ELSEVIER

Fitoterapia 78 (2007) 345–352

FITOTERAPIA

www.elsevier.com/locate/fitote

Induction of flavonoid production by UV-B radiation in *Passiflora quadrangularis* callus cultures

Fabiana Antognoni [a,*], Suiping Zheng [a,1], Cristina Pagnucco [a], Rita Baraldi [b], Ferruccio Poli [a], Stefania Biondi [a]

[a] *Department of Biology, University of Bologna, via Irnerio 42, 40126, Bologna, Italy*
[b] *IBIMET, Consiglio Nazionale delle Ricerche, via Gobetti 101, 40129 Bologna, Italy*

Received 7 February 2006; accepted 1 February 2007
Available online 11 April 2007

Abstract

Callus cultures from several species of *Passiflora* were initiated in vitro, and their capacity to produce four glycosyl flavonoids (orientin, isoorientin, vitexin, isovitexin) was analysed. The aim of the present work was to examine the possible role of UV-B irradiation and elicitation with methyl jasmonate (MJ) on the production of these compounds in callus cultures. All the species tested (*P. incarnata*, *P. quadrangularis*, *P. edulis*) formed friable callus from leaf explants after 4 weeks on medium supplemented with kinetin and 2,4-dichlorophenoxyacetic acid. Among them, *P. quadrangularis* turned out to have a faster growth rate and a more friable texture, and was therefore chosen for experiments with elicitors. In callus cultures only small amounts of isoorientin were found, while the concentration of the other flavonoids was below the detection limit. UV-B irradiation of calluses was able to increase the production of all four glycosyl flavonoids. After a 7-day exposure of cultures to UV-B light, the production of isoorientin reached concentrations similar to those found in fresh leaves from glasshouse-grown plants. Elicitation with methyl jasmonate also enhanced orientin, vitexin and isovitexin concentrations, even though the stimulation was about 6-fold weaker for orientin and vitexin and about 40-fold for isovitexin, than that exerted by UV-B treatment. Callus cultures treated with the UV-B dose which most enhanced flavonoid production showed a higher antioxidant activity compared to untreated calluses, with an increase ranging from 28% to 76%. Results show that the secondary metabolite biosynthetic capacity of *Passiflora* tissue cultures can be enhanced by appropriate forms of elicitation.

Keywords: *Passiflora quadrangularis*; Elicitation; Methyl jasmonate; Flavonoids; Cultures in vitro

1. Introduction

The genus *Passiflora* is indigenous to the tropical and semi-tropical zones of North, Central and South America. Although more than 480 species have been identified [1], 60 of which are edible and many of medicinal value, only *P. incarnata* and *P. edulis* have been extensively investigated for their chemical composition and biological activities. In particular, *P. incarnata* has long been known for its sedative activity, and other neuropharmacological properties [2].

* Corresponding author. Tel.: +39 051 2091294; fax: +39 051 242576.
E-mail address: fabiana.antognoni@unibo.it (F. Antognoni).
[1] Permanent address: Department of Bioengineering, South China University of Technology, Guangzhou, Guangdong, PR China.

0367-326X/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.fitote.2007.02.001

Phytochemical investigations have shown that C-glycosyl flavones of the apigenin and luteolin type, schaftoside, isoschaftoside, isoorientin, orientin, isovitexin and vitexin, are the main constituents [3,4].

The flavonoids have many functions including protection against UV-B radiation, defence against pathogen attack, as attractants to pollinators, and signal compounds in symbiotic relationships [5]. Due to their high antioxidant activity, they are thought to have health-promoting properties for humans, such as protection against cardiovascular disease, cancer, and age-related disorders [6–8].

The production of active constituents in plant cell and tissue cultures [9] has long attracted the attention of scientists due to the potential that such a biotechnological approach has in terms of year-long large-scale production under tightly controlled conditions. Unfortunately, the yield of the desired end-product is often too low to make this a viable alternative to extraction from field-grown plants. Consequently, many studies have been undertaken to unravel biosynthetic pathways and understand their regulation or, more simply, to find practical approaches to increasing yield. These include selection of high-producing cell lines, optimization of culture conditions and bioreactor design, and elicitation [10]. The latter generally involves exposure to biotic stress factors, such as pathogen culture filtrates, to environmental stress (light, heat/cold, heavy metals, etc.), or to compounds that are regarded as signalling molecules in plant stress responses, namely jasmonates [11]. Elicitation induces defence-related genes and proteins, as well as low molecular weight secondary metabolites. A broad range of secondary compounds have been elicited in cell cultures of several species by application of methyl jasmonate (MJ) [12,13], and strong enhancement of anthocyanin production was obtained by elicitation with MJ or jasmonic acid in cell cultures of *Vaccinium pahalae* [14] and *Vitis vinifera* [15].

Tissue cultures of *Passiflora* spp. have been mainly produced in order to obtain rapid clonal multiplication of superior cultivars or rootstock material in the context of breeding programmes and germplasm conservation [16]. To our knowledge, studies aimed at investigating the secondary metabolite biosynthetic capacity of *Passiflora* tissue cultures have not been carried out.

In this study, we report the feasibility of establishing tissue (callus) cultures from several *Passiflora* species. The production of four flavonoids (isoorientin, orientin, isovitexin, vitexin) by *P. quadrangularis* calluses, and the effect of elicitation by MJ and/or UV-B radiation are described.

2. Experimental

2.1. Plants

Passiflora edulis, *P. quadrangularis* and *P. incarnata* (Passifloraceae) leaves collected at a pre-flowering stage from plants grown in a glasshouse of the Botanical Garden of the University of Bologna, were authenticated by Dr. U. Mossetti (SMA, Univ. of Bologna); voucher specimens of each species (No. 5,000,001; 5,000,002; 5,000,003, respectively) are kept at the Herbarium of the University of Bologna (BOLO).

2.2. Tissue culture conditions

Leaves were surface sterilised by a brief immersion in 70% EtOH followed by 10 min in a commercial bleach solution (5% active chlorine) containing a few drops of Tween 80, and then rinsed three times with sterile distilled water.

Leaf explants (60 explants for each species, size approx. 0.5×1 cm) were excised either from the lamina or from the midrib. These were placed in 9-cm Petri dishes containing 25 ml MS basal medium [17] supplemented with 3% sucrose and solidified with 2.5% Phytagel (Sigma-Aldrich s.r.l, Milano, Italy). Various combinations of 2,4-dichlorophenoxyacetic acid (2,4-D, 0.25 to 3.0 mg/l), kinetin (0 to 2.0 mg/l) and benzylaminopurine (BAP, 0 to 2.0 mg/l) in the culture medium were tested (Sigma-Aldrich, Milano). Cultures were kept in the light (50 μmol m^{-2} s^{-1}) with a 16/8 h light/dark photoperiod at 22 ± 1 °C. Callus formation was monitored over a 1-month period and subsequent growth (in terms of fresh weight) was followed until the stationary phase was reached. Once established, calluses were sub-cultured at monthly intervals on the medium which gave the best growth rate, and under the same conditions described above.

2.3. Exposure to UV-B radiation

The lids of the plastic Petri dishes were removed and replaced with plastic film (Reynolds 910, Richmond, VA, USA) which is 85% transparent to UV-B radiation ≥ 300 nm. The dishes were transferred to a growth chamber where