Fitoterapia 84 (2013) 72–79

Contents lists available at SciVerse ScienceDirect

Fitoterapia

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

Review

Possible ways of fagopyrin biosynthesis and production in buckwheat plants

Oksana Sytar [a,b,*], Marian Brestic [a], Mahendra Rai [a,c]

[a] Department of Plant Physiology, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia
[b] Department of Plant Physiology and Ecology, Taras Shevchenko National University of Kyiv, Volodymyrs'ka St. 64, 01601 Kyiv, Ukraine
[c] Department of Biotechnology, SGB Amravati University, Amravati, PIN-444602, Maharashtra, India

ARTICLE INFO

Article history:
Received 9 May 2012
Accepted in revised form 4 September 2012
Available online 24 October 2012

Keywords:
Fagopyrin
Hypericin
Type III polyketide synthases (PKSs)
Naphthodianthrone
Biosynthesis
Buckwheat

ABSTRACT

The present work extends knowledge about possible biosynthesis of fagopyrin in buckwheat plants by providing possible candidate genes for its biosynthesis and the role of type III polyketide synthases (PKSs). Moreover, new information is presented about the possible connection between naphthodianthrones and phenolic biosynthesis. Possible regulation of fagopyrin biosynthesis and production under different growth conditions is also discussed.

Contents

1. Introduction

Buckwheat (*Fagopyrum esculentum*) as a traditional pseudocereal crop (*Polygonaceae*) is widely used as food and medicinal plant. Particularly buckwheat has gained its fame due to its broad spectrum of flavonoids characterized by health benefits, i.e. cholesterol reduction [1], tumor inhibition [2], hypertension regulation [3], control of inflammation, carcinogenesis [4], and diabetes [5]. Buckwheat-based products such as noodles, pancakes, and buckwheat corn muffins are consumed in many countries most especially in China, Japan, Korea, Nepal, and European countries. Tartary buckwheat (*F. tartaricum*) due to its

Abbreviations: HYP, hypericin; FAG, fagopyrin; EMDA, emodinanthrone; PTK, protein tyrosine kinases; type III PKS, polyketide pathway; PKS, polyketide synthase; EMD, emodinanthrone; OKS, octaketide synthase; CHS, chalcone synthase.

* Corresponding author at: Department of Plant Physiology and Ecology, Taras Shevchenko National University of Kyiv, Volodymyrs'ka St. 64, 01601 Kyiv, Ukraine. Tel.: +380 675807714.
E-mail addresses: o_sytar@ukr.net, s-pi-r@hotmail.com (O. Sytar), marian.brestic@uniag.sk (M. Brestic), mkrai123@rediffmail.com (M. Rai).

0367-326X/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.fitote.2012.10.008

health-beneficial compounds is used in oriental medicine. The profiles of individual metabolites in tartary buckwheat differ from the profile in common buckwheat. Tartary buckwheat has more bitter components than rutin and the common buckwheat (*F. esculentum*) [6].

Tartary buckwheat *F. tartaricum* Gaertn. and common buckwheat *F. esculentum* Moench. have been recognized as healthful foods because they contain the antioxidant rutin and other secondary metabolites with antioxidant and anticancerogenic effects as fagopyrin [7].

Fagopyrin is a naphthodianthrone with anticancerogenic effect, which was isolated for the first time in 1943 from the blossoms of the red flowering variety of *F. esculentum*, a plant known since 1833. The chemical structure of fagopyrin (red, fluorescent pigment) has been deduced only in 1979 [8] (Fig. 1). The structure of fagopyrin is very similar to that of hypericin, which is present in St. John's wort (*Hypericum perforatum* L.). Naphthodianthrone derivatives present in *Hypericum* sp. (Hypericin (HYP)), including *H. perforatum* L. and *H. erectum* and in buckwheat plants (fagopyrin (FAG)) also have a photosensitizing effect [9]. A derivative of HYP, FAG is found in much smaller quantities in the genus *Fagopyrum*. Brockmann and co-workers also extracted and purified HYP via acid precipitation from *H. perforatum* and determined the structure of the pigment itself which has a molecular weight of 504.43 [10]. The aromatic structure of FAG is similar to that of HYP, differing only in the presence of two symmetrically placed 2-piperidinyl groups in FAG.

FAG is a substance which causes light sensitivity after the ingestion of large amounts of the green parts of buckwheat [11]. Quinones, like HYP and FAG, express a light dependent activity; they may be used in medicine as potential sensitizers in photodynamic therapy [12]. HYP is a popular medicine for the treatment of depression. It has been used as a conventional medication for the treatment of depression and wound healing for a long time [13]. Recently, more and more interest has been given to some other important pharmaceutical potentials of this species, such as antivirus activity, anti-HIV, antibacterial and antitumor activities [14,15]. Most of the clinical functions are proposed to be related to the phototoxicity of HYP. For instance, the cytotoxic activity can be greatly enhanced after light activation [13,16]. The mechanism as to how it works is still to be explored, however, it is proposed that after being exposed in visible light at the wavelength of 540–600 nm, HYP will transfer light energy to oxygen and generate reactive oxygen species (ROS), which may further induce the apoptosis of cells [17].

Samel and Witte in 1994 supposed that FAG is a potent inhibitor of protein tyrosine kinases (PTK) in a similar way as HYP. The inhibitors of PTKs suppress the proliferative diseases and therefore possess a strong anticancerogenic effect. FAG shows light-dependent inhibition of epidermal growth factor receptor PTK activity. The possible mechanism of FAG photodynamic action was investigated [18].

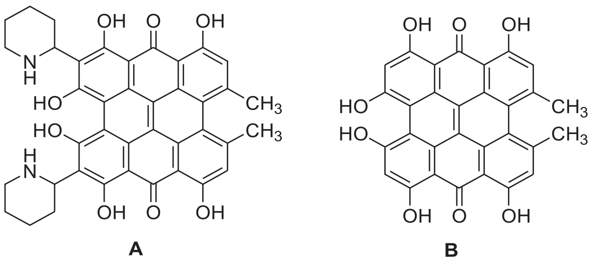

Fig. 1. Structure of fagopyrin (A) and hypericin (B) (Falk, 1999).

Nowadays, there are only a few reports about the accumulation site of FAG in buckwheat [19,20]. The highest amounts of FAG was found in inflorescences (0.08% FW) and smaller amount in leaves of common buckwheat (0.05% FW) [21] Ozbolt et al. (2008) found in leaves of common buckwheat (*Fagopyrum esculentum*) 44.5–63.6 mg/100 g d.m. of FAG, and in stems the content was 14.3–26.4 mg/100 g d.m. Leaves contained about two- to threefold the amount of FAG in comparison with stems [22].

The development of effective method determination of FAG was problematic as the buckwheat leaf extracts contain a considerable amount of chlorophyll [23]. FAG content can be determined with UV–vis spectrometer at 590 nm, i.e., the wavelength used for evaluating HYP concentrations [22,24] which makes the method not ideally suitable. Differential extraction of flavonoids and FAG from the green parts of buckwheat is possible by adjustment of the extraction conditions [24].

First in 2009 Eguschi et al. attempted to develop a HPLC screening method for determining the FAG content in buckwheat. Hinneburg and Neubert in 2005 in a study on development of extraction methods of FAG, revealed that raising the temperature increases FAG content when using ethanol in 70% or higher concentration. This can be attributed to the lipophilicity of FAG [24].

To obtain the buckwheat extract with antioxidants such as FAG, rutin, and chlorogenic acid, Hinneburg and Neuber, in 2005 investigated the influence of three parameters of extraction which include ethanol concentration, temperature, and extraction time. It provides evidence about good correlation between the antioxidant activity and the rutin content.

1.1. Possible regulation of naphthodianthrone biosynthesis and production under different growth conditions

Studying the impact of different factors (nutrition, stresses, light condition) with the aim to find alternative production methods, such as optimal conditions for plants in vivo growth or in vitro production of plant material and enzymatic in vitro synthesis of pure compounds, has attracted increasing attention.

FAG is not activated by UV-light but reacts to a different portion of the sunlight spectrum. No systematic influence of soaking the seeds in different solutions (sodium selenate and sodium selenite in different concentrations) or the level of UV-B radiation on FAG content in leaves or stems could be observed [22].

Briskin et al. (2000) indicated that naphthodianthrone production is modulated by nitrogen supply in a highly sensitive manner. A short-term low nitrogen stress in sand culture also resulted in increased production of HYP in *H. perforatum* leaves. While growth in a low nitrogen-containing condition resulted in elevated levels of HYP, its production was decreased by enriching of the growth condition with additional nitrogen. Moreover, altering the production of HYP in leaves occurred with changes in nitrogen supply [25]. Therefore it is possible to suggest that nitrogen starvation as different nitrogen supply can