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The inducible soybean glyceollin phytoalexins with multifunctional health-promoting properties

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ABSTRACT

The glyceollins are soybean-derived phytoalexins that accumulate in the seeds in response to various extrinsic factors. Several elicitors such as fungi, UV irradiation and physical injury have been demonstrated to alter the secondary metabolome of soybeans via induction of glyceollin synthesis and accumulation. The pterocarpan phytoalexins have exhibited antiestrogenic activity against estrogen receptor-mediated cellular processes. As a result, the glyceollins display anticancer activities against breast and ovarian cancers through inhibition of tumor growth and progression, and also by inducing cell cycle arrest in the cancer cells. Moreover, glyceollins were found to exhibit antimicrobial activity against pathogenic fungi, cellular antioxidant activities against endotoxin-induced oxidative stress, and regulatory effects on glucose metabolism and endotoxin-induced inflammatory response. The soybean-derived compounds warrant further studies to evaluate detailed molecular mechanisms during aberrant cellular processes, safety and efficacy in animal disease models and human subjects, and potential use for food preservation. The bioactivity of glyceollins can result in the enrichment of soybean with these phytoalexins for improved health functions and other novel applications.

1. Introduction

Secondary plant metabolism has provided structurally diverse groups of specialized chemical compounds with therapeutic properties, and several metabolites have also served as templates for the synthesis of drug candidates. Likewise, the secondary metabolome of plant-based food has attracted wide interest as a source of therapeutic agents that are often targeted against a variety of human health conditions such as cancer, hypertension, cardiovascular disease, diabetes, neurodegenerative and inflammatory diseases. Consequently, many phytochemicals are considered as the functional agents responsible for human health promotion associated with consumption of some whole foods. The plethora of published literature within the past two decades has clearly demonstrated the mechanisms and effectiveness of food-derived bioactive compounds in modulating aberrant physiological processes. Among several foods with established or potential health benefits, soybean (*Glycine max*) has been well-studied due to its constituent purportedly bioactive proteins and isoflavones, particularly daidzein and genistein (Dixon & Sumner, 2003; Sacks et al., 2006). The soybean isoflavones can also be transformed into their malonyl and gluco-malonyl derivatives, which have also displayed considerable bioactivity (Grün et al., 2001; Mathias, Ismail, Corvalan, & Hayes, 2006). Moreover, the reception of specific signals in the form of microbial challenge and physical stimulus by soybean tissues can result in the production of a group of compounds known as glyceollins, derivatives of 6α-hydroxypterocarpan (Banks & Dewick, 1983; Boué, Carter, Ehrlich, & Cleveland, 2000; Tilghman, Boué, & Burow, 2010). The glyceollins belong to a class of inducible phytochemicals known as phytoalexins and possess important biological properties relevant to human health promotion (Burow et al., 2001; Kim, Suh, Kim, Kang, et al., 2010; Kim, Suh, Kim, Park, et al., 2010; Kim, Suh, Lee, et al., 2010; Tilghman et al., 2010; Zimmermann et al., 2010). By definition, phytoalexins are low molecular weight compounds that are synthesized de novo and accumulated in plants following exposure to stressors (Paxton, 1980, 1981; Udenigwe & Aluko, 2012). Therefore, the process leading to the synthesis of glyceollins involves a reprogramming of metabolic activities in soybean as well as the expenditure of the plant's cellular energy (van Etten, Mansfield, Bailey, & Farmer, 1994).

Recent studies have demonstrated that the accumulation of phytoalexins in plant tissues can potentially enhance the overall health benefits of foods derived from the plants (Boué et al., 2008). Although glyceollins possess a massive potential for use in curative and preventive nutritional care, medicine and various food applications, the soybean phytoalexins are principally produced and intended by nature

Abbreviations: PAL, phenylalanine ammonia-lyase; C4H, cinnamic acid 4-hydroxylase; 4CL, 4-coumarate:coenzyme A ligase; CHI, chalcone isomerase; CHS, chalcone-synthase; CHR, chalcone reductase; G2-DT, glycinol 2-dimethylallyl transferase; G4-DT, glycinol 4-dimethylallyl transferase; IFR, isoflavone reductase; IFS, isoflavone synthase; IFOH, isoflavone 2′-hydroxylase; P6αH, pterocarpan 6α-hydroxylase; CDKN1A, cyclin-dependent kinase inhibitor 1A; ER, estrogen receptors; Nrf2, nuclear factor (erythroid-derived 2)-like 2; PPAR, peroxisome proliferator-activated receptor.

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as early defense molecules during plant–pathogen interactions in soybean (Ebel & Grisebach, 1988). The inducible defense system in soybean ensures the synthesis of glyceollins upon field infection or experimental microbial challenge against common fungal pathogens such as *Phytophthora megasperma* f. sp. *glycinea* (*Pmg*), the causative agent of soy root and stem rot (Ebel & Grisebach, 1988), as well as against food-grade fungi species such as *Aspergillus sojae*, *A. niger*, *A. oryzae* and *A. flavus* (Boué et al., 2000). Moreover, glyceollin production has also been observed in soybean cell suspension cultures treated with *Pmg* β-glucan elicitor (Ebel & Grisebach, 1988), and in symbiotic soy–microbe interactions such as the relationship of the plant with *Bradyrhizobium japonicum* (Schmidt, Parniske, & Werner, 1992).

2. Natural occurrence and structures of the glyceollins

Soybean is one of the most widely cultivated crops in the world and enjoys immense popularity for its use as food and feed including as meat and dairy substitutes. Moreover, soybean consumption has been credited with many health-promoting properties such as chemoprevention of osteoporosis, cardiovascular disease and certain postmenopausal disorders (Dixon & Sumner, 2003). To date, soybean is known to be a key source of the glyceollins in nature, in addition to a few other legumes belonging to the *Teramnus* species (Keen, Ingham, Hymowltz, Sims, & Midland, 1989). In addition to the glyceollin isomers, other important soybean isoflavonoids include genistein and daidzein. In fact, genistein has been the subject of extensive published literature in the past due to its purported health-related functions (see review by Dixon & Sumner, 2003). The soybean glyceollin predominantly exists in the form of three isomers namely glyceollins I, II and III (Fig. 1). The glyceollins possess basic pterocarpanoid skeletons that are linked to a cyclic ether ring originating from C_5 prenyl substitutions (Akashi, Sasaki, Aoki, Ayabe, & Yazaki, 2009). The pterocarpans are isoflavonoid derivatives generally found in the family Leguminosae and believed to be the phytoalexins with the most potent antifungal activity (Jiménez-González, Álvarez-Corral, Muñoz-Dorado, & Rodríguez-García, 2008). The pterocarpans are easily recognized by the presence of a tetracyclic system of fused benzofuran and chromene moieties containing two chiral centers in the 6α and 11α positions (Daniel & Purkayastha, 1995). Glycinol, the well-known nonprenylated 6α-hydroxylated daidzein derivative is a pterocarpanoid precursor of the glyceollins isomers (Boué et al., 2009). The three glyceollin isomers (I, II & III) each contain two chiral centers within their structure (Fig. 1) resulting in four possible enantiomers (RR, RS, SR, SS) for each of the compounds, with only the SS enantiomer configuration occurring naturally (Zimmermann et al., 2010). Apart from the three popular glyceollin isomers, other glyceollins have been recently reported to exist in challenged soybean, and these include glyceollin IV, glyceollin V and VI isomers, and glyceofuran (Simons et al., 2011). Moreover, similar phytoalexins with pterocarpan structure can also be found in other plant foods and these compounds include pisatin derived from stressed pea (*Pisum sativum*), phaseollin found in common beans (*Phaseolus vulgaris*), and medicarpin in alfalfa (*Medicago sativa*) (Udenigwe & Aluko, 2012).

The production of glyceollins in soybean tissues follows the exposure of the leguminous cotyledon to "elicitors", a term used to describe compounds that induce phytoalexin synthesis in plants (Ebel, 1986), although they are also known to stimulate the synthesis of other plant defense molecules (Roby, Toppan, & Esquerré-Tugayé, 1985; Showalter et al., 1985). Various biotic and abiotic elicitors that have demonstrated inducible effects on glyceollin synthesis include microorganisms (Boué et al., 2000), physical stimuli and stressors such as wounding, freezing

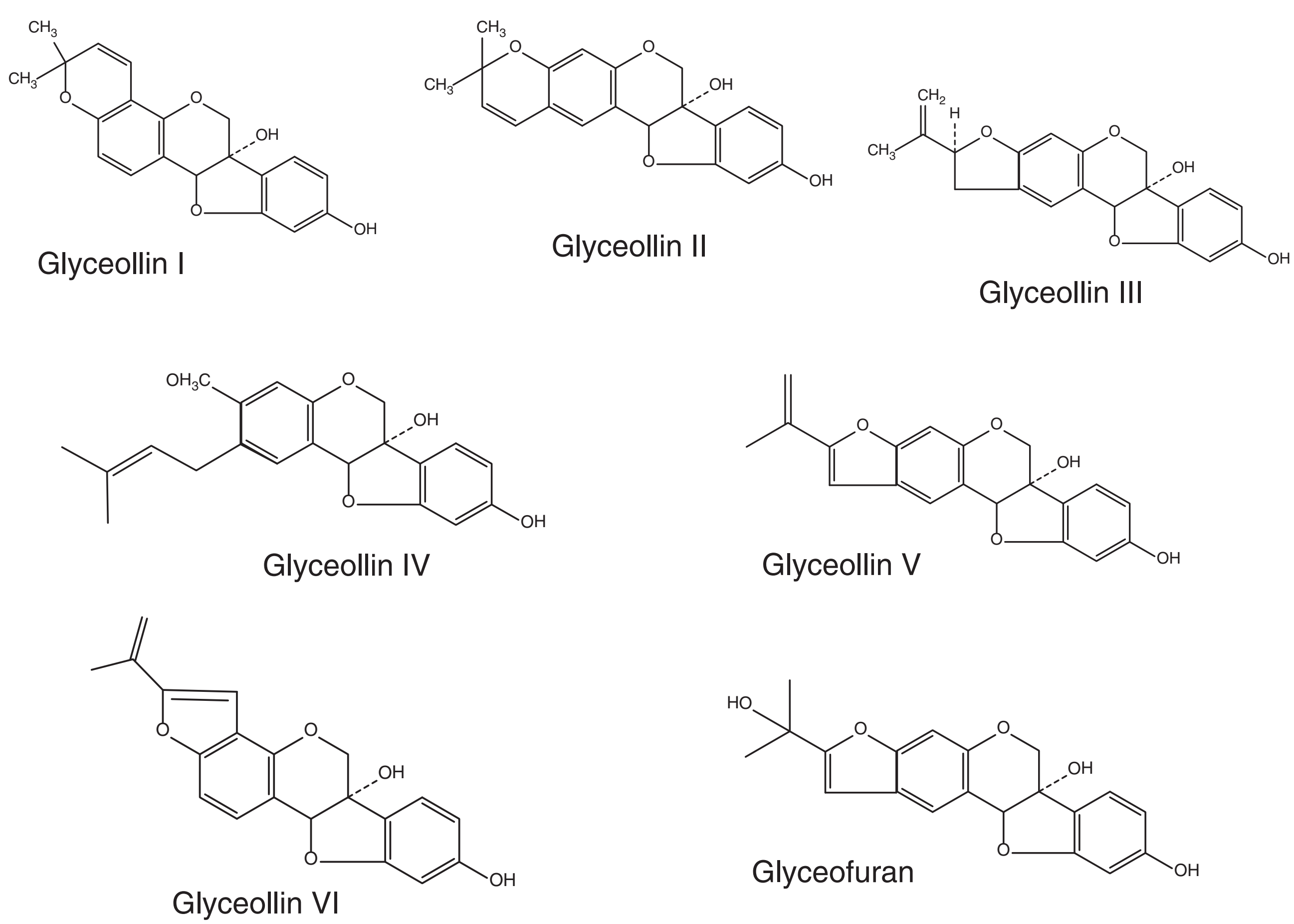

Fig. 1. Chemical structures of soybean phytoalexins; glyceollins I, II & III are the most common pterocarpan phytoalexin isomers found in the challenged soybean seeds.

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