Scientia Horticulturae 233 (2018) 407–411

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Short communication

Callus induction and establishment of cell suspension cultures of the halophyte *Armeria maritima* (Mill.) Willd.

L. Gourguillon[a,b], C. Rustenholz[c], A. Lobstein[a], L. Gondet[a,*]

[a] Université de Strasbourg, CNRS, LIT UMR 7200, F-67000 Strasbourg, France
[b] SEPPIC – BiotechMarine, ZI BP72, F-22260 Pontrieux, France
[c] Université de Strasbourg, INRA, SVQV UMR-A 1131, F-68000 Colmar, France

ARTICLE INFO

Keywords:
Armeria maritima
Callus induction
Cell suspension
Halophyte
Explant
Plant growth regulators

ABSTRACT

The objective of this work was to establish a cell suspension culture from *Armeria maritima* (Mill.) Willd., an halophyte so far only valuable for horticultural purposes, as a pre-requisite step towards the production of bioactives secondary metabolites *in vitro*. For the initiation of callogenesis, five types of explants were tested: leaves from mature plants; leaves, cotyledons and roots from axenic seedlings; seeds. Three criteria either from a quantitative (percentage of callus induction) or a qualitative basis (aspect and friability) were used to evaluate the effectiveness of callus formation. Seeds-derived calli grown in the dark on MS medium supplemented with 4.5 µM 2.4D and 0.93 µM KIN were successfully selected for the establishment of cell suspension cultures, characterized by a growth index of 3.4 after 14 days of culture.

1. Introduction

Halophytes are characterized by their ability to grow under saline stress, with concentrations of NaCl around 200 mM which is toxic to most crop species, leading to hyper osmotic shock and oxidative stress, nutrient imbalance, as well as to the inhibition of cell division and photosynthesis (Aslam et al., 2011). Salt tolerance in plants is determined by numerous physiological and biochemical processes, acting at molecular, cellular or morphological plant levels (Duarte et al., 2013). As an illustration for their adaptation to saline environment, halophytes produce osmo-protective compounds with quaternary ammonium or specific sugars such as glycine betaine and mannitol, respectively (Hanson et al., 1994), as well as polyphenols, in order to fight against oxidative stress notably caused by NaCl and UV radiations (Ksouri et al., 2007, 2013; Rodrigues et al., 2015; Stankovic et al., 2015). This molecular adaptation towards antioxidant capacity makes these halophytic plants particularly interesting as a raw material for bioactive molecules, with multifaceted potential for either medical, cosmetical or nutritional applications (Buhmann and Papenbrock, 2013; Lopes et al., 2016; Medini et al., 2015).

Moreover, their adaptive mechanisms to salt stress often confer tolerance to other toxic elements such as heavy metal ions. Therefore halophytes are gaining an increased interest for their potential in phytoremediation of heavy metals contaminated soils (Van Oosten and Maggio, 2015). Halophytes also appear as a possible alternative to glycophytic crops in the growing environmental context where salinization of soils increasingly contributes to land degradation worldwide, thus highlighting the need to develop saline agriculture (Akinshina et al., 2016; Panta et al., 2014).

Armeria maritima (Mill.) Willd., also called “sea thrift” or “sea pink”, is a common halophytic plant growing in occidental coasts of northern Europe, actually used for horticulture purposes, and already studied for its copper tolerance (Brewin et al., 2003). *A. maritima* belongs to the Plumbaginaceae family which is well-known for its halophytic genus *Limonium* (Hanson et al., 1994; Medini et al., 2015; Rodrigues et al., 2015). In the past, previous study described the characterization of flavonoids content in leaves of *A. maritima* (Lauranson et al., 1995). Flavonoids are well-known to have bioactive properties, especially interesting for cosmetic and pharmaceutical applications (Brand-Garnys et al., 2007). Moreover, Kumarasamy et al. (2002) showed an antibacterial effect of a methanol extract from *A. maritima* seeds against *Staphylococcus epidermidis*. Thus *A. maritima* could be considered as a multipurpose raw material.

Furthermore, the marine environment is a fragile ecosystem and the intensive harvest of wild seashore plants is not sustainable. Moreover, plants grown in field are submitted to seasonal and climatic fluctuations, inducing metabolic variations (Lauranson et al., 1995; Verma and Shukla, 2015) unsuitable with industrial valorization which needs standardized plant raw material. Thus, plant cell cultures of *A. maritima* could represent a suitable tool to supply high-value bioactive

* Corresponding author.
E-mail address: gondet@unistra.fr (L. Gondet).

http://dx.doi.org/10.1016/j.scienta.2017.08.001
Received 13 May 2017; Received in revised form 30 July 2017; Accepted 1 August 2017
Available online 16 February 2018

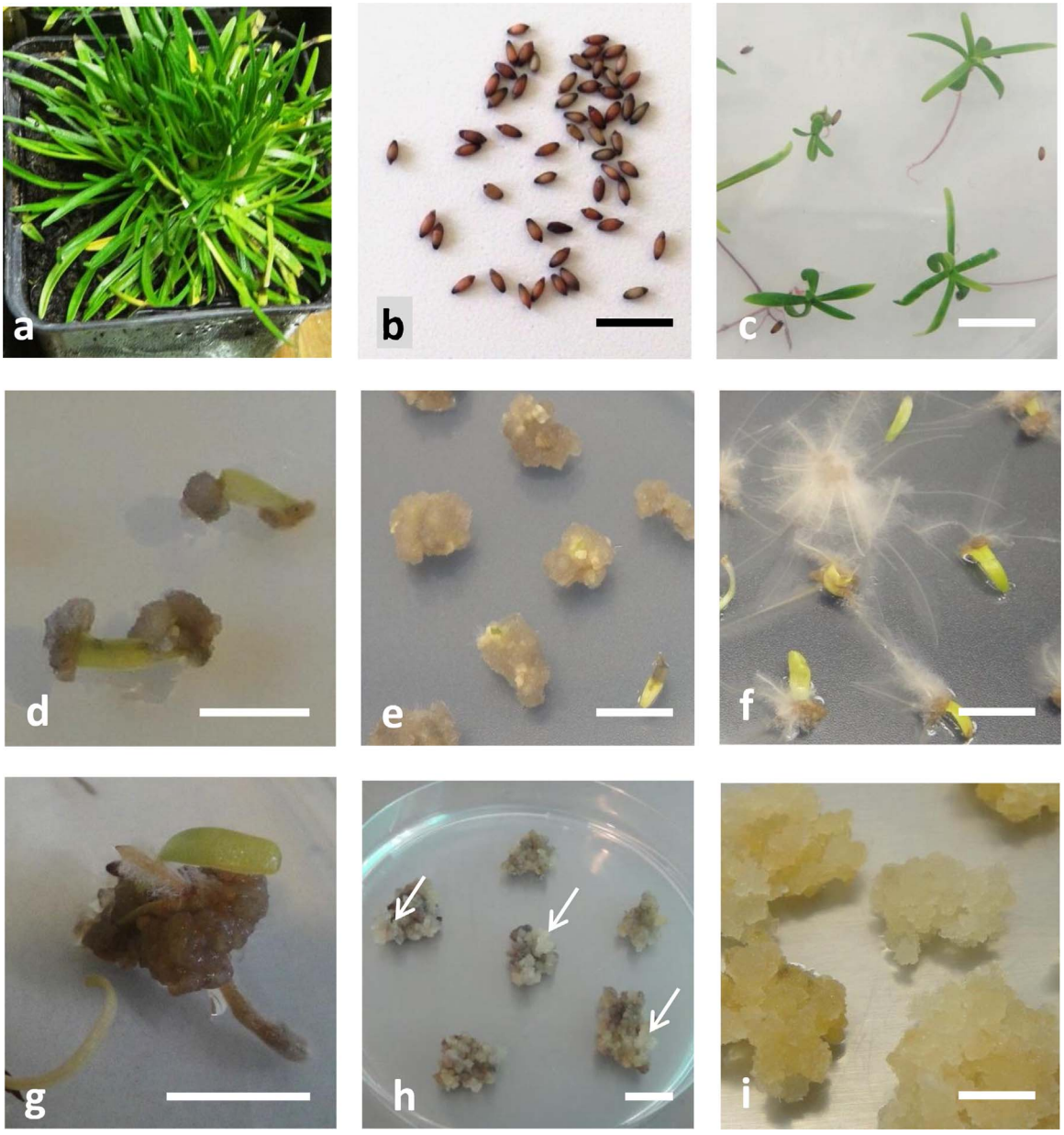

Fig. 1. Callus induction of *A. maritima*. (a) Plant cultivated in pots; (b) seeds; (c) 6 weeks-old axenic seedlings; (d) 6 weeks and (e) 8 weeks leaf-derived calli on MS supplemented with 4.5 µM 2.4D, 0.93 µM KIN and 3% sucrose; (f) 8 weeks leaf-derived calli and rhizogenesis on MS supplemented with 2.7 µM NAA, 0.4 µM BA and 3% sucrose; (g) 8 weeks seed-derived callus on MS supplemented with 4.5 µM 2.4D, 0.93 µM KIN and 3% sucrose; (h) friable light yellow areas (arrows) developing on the brown primary seed-derived calli (i) multiplication of friable seed-derived calli. Bar = 1 cm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

molecules, as it was already described for numerous other plants (Arias et al., 2016; Dias et al., 2016; Szopa et al., 2017). The main objective of this work was to establish an efficient protocol for friable callus induction and multiplication of *A. maritima* as a source for initiation of cell suspension culture, which is the first step towards *in vitro* metabolites production.

2. Material and methods

2.1. Plant material

Seeds of *A. maritima* var. *splendens* were purchased from Garden Seeds B.V. (Postbus 296, 1600 AG, Enkhuizen, Holland). For establishment of axenic seedlings, seeds were surface-sterilized for 5 h with agitation in 0.5% (v/v) Plant Preservative Mixture (PPM™, Plant Cell Technology), then washed three times in water and kept for 24 h at 4 °C prior to sowing on MS medium (Murashige and Skoog, 1962) at pH adjusted to 5.8, containing 3% (w/v) sucrose, solidified with 0.8% (w/v) agar and sterilized by autoclaving for 15 min at 121 °C. Seeds were incubated for 6 weeks in a growth chamber at 25 °C under a continuous photosynthetic photon flux density (PPFD) of 50 µmol.m^{-2}.s^{-1} provided by cool-white fluorescent lamps.

A. maritima var. *splendens* plants cultivated in pots were purchased by Le Jardin du Pic Vert (26 Carrière Dorée, 59310 Orchies, France).

2.2. Callus induction

Five types of *A. maritima* explants were used for callus induction: 1 cm segments of leaves from mature plants cultivated in pots (Fig. 1a); seeds (Fig. 1b); young leaves, cotyledons and roots from 6 weeks-old axenic seedlings (Fig. 1c). Leaves from plants grown in pots were first washed in ethanol for 30 s, then surface-sterilized for 5 min with