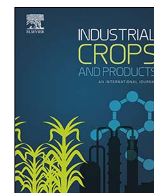
Industrial Crops & Products 108 (2017) 183–189

Contents lists available at ScienceDirect

Industrial Crops & Products

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



Changes induced by gamma ray irradiation on biomass production and secondary metabolites accumulation in *Hypericum triquetrifolium* Turra callus cultures

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A R T I C L E I N F O

Keywords:
Hypericum triquetrifolium
Callus culture
Elicitors
Gamma rays
Bioactive compounds

A B S T R A C T

The present study investigated the effect of gamma irradiation on biomass formation and yield of pharmacologically relevant secondary metabolites in callus cultures induced from different seedling parts (leaf, stem and root). Calli induction and maintenance were performed on MS medium supplemented with 0.5 mg L^{-1} IAA and 0.4 mg L^{-1} TDZ and all parameters were recorded after the third 21 days successive subculture post-elicitation. Fresh callus biomass (FCB) expressed as growth index (Gi) was investigated and the higher Gi value (1109%) was recorded on callus culture from leaf explants and irradiated with 10 Gy dose, as compared to untreated control (757%). *H. triquetrifolium* callus cultures exhibited quantitatively different phenolic compounds (*p*-OH-benzoic and chlorogenic acid, epicatechin) and naphtodiantrones (hypericin and pseudohypericin). The chromatographic analysis of callus extracts revealed that after gamma rays elicitation with 10 Gy dose, the higher amount of *p*-OH-benzoic acid was recorded on calli from leaf explant (4.35 mg 100 g^{-1} DW material) while root callus recorded the highest amount of chlorogenic acid (12.91 mg 100 g^{-1}) over the control (3.55 mg 100 g^{-1} and 10.22 mg 100 g^{-1}, respectively). The 10 Gy and 20 Gy irradiation doses stimulate the epicatechin accumulation on calli from leaf and stem (126.39 and 148.80 mg 100 g^{-1}) compared to the control samples (98.81 and 101.72 respectively).

In this study, small amount of hypericin and pseudohypericin were identified and quantified on irradiated callus cultures initiated from stem and leaf of *Hypericum triquetrifolium*, but not in root calli. Callus induced from leaf and irradiated with 10 Gy showed the higher amounts of hypericin and pseudohypericin content (0.29 and 4.00 mg 100 g^{-1} DW material). Phytochemical screening carried out on different elicited callus cultures variants reveal, for the first time, the stimulatory effects of gamma irradiation on the production of phenolic compounds and naphtodiantrones in *Hypericum triquetrifolium* Turra. The results of this study are interesting and offer an innovative approach of elicitation, not yet widely studied for the *Hypericum* genus.

1. Introduction

Statistics of World Health Organization shows that nowadays still 80% of world population depends on traditional medicine using medicinal plants and more than that, most of the anticarcinogenic and anti-infectious drugs are derived from plants (Peter et al., 2005). The genus *Hypericum* L. belongs to the family *Hypericaceae* (Crockett and Robson, 2011), including about 484 species of annuals, perennials, shrubs and infrequently trees, widespread in warm-temperate areas throughout the world (Guedes et al., 2012). Several species of this genus were used in diseases treatment in many countries and have an important place among useful medicinal herbs (Yamaner et al., 2013; Bertoli et al., 2015).

Hypericum triquetrifolium Turra is an herbaceous perennial plant and has been used in folk medicine for skin treatment and gastrointestinal diseases (Çirak et al., 2011). Recent studies highlight the medicinal

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http://dx.doi.org/10.1016/j.indcrop.2017.06.040
Received 3 February 2017; Received in revised form 20 May 2017; Accepted 16 June 2017
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potential of this species, mainly due to its anti-nociceptive (Apaydin et al., 1999), anti-inflammatory (Oztürk et al., 2002), antibacterial (Pistelli et al., 2005), antifungal (Fraternale et al., 2006), antitumor (Ferraz et al., 2005), antioxidant (Kizil et al., 2008), antidepressant (Rodriguez-Landa and Contreras, 2003; Tian et al., 2014) and cytotoxic (Rouis et al., 2013) properties.

Previous phytochemical studies on *H. triquetrifolium* Turra have led to the isolation and identification of several groups of phytochemicals with pharmaceutical value, including phenolic and flavonoid compounds (Toker, 2009 Çirak et al., 2011) and essential oils (Kizil et al., 2004; Hosni et al., 2011).

The quality and content of secondary metabolites in plants are grately influenced by environmental, ecological, genetic and physiological factors or in some cases contamination by pathogens and insect pests (Murch and Saxena, 2006). It has generally been accepted that biologically active substances from *Hypericum* sp. are synthesized and/or accumulate in different secretory structures, including translucent glands, dark glands and secretory canals located in different organs of the plants (Soelberg et al., 2007 Çirak et al., 2011). In the absence of secretory structures and in some experimental conditions such as *in vitro* culture, cells and callus cultures have had the capacity to accumulate the products with pharmaceutical value at the same level or higher than intact plants cultivated in field conditions (Kartnig et al., 1996; Pasqua et al., 2003; Charchoglyan et al., 2007; Gadzovska et al., 2015).

The increasing demand of plants as raw materials for different drugs may lead to loss of plant populations or genetic diversity, degradation of natural habitat or species disappearance (Dal Taso and Melandri, 2011).

In vitro culture of important medicinal plants became a reliable technique for high amounts of plant material production (Kolewe et al., 2008).

Production of secondary metabolites via plant cell cultures, yields various advantages, including the extensive manipulation of the biosynthesis of bioactive compounds using large quantities of vegetal material under sterile and controlled conditions (Docimo et al., 2015; Dias et al., 2016) and higher productivity without seasonal harvesting and potential cross-contamination compared to field grown plants (Murch and Saxena, 2006).

Among different culture systems, callus culture may be considered an important initial biotechnological step for large scale production of biomass and starting material for cell suspension cultures and shoots regeneration, being exploited for the accumulation of active compounds (Khalil et al., 2015).

However, the commercial value of secondary metabolites production of plant cell cultures was limited in some situations by low product yield, biosynthetic instability of cells and fluctuations in accumulation and storage capacity of secondary metabolites at the cellular level (Gadzovska Simic et al., 2015).

In order to overcome of these drawbacks and due to the growing economic value of *Hypericum* products, several biotechnological strategies have been applied to increase *in vitro* yield of bioactive secondary metabolites. The elicitation strategy represented an attractive biotechnological approach for enhancement of valuable compounds, mainly naphtodianthrones (hypericin and pseudohypericin), phloroglucinols (hyperforin and adhyperforin), flavonoids (hyperoside, rutin or quercitrin), xanthones and essential oils from *in vitro* cultures of *Hypericum* species (Danova et al., 2011; Coste et al., 2011; Gadzovska Simic et al., 2015; Gutiérrez Mañero et al., 2012; Wang et al., 2015).

Secondary metabolites are usually produced in plant cells as a response to protect plants from various environmental stresses generated by biotic (e.g., diseases and insects) and abiotic (e.g., temperature, humidity, UV light, radiation, heavy metal, and minerals) factors known as elicitors (Ramakrishna and Ravishankae, 2011). Elicitors are represented by molecules implied in defense system or stress-induced response in plant cells (Weathers et al., 2010).

Electromagnetic radiation (gamma rays, X-rays, visible light and ultra violet) are known to influence the plant growth and development by inducing morphological, structural and functional changes in cells and tissues (Hossam et al., 2011).

In the last decades, gamma ray irradiation drawn attention as a new and rapid method to enhance secondary metabolites concentration in plant cell cultures (Jaisi et al., 2013).

The irradiation of plant tissues with gamma rays stimulates the intracellular synthesis of active oxygen radicals and hydrogen peroxide, which are known to play an important role in plant defense response and secondary compounds accumulation. These radicals can modify or damage plant cells and affect certain important biochemical processes that might be vital for organism survival (Apostol et al., 1989). These modifications depend on the strength and duration of irradiation doses after exposure (Marcu et al., 2012).

To the best of our knowledge, the effect of gamma ray elicitation on callus cultures of *H. triquetrifolium* Turra has not been reported yet, even though the effect of gamma rays irradiation on callus cultures has been studied in other species.

For example, the low doses (16 Gy) of gamma irradiation significantly stimulated the production of shikonin derivatives in callus cultures of *Lithospermum erythrorhizon* but higher doses than 32 Gy did not enhance the shikonin derivatives (Chung et al., 2006).

Low doses of gamma irradiation (15 and 20 Gy) enhanced the total phenolic and flavonoid accumulation in rosemary (*Rosmarinus officinalis* L.) callus cultures (El-Beltagi et al., 2011).

Gamma radiation induced variation in growth characteristics and production of stevioside during callogenesis in *Stevia rebaudiana* (Khalil et al., 2015). Therefore, higher fresh callus biomass production was observed in cultures irradiated with 15 Gy dose, as compared to untreated cultures after 30 days. Similarly, 15 Gy dose slightly enhanced stevioside content over the control.

In the current work, the influence of gamma ray irradiation on biomass production and secondary metabolites accumulation in *Hypericum triquetrifolium* Turra callus cultures induced from different parts of the seedlings, after irradiation process, was studied.

2. Materials and methods

2.1. Plant material

Hypericum triquetrifolium seeds were collected during January 2012 from a well-growing plant in a wild population in Tasluja, Sulaimania, at the altitude of about 1000 m asl. Voucher specimen number (002614) of *H. triquetrifolium* is deposited in Herbarium of the Department of Biology, Sulamani University. The seeds were washed with 70% ethanol for 1 min, submerged in 1% NaOCl solution + 0.1% Tween-20 for 20 min, followed by rinsing 4 times in autoclaved distilled water and cultured in test tubes (5 cm × 9.5 cm) with 1/2 MS (Murashige and Skoog, 1962) medium solidified with 0.6% agar (Sigma-Aldrich Inc.). Seeds were maintained in a growth chamber at 25 °C under light and dark conditions (16/8 h) for germination.

In vitro germinated seedlings (21 days old) were cut into 10 mm long explants from different plant parts (leaf, stem and root) and transferred under aseptic conditions into culture jars. Calli induction and maintenance were performed on MS medium, solidified with 0.6% agar and supplemented with 5% sucrose, 0.5 mg L^{-1} IAA and 0.4 mg L^{-1} TDZ. The pH of culture medium was adjusted to 5.9 before autoclaving (20 min at 121 °C). The cultures were incubated at 26 ± 1 °C for 16 h photoperiod at light intensity of 3000 lx and relative humidity 70% (Vardapetyan et al., 2006).

2.2. Gamma ray irradiation and calli subculturing

Calli induced from different explant types of *H. triquetrifolium* T. were transferred on MS medium supplemented with 0.5 mg L^{-1} IAA

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