 0.1 gl^{-1} α-cysteine , 10 mgl^{-1} calcium pantothenic acid, 2.0 mgl^{-1} asparagine, 2.0 mgl^{-1} arginine and 6 gl^{-1} agar (Pronadisa, Madrid, Spain) was used throughout this study. The pH of the medium was adjusted to 5.8 prior to autoclaving at 1.1 kg cm^{-2} (121 ºC) for 20 minutes. Newly-inoculated explants were incubated in dark for one week followed by exposure to 16 hours of daylight (supplied by two pieces of 40-W fluorescent tubes) at 26 ± 1 ºC.

Nodal stem culture of purple D. alata and adventitious shoot formation

To break dormancy of lateral bud, nodal stems were inoculated individually in glass vial (25 mm × 95 mm) containing 10 ml of medium. The medium was supplemented with 1.0 mgl^{-1} BAP and 0.1 mgl^{-1} NAA (T_1); 2.0 mgl^{-1} BAP and 0.1 mgl^{-1} NAA (T_2); 80 mgl^{-1} adenine sulfate ($AdSO_4$) and 0.1 mgl^{-1} NAA (T_3); 100 mgl^{-1} $AdSO_4$ and 0.1 mgl^{-1} NAA (T_4). The medium lacking plant growth regulators (PGR) served as control (T_0). Twenty-five explants were inoculated for each treatment and replicated four times. All nodal cultures were kept in dark for one week and then exposed to 16 h photoperiod for further eight weeks. The explants were transferred to fresh medium at one week interval until lateral bud break and shoot emergence.

Induction of multiple adventitious shoots

Nodal stem-derived shoots containing at least four nodes were cut into single node stem pieces and inoculated vertically in one-half strength