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Xanthone biosynthesis in *Hypericum perforatum* cells provides antioxidant and antimicrobial protection upon biotic stress

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ABSTRACT

Xanthone production in *Hypericum perforatum* (HP) suspension cultures in response to elicitation by *Agrobacterium tumefaciens* co-cultivation has been studied. RNA blot analyses of HP cells co-cultivated with *A. tumefaciens* have shown a rapid up-regulation of genes encoding important enzymes of the general phenylpropanoid pathway (PAL, phenylalanine ammonia lyase and 4CL, 4-coumarate:CoA ligase) and xanthone biosynthesis (BPS, benzophenone synthase). Analyses of HPLC chromatograms of methanolic extracts of control and elicited cells (HP cells that were co-cultivated for 24 h with *A. tumefaciens*) have revealed a 12-fold increase in total xanthone concentration and also the emergence of many xanthones after elicitation. Methanolic extract of elicited cells exhibited significantly higher antioxidant and antimicrobial competence than the equivalent extract of control HP cells indicating that these properties have been significantly increased in HP cells after elicitation. Four major *de novo* synthesized xanthones have been identified as 1,3,6,7-tetrahydroxy-8-prenyl xanthone, 1,3,6,7-tetrahydroxy-2-prenyl xanthone, 1,3,7-trihydroxy-6-methoxy-8-prenyl xanthone and paxanthone. Antioxidant and antimicrobial characterization of these *de novo* xanthones have revealed that xanthones play dual function in plant cells during biotic stress: (1) as antioxidants to protect the cells from oxidative damage and (2) as phytoalexins to impair the pathogen growth.

1. Introduction

Hypericum, a genus of the family Clusiaceae, is widely used in traditional medicine throughout the world since ancient times. The genus is known to produce several xanthones (Dias et al., 2000, 2001; Dias, 2003; Ferrari et al., 2005; Tanaka and Takaishi, 2006).

Xanthones are a class of polyphenolics that exhibit well-documented pharmacological properties, mainly due to their oxygenated heterocyclic nature and diversity of functional groups. They have been described as strong scavengers of free radicals (Jiang et al., 2004). In addition, many of the xanthones have been reported to be active against bacteria including methicillin/multidrug resistant *Staphylococcus aureus* (Rukachaisirikul et al., 2003, 2005; Sukpondma et al., 2005; Xiao et al., 2008), vancomycin resistant *Enterococci* (Sakagami et al., 2005), *Mycobacterium tuberculosis* (Suksamrarn et al., 2003), etc. Some xanthones even surpass the antimicrobial activity of traditional antibiotics (Iinuma et al., 1996; Xiao et al., 2008). Other pharmacological properties of xanthones include anti-inflammatory (Banerjee et al., 2000), cancer-chemopreventive (Ito et al., 2003), hepatoprotective (Tian et al., 2005), cardiovascular protective (Jiang et al., 2004), selective inhibition of cyclooxygenase-2 (Zou et al., 2005), inhibition of platelet-activating factor (PAF)-induced hypotension (Ishiguro et al., 2002; Oku et al., 2005) and cytotoxic activities (Yimdjo et al., 2004; Boonsri et al., 2006; Suksamrarn et al., 2006).

Plant cells respond to pathogens predominantly by mobilizing their secondary metabolites, which tend to protect the plant cells from pathogen attack. In this context, Beerhues and Berger (1995) observed an increase in xanthone accumulation in suspended cells of *Centaurium* species upon elicitation with yeast extract and methyl jasmonate (MeJ). Conceição et al. (2006) observed a similar response (xanthone accumulation) in *Hypericum perforatum* suspended cells elicited with *Colletotrichum gloeosporioides* cell wall extracts. The induced xanthone accumulation was further increased (by at least 12-fold) when the HP cells were primed with either MeJ or salicylic acid (well known defense signaling compounds in plants), an important observation, which explains possible role of xanthones in plant cells under biotic stress.

Here, we show that the antioxidant and antimicrobial potentials have been significantly increased in HP cells after elicitation with *Agrobacterium tumefaciens* due to the rapid up-regulation of xanthone metabolism. Putative roles of xanthone metabolism in HP cells under conditions of biotic stress are discussed.

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2. Results and discussion

2.1. Induction of xanthone biosynthesis is a defense response

2.1.1. Xanthone profile of HP cells was altered after co-cultivation with *A. tumefaciens*

Major classes of phenolic compounds produced by cultured HP cells under normal conditions are flavonoids and xanthones (Fig. 1a, F, flavonoid and X, xanthone). Though the flavonoid profile remained unaltered after elicitation, xanthone profile has significantly changed within 24 h (Fig. 1b). With new xanthones, the total xanthone content increased 12-fold (Fig. 2) indicating that xanthone biosynthesis has potential role in the biotic interaction. Similar patterns of xanthone accumulation has also been observed in HP cells that were elicited with fungal extracts or primed with plant defense signaling compounds such as MeJ and salicylic acid (Conceição et al., 2006). Likewise, xanthones were seen accumulated when cell cultures were treated with MeJ and yeast-extract in *Centaurium* species (Beerhues and Berger, 1995), *H. androsaemum* and *Centaurium erythraea* (Abd El-Mawla et al., 2001); the latter cell cultures exhibited new xanthones after MeJ treatment. All these observations support the participation of xanthones in plant defense response.

Although mangiferin (peak denoted as 'M') found along with few other unidentified xanthones in both control and treated cells, new xanthones appeared in the HP cells treated with *A. tumefaciens* (Figs. 1a and b). Amongst the up-regulated xanthones, four were identified (Fig. 1b, peaks 1–4, see Fig. 1c for structural formulae)

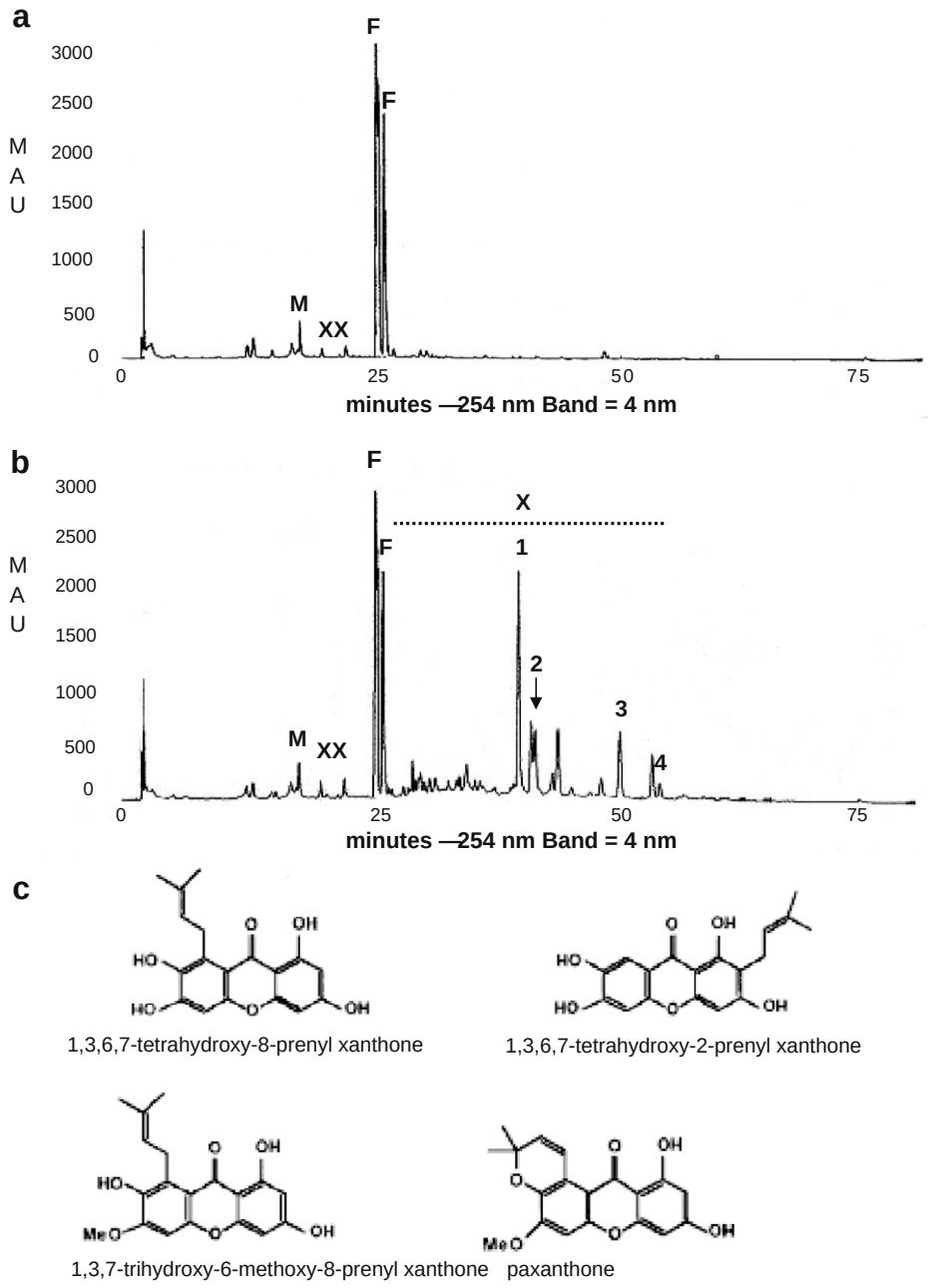

Fig. 1. Phenolic profile of *H. perforatum* cells before and after co-cultivation with *A. tumefaciens*. HPLC chromatogram showing the major phenolic compounds (F, flavonoids; X, xanthones and M, mangiferin) in control (a) and *H. perforatum* cells co-cultivated with *A. tumefaciens* for 24 h (b). Note the appearance of many new compounds after elicitation. Peaks 1, 2, 3 and 4 were identified as 1,3,6,7-tetrahydroxy-8-prenyl xanthone, 1,3,6,7-tetrahydroxy-2-prenyl xanthone, 1,3,7-trihydroxy-6-methoxy-8-prenyl xanthone and paxanthone, respectively. Structural formulae of these xanthones are shown in (c).