South African Journal of Botany 109 (2017) 269–274

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Ultrastructure of somatic embryo development and plant propagation for *Lachenalia montana*

P. Baskaran, J. Van Staden *

Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, Scottsville 3209, South Africa

ARTICLE INFO

Article history:
Received 10 May 2016
Received in revised form 1 December 2016
Accepted 8 January 2017
Available online 28 January 2017

Edited by D Sarkar

Keywords:
Cellular development
Horticultural plant
Phloroglucinol
Somatic embryogenesis
Thidiazuron
Transmission electron microscopy

ABSTRACT

Embryogenesis for plant propagation by cell suspension culture was achieved for the first time using friable embryogenic callus (FEC) from leaf explants of *Lachenalia montana* Schltr. ex W.F. Barker. FEC was established with solid (8 g L^{-1} agar) MS (Murashige and Skoog, 1962) medium containing various concentrations and combinations of sucrose and plant growth regulators (PGRs). The somatic embryos (SEs) were developed from FEC in liquid MS (MS_L) medium with or without PGRs. A higher number of SEs of different developmental stages (26.0–19.4, globular to cotyledonary-stages respectively) were obtained on MS_L medium with 0.5 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and 1 μM thidiazuron (TDZ). The germination frequency (33.7%) was highest in MS_L medium containing 1 μM 2,4-D and 2 μM TDZ. The different stages of SEs germinated (92%) on solid full-strength MS medium containing 15 g L^{-1} sucrose and 10 μM phloroglucinol (PG). The plantlets were successfully acclimatized in the greenhouse. Developmental stages and features of embryoids were confirmed by histological and ultrastructural studies using light and transmission electron microscopy (TEM). Many mitochondria, lipid bodies together with starch grains, chloroplasts, Golgi apparatuses, vacuoles and nuclei were detected. The system developed offers controlled large-scale clonal propagation which ensures germplasm conservation, confirms viability of embryogenesis by ultrastructural studies and provides a system for genetic transformation studies for horticultural and ornamental improvement.

1. Introduction

Lachenalia (*Hyacinthaceae*) is an important genus of southern African bulbous horticultural and ornamental plants, especially in the Cape Floral Kingdom (Duncan, 1996). The genus has a narrow range of distribution and many species are endangered or threatened requiring conservation efforts (Duncan, 1988; Doutt, 1994; SANBI, 2014). Many species are very popular in the international flower industry (Kleynhans, 2009). An 'African Beauty' has been developed in a new series of cultivars in the *Lachenalia* breeding programme. It displays spotted leaves, combined with variously colored flowers, making the cultivars ideal garden and particularly pot plants (Kapczyńska, 2013). Conventional methods have been used widely for new cultivars of *Lachenalia* (Du Preez et al., 2002; Kleynhans et al., 2002). Nevertheless high efficiency rapid clonal selection and development for horticultural varieties remains a major challenge (Stirton, 1980; Jansen van Vuuren, 1995; Niederwieser et al., 2002; Kapczyńska, 2013). Although *in vitro* micropropagation of *Lachenalia* cultivars (Namakwa, Ronina and Rupert) and somatic embryogenesis of *Lachenalia viridiflora* have been described (Slabbert and Niederwieser, 1999; Fennell and Van Staden, 2004; Niederwieser, 2004; Kumar et al., 2016), the confirmation of somatic embryogenesis with different developmental stages for clonal propagation of *Lachenalia* species or cultivars remains a major bottleneck. Improvement programmes based on a somatic embryogenesis with different developmental stages protocol *via* cell suspension culture would be helpful for *Lachenalia* species and cultivars. There is no report on rapid mass *in vitro* plant regeneration and confirmation of somatic embryogenesis with different developmental stages for *Lachenalia montana*. An efficient *in vitro* plant regeneration *via* somatic embryogenesis (cell suspension culture) is therefore required for selection of clonal plants, mass propagation, conservation, gene transfer and studying ecological and physiological aspects of *L. montana*.

Somatic embryogenetic systems are of growing interest for ornamental and horticultural plants (Pareek and Kothari, 2003; Fennell and Van Staden, 2004; Rout et al., 2006; Ji et al., 2011). Dedifferentiation of cells, activation of cell division, reprogramming of cell physiology, metabolism, and gene expression patterns occurred during unique developmental pathways of somatic embryogenesis. However, morphological abnormalities such as embryo fusion and lack of suitable apical meristems or loss of bipolarity have occurred resulting in poor yields (Benelli et al., 2010). A morpho-histological and cytological study during embryogenesis is therefore required to confirm the embryogenic capacity and for clonal propagation of *Lachenalia* species.

* Corresponding author.
E-mail address: rcpgd@ukzn.ac.za (J. Van Staden).

http://dx.doi.org/10.1016/j.sajb.2017.01.006
0254-6299/

Table 1
Effect of sucrose and growth regulators on friable embryogenic callus (FEC) production for somatic embryo (SE) development with 6 weeks of incubation in cell suspension culture of *L. montana*.

Sucrose (g L^{-1}) + PGR (μM) in solid MS medium	Number of SEs/SCV developmental stage		Germination (%)
6 weeks of culture	Globular	Different stages of embryo*	
Control	0	0	0
30 + 10 2,4-D	4.0 ef	3.0 gh	0
35 + 10 2,4-D	6.2 de	4.6 ef	0
40 + 10 2,4-D	7.8 cd	6.4 de	0
35 + 10 2,4-D + 2 BA	9.4 c	8.0 c	0
35 + 10 2,4-D + 2 TDZ	14.8 ab	9.8 a	0
30 + 10 picloram	3.6 f	2.0 h	0
35 + 10 picloram	7.2 cd	5.0 ef	0
40 + 10 picloram	10.4 c	6.6 de	0
35 + 10 picloram + 2 BA	16.2 a	9.2 ab	0
35 + 10 picloram + 2 TDZ	9.8 c	7.0 cd	0

FEC = Friable embryogenic callus. PGR = Plant growth regulator. SEs = Somatic embryos. SCV = Settled cell volume. *Different stages of embryo = pear-shaped, early torpedo-shaped, torpedo-shaped and cotyledonary-stage embryos. Values with the means derived from 5 replicate (each 500 mg FEC) with 1000 μL SCV of embryogenic suspension cells per replicate. Mean values followed by same letters in each column are not significantly different according to the Duncan's multiple range test at 5% level.

The aim of the present study was to develop an efficient protocol for plant regeneration *via* somatic embryogenesis of cell suspension cultures from FEC of leaf explants of *L. montana*, using different concentrations and combinations of sucrose, plant growth regulators and phloroglucinol. In addition, histological and ultrastructural studies were made to confirm the bipolar structure, viability and features of somatic embryos of *L. montana* for production of clonal plants.

2. Materials and methods

2.1. Plant material and somatic embryogenesis

The bulbs of *L. montana* were generously supplied by Dr. Graham Duncan, Kirstenbosch, South Africa. Young leaves of *L. montana* were excised from three-month-old greenhouse grown bulbs. Leaves were washed with Tween® 20 for 1 min and then decontaminated with 0.1% aqueous $HgCl_2$ for 10 min. Leaves were then rinsed five times with sterile distilled water. Leaf explants (approximately, 10 × 5.0 mm) were excised and cultured on solid (8 g L^{-1} agar, Sigma, USA) MS (Murashige and Skoog, 1962) medium with 30–40 g L^{-1} sucrose and different concentrations and combinations of plant growth regulators [PGRs: 2,4-dichlorophenoxyacetic acid (2,4-D), picloram, benzyladenine (BA) and thidiazuron (TDZ)] for production of friable embryogenic callus (FEC) for 6 weeks. All experiments were repeated three times with 25 replicates per treatment; each replicate consisted of 5 explants. Six-week-old FEC (approximately 500 mg fresh weight) were transferred to 20 mL liquid MS medium (MS_L) containing 30 g L^{-1} sucrose in 100 mL Erlenmeyer flasks for 6 weeks to determine optimal FEC for formation and germination of somatic embryos (SEs). Optimized FEC from solid medium containing 35 g L^{-1} sucrose, 10 μM picloram or 2,4-D and 2 μM BA or TDZ were inoculated into MS_L medium containing different concentrations and combinations of PGRs [2,4-D, picloram, BA, *meta*-topolin riboside (*m*TR) and TDZ] for 4 weeks and then transferred to MS_L medium for 2 weeks to improve somatic embryogenesis and germination frequency. The precise concentrations and combinations are outlined in Tables 1–3. Suspension culture of *L. montana* was performed as previously described (Baskaran and Van Staden, 2012) and modified with settled cell volume (SCV: the volume of cells in 100 mL flasks was maintained as 1000 μL). In all experiments, the medium lacking plant PGRs served as control. The chemicals used were of analytical grade (Biolab, South Africa; Oxoid, England and Sigma, USA). All media were adjusted to pH 5.8 with 0.1 N NaOH and/or 0.1 N HCl before gelling with 8 g L^{-1} agar and autoclaved at 121 °C for 20 min. The cultures were maintained at 25 ± 2 °C and light intensity of 40 μmol m^{-2} s^{-1} provided by cool white fluorescent light (OSRAM L 58 W/740, South Africa) under a 16 h photoperiod. All experiments were repeated three times with 5 replicates per treatment. Different developmental stages of SEs (globular embryo, pear-shaped, early torpedo-shaped, torpedo-shaped and cotyledonary stages) and germination frequency (number of germinated SE / total number of SE × 100) were calculated after 6 weeks of culture.

Four-week-old developmental stages of SEs (globular to cotyledonary) were cultured on half-strength solid MS medium plus 15 g L^{-1} sucrose, solid MS medium plus 30 g L^{-1} sucrose, solid MS medium plus 15 g L^{-1} sucrose and solid MS medium containing 15 g L^{-1} sucrose and 1–15 μM phloroglucinol (PG) for germination. The cultures were maintained in the dark for 3 days and then incubated under 40 μmol m^{-2} s^{-1} light intensity and a 16 h photoperiod. All experiments were repeated three times with 15 replicates per treatment; each replicate consisted of 3 SEs of each stage. Embryo germination percentage was calculated after 8 weeks (number of germinated SE / total number of SE × 100). All the plantlets (approximately, 60–80 mm) from plant induction media were acclimatized successfully in a greenhouse as described previously (Baskaran and Van Staden, 2012).

2.2. Microscopic studies

Developmental stages of SEs (globular, pear-shaped, early torpedo-shaped, torpedo-shaped and cotyledonary embryos) were photographed using a Leica MZ 16 Stereo Microscope (Leica: OFC450 C, Digital Camera).

Table 2
Enhancement of somatic embryos and germination frequency in cell suspension culture from friable embryogenic callus (FEC) of *L. montana*.

Sucrose (g L^{-1}) + PGR (μM) in solid MS medium	PGR (μM) in liquid MS medium	Liquid MS medium (MS_L)	Number of SEs/SCV developmental stage		Germination (%)
6 weeks of culture	4 weeks of culture	2 weeks of culture	Globular	Different stages of embryo*	
35 + 10 picloram + 2 BA	1 picloram	MS_L	10.0 e	6.4 e	0
	1 2,4-D	MS_L	14.4 cd	9.0 cd	0
	1 2,4-D + 0.5 BA	MS_L	15.0 cd	9.8 cd	7.2 e
	1 2,4-D + 0.5 *m*TR	MS_L	7.8 f	6.0 e	8.6 de
	1 2,4-D + 0.5 TDZ	MS_L	18.0 b	12.4 b	14.0 ab
35 + 10 2,4-D + 2 TDZ	1 picloram	MS_L	6.8 fg	3.0 f	0
	1 2,4-D	MS_L	16.0 cd	6.8 e	0
	1 2,4-D + 0.5 BA	MS_L	18.4 b	10.0 c	11.4 c
	1 2,4-D + 0.5 *m*TR	MS_L	10.2 e	6.8 e	14.3 ab
	1 2,4-D + 0.5 TDZ	MS_L	21.4 a	14.6 a	15.2 a

PGR = Plant growth regulator. MS_L = Liquid MS medium. SEs = Somatic embryos. SCV = Settled cell volume. *Different stages of embryo = pear-shaped, early torpedo-shaped, torpedo-shaped and cotyledonary-stage embryos. Values with the means derived from 5 replicate (each 500 mg FEC) with 1000 μL SCV of embryogenic suspension cells per replicate. Mean values followed by same letters in each column are not significantly different according to the Duncan's multiple range test at 5% level.