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Extended Abstract

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Establishment of in vitro regeneration protocol and development of hairy root culture in Aerva lanata with Agrobacterium rhizogenes

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The Aerva lanata belongs to the family Amaranthaceous and has the high medicinal values. The present study focused on in vitro regeneration of plant through tissue culture techniques and induction of hairy roots from stem internode explants by using various Agrobacterium rhizogenes strains such as A4, A4T and A4RS. Multiple shoots were induced from the stem explants on the MS medium containing BAP 0.5 mg/l and IAA 0.25 mg/l. Around 5.42 ±2.11 shoots were observed per explant with average shoot length of 2.19 ±1.2 cm. After 3-4 weeks of incubation the cultures were transferred to root induction medium containing 0.5 to 1.0 mg/l of IBA. Root initiation was occurred in 7-10 days on a half strength MS medium supplemented with 0.5 mg/l IBA. Healthy plants were transferred to greenhouse, were infected with bacterial strains A4, A4T and A4RS for hairy root formation from internodes. The explants infected with A4RS strain showed maximum hairy root emergence within 8-10 days whereas, A4 and A4T strains fail to influence hairy root emergence. Strain A4RS was proved to be more virulent than A4 and A4Twith highest transformation frequency of 83.33 %. Symbols: A4, A4T, A4RS - Agrobacterium rhizogenes strains; MS medium – Murashige and Skoog medium; BAP - 6-benzyl aminopurine; IAA/IBA – Indole acetic acid/ Indole butyric acid. Isatis tinctoria L. (woad), the biennial herb of Brassicaceae family, is a popular medicinal crop widely cultured in Europe and Asian countries. Its roots (Radix isatidis) known as Ban-Lan-Gen has been used in Traditional Chinese Medicine (TCM) for hundreds of years for the clinical treatment of pestilence, epidemic hepatitis and infections, especially for influenza such as severe acute respiratory syndrome (SARS) and H1N1. Alkaloids, phenylpropanoids and terpenoids are recognized as the principle active ingredients of Radix isatidis. Among them, alkaloids always attract much attention, and are validated to be responsible for various bioactivities. Nevertheless, phenylpropanoids mainly comprised of flavonoids (FL) have been identified as anti-inflammatory and antiviral constituents, and also are involved in the major drug actions of Radix isatidis. In this context, the interest and market demand of FL from Radix isatidis is increasing more and more. Due to the unreliability on harvest of phytochemicals from natural resources and the complexity in producing natural products through chemical synthesis, one has to look for an environment friendly and renewable production system to fulfill the need of food and pharmaceutical industries. Plant cell culture technology emerging as an attractive alternative system, can continuously provide high-value ingredients independent of geographical, climatic or environmental variations and constraints. Over the past years, plant cell suspension cultures for the production of secondary metabolites have been hampered by several limitations, such as low yields of desired compounds, expensive culturing process, application of phytohormones, heterogeneous cell types, lack of storage tissue, and products easily degraded by the enzymes released in the media. One way around this problem has been the development of specialized differentiated or plant organ cultures instead of cell suspension cultures, best exemplified by Agrobacterium rhizogenes-based hairy root cultures (HRCs). HRCs induced by the infection of wounded plant tissues with A. rhizogenes bearing the root-inducing (Ri) plasmid, possess comparable biosynthetic capacity of secondary metabolites to native plant roots with advantages of fast growth rates independent of phytohormones, genetic and biochemical stability, long-term preservation, and sizable biomass production. More importantly, HRCs often accumulate phytochemicals at a higher level as against undifferentiated cell suspension cultures. Herein, it is believed that the transformation of I. tinctoria by A. rhizogenes could result in hairy root lines with the potential to biosynthesize FL for research or food, agricultural and pharmaceutical applications. The present study demonstrated a protocol for the development of A. rhizogenes-mediated hairy root system in I. tinctoria to produce valuable FL. The high-productive I. tinctoria hairy root line (ITHRL) was initially screened followed by the molecular characterization. Afterwards, the culture conditions of I. tinctoria HRCs (ITHRCs) were optimized systematically for the efficient production of FL, which may provide valuable data for industrial scale-up applications in bioreactors. Subsequently, eight FL constituents from ITHRCs including rutin (RUT), neohesperidin (NEO), buddleoside (BUD), liquiritigenin (LIQ), quercetin (QUE), isorhamnetin (ISR), kaempferol (KAE) and isoliquiritigenin (ISL) were quali-quantitatively determined by LC-MS/MS. Moreover, considering that antioxidant activities of FL are of great interest in food, cosmetic and pharmaceutical fields, antioxidant capacities of ITHRCs extracts were also evaluated. Furthermore, the predominance of ITHRCs was eventually summarized as against I. tinctoria field grown roots (ITFGRs). In light of the presented results, ITHRCs may offer a promising and continuous product platform for naturally-derived, high quality and valuable nutraceuticals.

Bottom Note: *This work is partly presented at Joint Event on Biotechnology, Biochemistry and Aquaculture* August 08-09, 2019 | Paris, France