Production of somatic embryos and plantlets from root cells of the Greater Yam

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Abstract

Morphogenetically competent cell clusters were induced from 3-4 mm-long root segments excised from in vitro shoot cultures of the greater yam (Dioscorea alata L. ‘Oriental Lisbon’) plated in a liquid modified Murashige and Skoog (1962) medium (PMS) supplemented with 1 mg l\(^{-1}\) 2,4-dichlorophenoxy-acetic acid and 3% sucrose at 25 °C in the light. Embryogenic cell clusters released from subcortical regions of the root explants proliferated further into proembryonic cell masses which, when transferred to auxin-free medium, differentiated into somatic embryos. A proportion of the somatic embryos (as high as 80% in some experiments) converted to plantlets within two months after initial root segment culture. A description of the different stages of yam somatic embryogenesis is presented. Cultural factors which affected somatic embryo maturation in liquid medium to the greatest extent were embryogenic cell cluster density and gibberellic acid (GA\(_3\)) level present in the medium. Optimal somatic embryo maturation occurred when the density of embryogenic clusters was adjusted to 2–4 per 5 ml PMS and when GA\(_3\) was present in conditioned PMS medium at a concentration of 1.5 mg l\(^{-1}\)

Abbreviations: 2,4-D – 2,4-dichlorophenoxy-acetic acid; BA – benzyladenine; ABA – abscisic acid; 2iP – 2-isopentyladenine; GA\(_3\) – gibberellic acid; NAA – naphthyleneacetic acid; PMS – modified Murashige and Skoog (1962) medium; PAR – photosynthetically active radiation

Introduction

Dioscorea alata L. (the Greater Yam) is an important food yam cultivated in the tropics and wet sub-tropics for its starchy tubers which are a primary carbohydrate food source for many people in west Africa, southeast Asia, Oceania and the Caribbean (FAO, 1990). The cultivar ‘Oriental Lisbon’ is cultivated in eastern Caribbean countries as an early cropping yam which has a short tuberization cycle and some degree of resistance to anthracnose disease. Despite the importance of D. alata yams as both a subsistence and an export crop, their genetic improvement through conventional breeding has been hampered by unreliable and unknown fertility caused by dioecious flowering in the more commonly cultivated cultivars (viz. Degras, 1994; Vijaya Bay and Jos, 1986).

The use of biotechnological methods of in vitro breeding and genetic transformation to improve the genetic bases of this crop have been limited so far by the absence of reliable regeneration protocols (Mantell, 1993). There has been only one report in the literature of de novo regeneration in D. alata, this being from callus derived from petiole explants (Fautret et al., 1985). In fact, there is a notable lack of detailed information on the cultural requirements influencing plant regeneration processes in Dioscorea. The one exception is the description of plantlet regeneration in D. opposita by Nagasawa and Finer (1989). Moreover there are few detailed descriptions of the morphological events leading to somatic embryogenesis of yams.

In this paper, we report the results of investigations in which D. alata somatic embryos and plantlets were produced from morphogenetically competent cells
derived from subepidermal cells of root explants exposed for short periods of 7–10 days to low levels of 2,4-D.

Materials and methods

Plant material

The *D. alata* cultivar ‘Oriental Lisbon’ was used for this study. Tubers were originally provided by Dr Frances Chandler of the Caribbean Agricultural Research and Development Institute and had been harvested from a yam crop grown at Friendship Plantation, St Lucy, Barbados. All root tips used were excised from *in vitro* plantlets of clone ‘C26’, which had been regenerated previously from embryogenic calluses of Wye Yam Germplasm Accession 78/104 (Twyford, 1993). Stock plants of clone ‘C26’ were maintained as single node cultures (Mantell et al., 1978) in 50 ml glass jars, each containing 5 ml of modified Murashige and Skoog medium (1962) consisting of MS basal salts and 2% (w/v) sucrose with (solidified) or without (liquid) 0.3% (w/v) Phytagar and adjusted to pH 5.7 prior to autoclaving at 1.1 kg cm$^{-2}$ (121 °C) for 20 min (Medium PMS). All shoot cultures were maintained at 25 °C under 16 h per day photoperiods (produced by TLD93 Philips fluorescent strip lights giving 63 μmol m$^{-2}$ s$^{-1}$ PAR at culture level). Yam shoot cultures were subcultured regularly every 10–14 days.

Root explants were excised for embryogenic induction from the tips of roots (15 mm in length) present on shoot cultures 10 days following routine subculture. Embryogenic cell clusters were produced from 3–4 mm root segment explants, from which the distal 1 mm (containing the root meristem) had been deliberately removed, after an initial exposure for 10 days to a liquid medium consisting of MS salts supplemented with 3% sucrose and 1 mg l$^{-1}$ 2,4-D (D1 Medium) on a rotary shaker (90 rpm) in the light at 25 °C and transfer of the induced root explants to auxin-free liquid PMS medium for 21–28 days. In order to determine which level of 2, 4-D was the most suitable for the long-term multiplication of embryogenic calluses, ca 5 mg fresh weight embryogenic cell cluster/proembryonic masses produced from root explants by the above-mentioned method were subcultured in 2.5×10 cm glass tubes containing semi-solidified PMS medium supplemented with 0.05, 0.1, 0.5, 1.5, 2.5 or 4.0 mg l$^{-1}$ 2,4-D (at initial pH 5.7) and 0.3% (w/v) Phytagar. Each replicate tube contained 10 ml medium and 15 replicates were used per treatment. After 6 months, cultures were assessed for differences in fresh weights and appearances of calluses (textures, colour, and embryogenic characteristics).

Conversion and maturation of somatic embryos

The various stages of yam somatic embryo maturation were studied in detail by inducing root segments in a standard volume (2 ml) of liquid D1 medium in 5-cm Petri dishes. Positively responding root segments, i.e. ones in which new cell divisions were induced as observed, were collected and randomly assigned to treatments. Firstly, the effects of root explant density on somatic embryo maturation were studied by culturing induced explants (10 days in liquid D1 medium) at densities of either 1, 2, 3, 4, 8, 10, 11, or 13 root tip explants per 5 ml of liquid PMS. After 25 and 42 days of culture, numbers of somatic embryos present in each Petri dish were scored for one of six developmental stages (as illustrated in Fig. 3). Secondly, the effects of GA$_3$ on the conversion of cotyledonary initiation-stage somatic embryos were evaluated by culturing 50 somatic embryos at this stage of development (<2 mm in length) per 5-cm diameter Petri dish, each containing 5 ml filter sterilized liquid PMS medium supplemented with GA$_3$ at either 0.5, 1.0 or 1.5 mg l$^{-1}$. Appropriate somatic embryos were selected with the aid of a dissecting microscope (10x magnification) from 45-day-old cultures containing previously induced explants. After 10 days, somatic embryos were scored for hypocotyl elongation by measuring total somatic embryo length. Lastly, the effects of a range of plant growth regulators on yam somatic embryo maturation were tested. Root explants were induced in the standard way (on D1 medium for 10 days), transferred to individual wells in 25-compartment dishes each containing 0.2 ml of conditioned liquid PMS medium (in which root explants and somatic embryos had previously been cultured for at least 7 days). After 27 days, groups of six wells were randomly allocated to one of the following plant growth regulator treatments (previously filter-sterilized and added as volumes no greater than 0.75 μl): 1.5 mg l$^{-1}$ GA$_3$, 0.15 mg l$^{-1}$ ABA, 0.005 mg l$^{-1}$ BA or 0.005 mg l$^{-1}$ 2iP. One set of six wells received no further addition apart from 0.75 μl fresh PMS medium and this acted as a control. Treatments were assessed after 20 days of culture for mean numbers of embryos per 10x magnification field showing the six stages of somatic embryo development of proembryonic masses, cotyledon-initiation, cotyle-