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Chitosan elicitation of *Isatis tinctoria* L. hairy root cultures for enhancing flavonoid productivity and gene expression and related antioxidant activity

Jiao Jiao[a,*,1], Qing-Yan Gai[a,1], Xin Wang[a], Qi-Ping Qin[a], Zi-Ying Wang[a], Jing Liu[a], Yu-Jie Fu[b,*]

[a] Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, Harbin 150040, PR China
[b] Beijing Advanced Innovation Center for Tree Breeding by Molecular Design, Beijing Forestry University, Beijing 100083, PR China

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ABSTRACT

Elicitation for phytochemical enhancement *via* cost-effective elicitors can overcome the limitation of commercial application faced by plant cell and organ culture technology. Chitosan is a natural, low-cost, and nontoxic elicitor that can trigger plant defense responses with the concomitant enhancement in phytochemical biosynthesis. In this work, the elicitation of *Isatis tinctoria* L. hairy root cultures by chitosan was conducted to enhance the production of pharmacologically active flavonoids. In comparison with control (2.31 ± 0.29 mg/g DW), a 7.08-fold enhancement of total flavonoids (16.35 ± 0.88 mg/g DW) was achieved in 24 day-old *I. tinctoria* hairy root cultures elicited by 150 mg/L chitosan for 36 h. Interestingly, the multiple hydroxyl-substituted flavonoids (rutin, quercetin, isorhamnetin, and isoliquiritigenin) were noticed to increase significantly in chitosan-elicited *I. tinctoria* hairy root cultures. Moreover, the transcription of associated genes involved in flavonoid biosynthesis pathway was significantly up-regulated underlying chitosan elicitation, among which *chalcone synthase* and *flavonoid 3′-hydroxylase* might play an important role in flavonoid enhancement. Additionally, extracts from chitosan-elicited *I. tinctoria* hairy root cultures exhibited higher antioxidant activities with lower IC_{50} values as compared with control. Overall, a cost-effective strategy *via* the simple chitosan elicitation is provided here to enhance the production of high-added value flavonoids in *I. tinctoria* hairy root cultures, which paves the way toward the successful commercialization of this *in vitro* culture system in the future.

1. Introduction

Isatis tinctoria L. is an economically important crop widely cultivated in the northern region of China (Ni et al., 2012). Its dried root named "Banlangen" in Traditional Chinese Medicine (TCM), is one of the top-selling herbs in East Asian, due to the notable clinical treatment efficacy of influenza, such as severe acute respiratory syndrome (SARS) and novel swine-origin influenza A (H1N1) (Xiao et al., 2015). *I. tinctoria* root is commonly processed into two commercial pharmaceutical preparations, namely "Banlangen Keli" and "Banlangen Tangjiang", which can be sold as over-the-counter (OTC) drugs at the pharmacy (Zhou and Zhang, 2013). Phenylpropanoids (flavonoids and lignans) are thought to be primarily related to the outstanding pharmacological activity of *I. tinctoria* (Nguyen et al., 2017). During the long-term evolution, plants can produce a huge number of secondary metabolites that play a crucial role in interaction with environmental factors and defense against unfavorable conditions (Ramirez-Estrada et al., 2016). Thus, the field cultivation of *I. tinctoria* is associated with an important challenge that the phytochemical profile is highly affected by geographical regions, climatic fluctuations, and soil conditions, which can significantly affect the therapeutic efficacy of *I. tinctoria* root (Chen et al., 2015). To overcome this issue, an *in vitro* culture system has been developed, *i.e. I. tinctoria* hairy root cultures (ITHRCs), as an alternative to the field cultivation for the sustainable and standard production of bioactive flavonoids (Gai et al., 2015).

Plant cell and organ cultures offer promising biotechnological platforms for the production of diverse phytochemicals of pharmaceutical and nutraceutical interest (Dias et al., 2016; Murthy et al., 2014). However, very few successful cases are commercially available due to the low phytochemical productivity not being sufficient to cover the culture cost (Davies and Deroles, 2014; Yue et al., 2016). In this context, research efforts need to be directed towards the enhancement of phytochemical productivity without significantly increasing production cost, which can make plant *in vitro* culture technology more

* Corresponding authors.
E-mail addresses: jj_nefu@163.com (J. Jiao), yujie_fu@163.com (Y.-J. Fu).
[1] These authors contributed equally to this work.

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