South African Journal of Botany 112 (2017) 40–47

Contents lists available at ScienceDirect

South African Journal of Botany

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

Establishment of callus and cell suspension cultures of *Eysenhardtia polystachya* (Ortega) and fungistatic activity of their extracts

A. Bernabé-Antonio [a,*], A. Maldonado-Magaña [b], C.B. Ramírez-López [a], E. Salcedo-Pérez [a], J.C. Meza-Contreras [a], Y. González-García [a], F.A. López-Dellamary Toral [a], F. Cruz-Sosa [c]

[a] Departamento de Madera, Celulosa y Papel, Centro Universitario de Ciencias Exactas e Ingenierías, Universidad de Guadalajara, Km. 15.5 Carretera Guadalajara-Nogales, C.P. 45100, Zapopan, Jalisco, Mexico
[b] Centro de Investigaciones Químicas, Universidad Autónoma del Estado de Morelos, Universidad 1001, Col. Chamilpa, C.P. 62209, Cuernavaca, Morelos, Mexico
[c] Departamento de Biotecnología, Universidad Autónoma Metropolitana-Iztapalapa, San Rafael Atlixco 186, Col. Vicentina, C.P. 09340, Ciudad de México, Mexico

ARTICLE INFO

Article history:
Received 5 January 2017
Received in revised form 12 April 2017
Accepted 11 May 2017
Available online xxxx

Edited by AO Aremu

Keywords:
Plant growth regulator
Fungistatic activity
Total phenols
Total flavonoids

ABSTRACT

Eysenhardtia polystachya (Fabaceae) is a valuable medicinal plant from Mexico. There have been no cell culture studies of this plant to produce secondary metabolites. The aim of this work was to establish callus and cell suspension cultures of *E. polystachya* (Ortega) and to evaluate the content of phenols, total flavonoids, and fungicidal activity against *Sclerotium cepivorum* and *Rhizoctonia solani* from extracts of wild plant and cell cultures. An efficient protocol for callus induction and cell cultures was established. The maximum percentage of explants inducing callus (100 and 98%) was obtained using 1-naphthaleneacetic acid (8.28 μM) plus kinetin (0.41 μM) or picloram (4.14 μM) plus kinetin (0.41 μM), respectively. Only the cell suspension cultures from callus with picloram plus kinetin grew appropriately and the maximum dry biomass accumulation (14 g L^{-1}) occurred at 12 days of culture. The leaves from wild plants yielded larger amounts of total phenols (155.17 mg gallic acid equivalents g^{-1} dry weight) and total flavonoids (124.3 mg quercetin equivalents g^{-1} dry weight) compared to cell suspension cultures, which exhibited total phenols amounts of 73.98 mg gallic acid equivalents g^{-1} dry weight and total flavonoids of 16.29 mg quercetin equivalents g^{-1} dry weight. The fungicide Cercobin® inhibited 100% the *S. cepivorum* mycelial growth, while 80.0 and 73.0% inhibition was achieved with the ethyl acetate fraction of the dichloromethane extracts from *E. polystachya* sapwood and heartwood, respectively. However, cell suspension cultures showed only 39% inhibition of *S. cepivorum* mycelial growth with methanol extracts. The maximum percentage inhibition of *R. solani* (66.0%) occurred using the hexane fraction of methanolic extracts from cell suspension cultures; however, Cercobin® presented only 33.0% growth inhibition. Extracts of *E. polystachya* cell suspension cultures can be used as a biotechnological option against phytopathogenic fungi.

1. Introduction

For centuries, plants have been widely used to treat microbial diseases due to their extensive antimicrobial properties (Hemalatha et al. 2013). Fungi, for example, can result in enormous economic losses in agriculture, a reduction of food for consumption, and serious, often fatal, diseases in humans and animals (De Lucca 2007). In this regard, plants may be a source of antifungal compounds since they have had to develop compounds to resist infections by fungi present in their environment (De Lucca 2007). Interest in using plant extracts as a potential natural alternative to synthetic fungicides has increased. Extracts are largely used to combat phytopathogenic fungi such as *Rhizoctonia solani* and *Sclerotium cepivorum*, which are soil-borne pathogens with a broad host range that cause significant losses in crop quantity and quality (Naiki 1985; Martin 1988; Amin et al. 2014). *R. solani* is capable of attacking a tremendous range of crop plants, including Solanaceae, Fabaceae, Asteraceae, Poaceae, and Braccicaceae as well as ornamental plants and forest trees (Ogoshi 1996; Lewis and Lumsden 2001; Anees et al. 2010). Furthermore, this fungus can cause seed decay, stem canker aerial leaf blight storage rot, and seedling damping-off in crops including carrots, rice, soybeans, tomatoes, potatoes, and cowpeas (Lahkim et al. 2000; Harikrishnan and Yang 2002). On the other hand, *Sclerotium cepivorum* is the predominant disease of onion crops worldwide and is found in practically all regions where species of *Allium*—including onions, garlics, leeks, shallots, and chives—are grown. It attacks leaves, roots, and bulbs (Schwartz and Krisna 1995; Metcalf and Wilson 1999; Dilbo et al. 2015). Some studies have reported fungicidal and fungistatic activity of some plant extracts against phytopathogenic fungi (Dellavalle et al. 1999; Masoko et al. 2007; Bahraminejad et al. 2015). In this regard, *Eysenhardtia polystachya* (Fabaceae), commonly known in Mexico as "Palo Dulce," is an important wild shrub or small tree

* Corresponding author.
E-mail address: bernabe_aa@hotmail.com (A. Bernabé-Antonio).

http://dx.doi.org/10.1016/j.sajb.2017.05.023
0254-6299/

used for its wood in traditional chairs "equipales" and furniture and in Mexican folk medicine. In folk tradition, it is used to fight digestive system diseases and inflammation in animals. For humans, infusions obtained from the wood are traditionally used against kidney, liver, and gallbladder diseases (Rzedowsky and Equihua 1987; Villavicencio et al. 2002; Pérez et al. 2005). In addition, *E. polystachya* has been reported to possess antispasmodic, antipyretic, healing, and antidiuretic properties and be effective against some eye diseases (Martínez 1996). Despite the importance of the *Eysenhardtia* genus, few studies have been conducted to determine the structure of the secondary metabolites responsible for its bioactive properties. For instance, the *C*-glucosyl-α-hydroxydihydrochalcones: coatline A and B were identified and isolated from *E. polystachya* (Beltrami et al. 1982). Additionally, the isoflavone (7-hydroxy-2′,4′,5′-trimethoxyisoflavone) was isolated from the heartwood and the coumestan (9-methoxy-2,3-methylenedioxycoumestan) was isolated from the bark stem as the major components of methanol (ME) extracts of *E. polystachya* (Burns et al. 1984). Other studies have been performed to investigate its traditional uses, and different biological activities of *E. polystachya* have been found. For instance, two cytotoxic isoflavans ((3*S*)-7-hydroxy-2′,3′,4′,5′,8-pentamethoxyisoflavan and (3*S*) -3′,7-dihydroxy-2′,4′,5′,8-tetramethoxyisoflavan) were isolated from the bark and trunk; all compounds exhibited moderate cytotoxicity (ED_{50} 3.8 and 3.0 μg mL^{-1}, respectively) against KB nasopharyngeal tumor lines (Álvarez et al. 1998). One α-hydroxdihydrochalcone (α*R*)-3′-*O*-D-xylopyranosyl-α-3,4,2′,4′-pentahydroxydihydrochalcone had insecticidal activity at a lethal concentration of $LC_{100} < 100$ ng cm^2 (Álvarez and Delgado 1999). Some studies have reported the presence of isoflavones highly effective against urolithiasis (Pérez et al. 1998, 2000, 2002). The most recent study of *E. polystachya* acaricidal activity of woody branch aqueous extracts was reported by Alcalá et al. (2015). To the best of our knowledge, however, studies in the literature have been conducted directly using wild plant extracts. Nowadays, due to overexploitation, deforestation, and pollution, it is often not convenient to obtain extracts or secondary metabolites directly from wild plants. Therefore, through plant biotechnology, it is possible to sustainably produce extracts bioactive using cell suspension culture systems (Mulabagal and Hsin-Sheng 2004). Nonetheless, no reports have been published about deriving natural fungicides from plant cell cultures of *E. polystachya*. The aims of this study were to establish a protocol for callus induction, establish a cell suspension culture system from *E. polystachya*, determine the total phenol content and total flavonoid content, and evaluate the fungistatic activity of the wild plant and cell culture extracts against phytopathogenic fungi.

2. Material and methods

2.1. Plant material

To obtain plant material for callus induction experiments, 6-month-old plants (Fig. 1A) were provided by the Vivero Forestal del Valle de Ameca S.A. nursery in the State of Jalisco, Mexico. At the same time, the leaf, heartwood, and sapwood from wild plants used to obtain extracts were collected in Las Trojes, Jocotepec, State of Jalisco, in July 2014.

2.2. Callus induction and incubation conditions

The leaves from the nursery plants were disinfected with a soap solution for 5 min and then with a sodium hypochlorite solution (0.6% v/v) under constant stirring for 15 min. The disinfectant solution was complemented with three drops of Tween 20 per 100 mL as a wetting agent. Finally, the leaf explants were washed with sterile distilled water and used for the callus induction experiments. The basal culture medium consisted of semisolid MS (Murashige and Skoog 1962) and 3% (w/v) sucrose. Different concentrations (0.0, 0.41, 4.14, and 8.28 μM) of plant growth regulators (PGRs) such as 1-naphthaleneacetic (NAA) or picloram (PIC) were combined with kinetin (KIN) to induce callus formation on the leaf explants. The pH was adjusted to 5.8, and the culture medium was gelled with 0.2% (w/v) Phytagel (Sigma-Aldrich Co., USA). Autoclave sterilization was carried out at 121 °C and 124.106×10^3 Pa for 18 min. Four disinfected leaf segments (5 mm in length) were transferred to Gerber flasks containing 25 mL MS semisolid medium that had been previously sterilized (Fig. 1B). Each treatment (including the control, which was PGRs-free) consisted of four

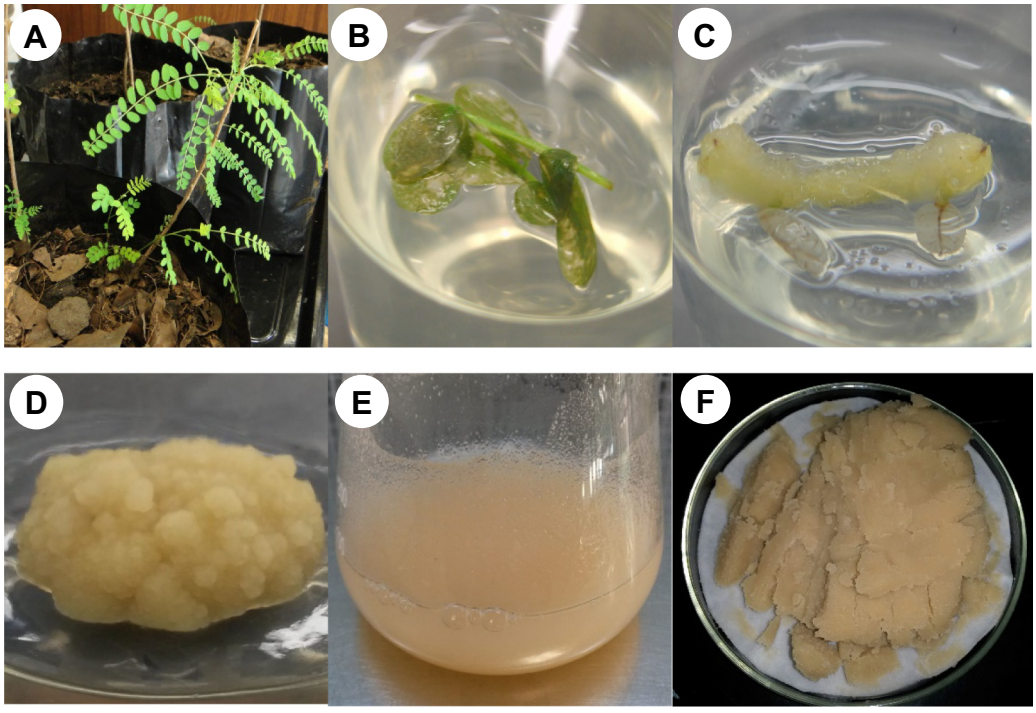

Fig. 1. Morphogenetic response using PIC (4.14 μM) and KIN (0.41 μM) on leaf explants from *Eysenhardtia polystachya*: (A) plant 6-month-old; (B) leaf explant without PGRs; (C) rachis leaf inducing callus at 10 days of culture; (D) callus grown at 40 days of culture; (E) cell suspension cultures at 15 days of culture; (F) biomass obtained from cell suspension cultures.