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Media culture factors affecting somatic embryogenesis in *Agave angustifolia* Haw

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ABSTRACT

A complete protocol of somatic embryogenesis involves induction of embryogenic callus, embryo development, embryo maturation, and their conversion or germination to form complete plants; in this sense judicious selection of nutrient medium, growth regulators, and physical culture environment is required. In this work, culture medium factors that influence somatic embryogenesis in *Agave angustifolia* were investigated. In an induction medium (IM) we evaluated the effect of three sucrose concentrations, three plant growth regulator (PGR) combinations, two groups of vitamins and two sources of amino acids. We observed that somatic embryos (SE) in medium containing 6% sucrose concentration grew vigorously, while those induced in medium with 8% sucrose were abnormally shaped and did not develop fully. In contrast, a higher sucrose concentration (10%) inhibited development of explants. Embryogenic callus cultured in IM containing 13.59 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and 4.44 μM 6-benzyladenine (BA) only produced SE, while those explants assayed in IM with 11.34 μM abscisic acid (ABA) did not improve embryogenesis response. Phillips-Collins (L2) vitamins induced a higher number of SE (34 ± 0.4) than Murashige–Skoog (MS) vitamins (1.7 ± 0.7). High levels of amino acids (500 mg l^{-1} L-glutamine or casein hydrolysate) were not effective in promoting embryogenesis. Conversion frequency to plantlets ranged from 95 to 100% with 100% survival under *ex vitro* conditions.

1. Introduction

The *Agave* species have a great industrial potential for the production of food, cellulose, fibres, sugars, pharmaceutical compounds, syrups, sapogenins, and ornamental plants (Portillo et al., 2007), as an energy crop to produce biofuel and as raw material to produce alcoholic beverages such as tequila or mescal. This economic value results in a tremendous national and international demand of the *Agave* species, making the genus an important target for *in vitro* mass propagation and genetic improvement.

For the genus *Agave*, somatic embryogenesis protocols have been reported *for A. victoria-reginae, A. sisalana, A. tequilana, A. Veracruz* and *A. angustifolia* (Rodríguez-Garay et al., 1996; Martínez-Palacios et al., 2003; Nikam et al., 2003; Portillo et al., 2007; Tejavathi et al., 2007; Arzate-Fernández and Mejía-Franco, 2011). *A. angustifolia* is one of most important raw materials for production of high quality mescal.

Somatic embryogenesis forms the basis of cellular totipotency in higher plants. Under *in vitro* conditions, one or a few somatic cells of the explant should be competent to receive a signal (endogenous or exogenous) for the developmental restructuring towards the embryogenic pathway. It triggers the pathway of embryogenic development (commitment) leading to somatic embryo formation. In this fate, plant cells attempt to establish a new programme through changes in pH gradients of all cell compartments, arresting differentiated functions, reactivating the cell cycle and re-organising gene expression as well as metabolism (Feher et al., 2003). However, for a particular genotype, the *in vitro* forms of somatic embryogenesis, the optimum conditions (potential, competence, induction, and commitment) have to be experimentally optimised (Feher et al., 2003).

Thus, the establishment of improved micropropagation procedures

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; BA, 6-benzyladenine; ABA, abscisic acid; MS, Murashige–Skoog; PGR, plant growth regulator

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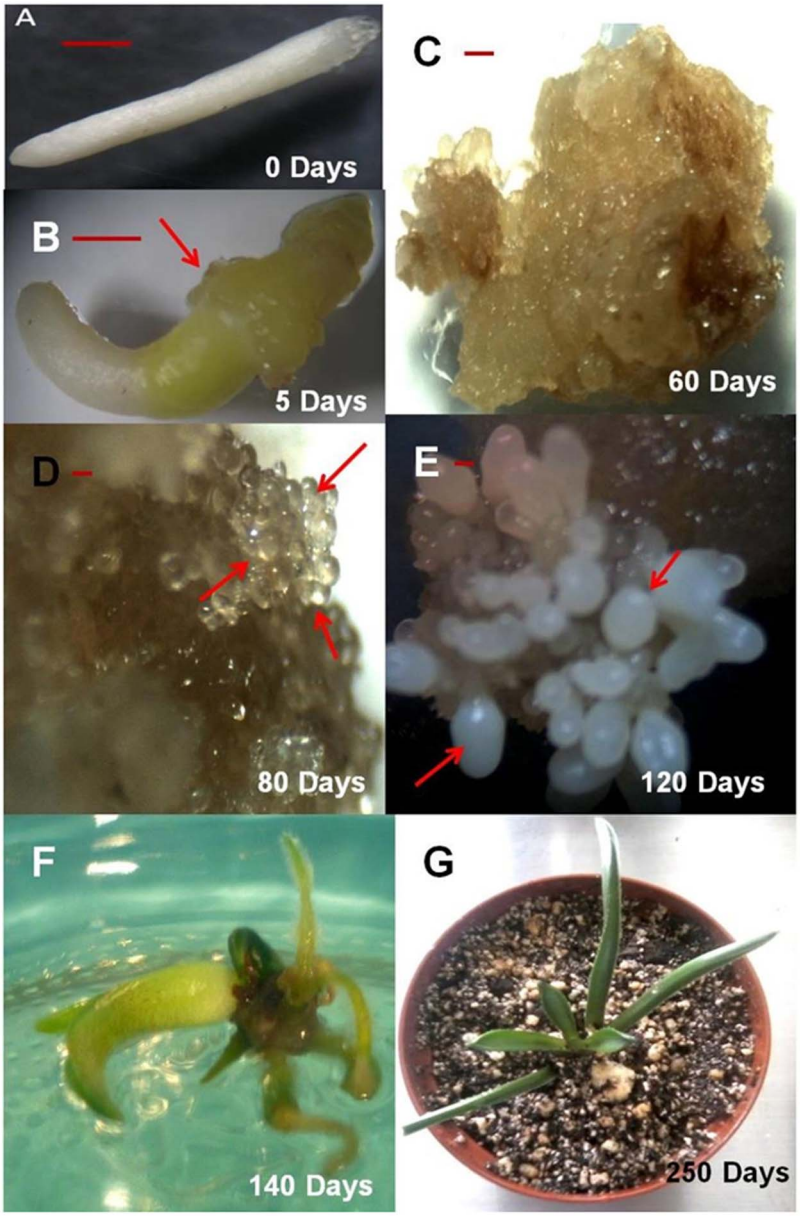

Fig. 1. Somatic embryogenesis in *Agave angustifolia* Haw. obtained with vitamins L2, 14 μM 2,4-D, 5 μM BA and 60 g l^{-1} of sucrose. (A): explant, (B): callus initiation, (C): creamy-white friable callus, (D): globular masses, (E): mature somatic embryos, (F): rooted plantlet, (G): plantlet exposed to natural conditions. Bar: 1 mm.

by testing media culture factors is desirable and could help to increase the efficiency of protocols. In this work, we present data on the influence of some culture medium components (highlighting carbon concentration, plant growth regulators and nitrogenous substances) on growth and development of embryogenic callus to optimize *in vitro* regeneration potential in *A. angustifolia*. The results showed that these factors affected induction frequency of embryogenic calluses, size of embryogenic calluses then differentiation potential of calluses for somatic embryo formation.

2. Materials and methods

2.1. Explant source and surface sterilization

The experimental materials were *Agave angustifolia* Haw seeds, collected from wild plants in Zumpahuacan, State of Mexico. The seeds were washed in running water with detergent for 10 min, surface-sterilized in 96% ethanol for 1 min, followed by immersion in 5% calcium hypochlorite solution (CaClO) for 15 min, and rinsing three times with sterile distilled water (SDW). Later, they were soaked in SDW for 96 h. Under laminar flow beach, aseptic mature zygotic embryos (Fig. 1A) were dissected from seeds and used as initial explants for callus induction.

2.2. Callus induction

Initial explants were placed in callus induction medium (IM) consisting in quarter-strength MS salt basal medium (Murashige and Skoog, 1962). In this IM, the following external factors were assessed: sucrose (60, 80 and 100 g l^{-1}), plant growth regulators (PGR) (13.59 μM 2, 4-D and 4.44 μM BA with or without ABA and only 11.34 μM ABA), supplemented or not with 500 mg l^{-1} L-glutamine or casein hydrolysate (as amino acids source), with or without MS (Murashige and Skoog, 1962) or L2 (Phillips and Collins 1979) vitamins. These factors were added to evaluate their influences on embryogenic callus induction (Table 1). Thus, 81 treatments were assayed. The pH of the medium was adjusted to 5.6–5.8 before adding the gelling agent (8 g l^{-1} agar) and autoclaving at 121 °C for 20 min. Petri dishes (9 × 1.5 cm) were filled with 25 ml of medium and sealed with Allupack®.

Each treatment consisted of 12 replicates each with ten explants.