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Polymorphisms of *IFS1* and *IFS2* gene are associated with isoflavone concentrations in soybean seeds

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ABSTRACT

Soybean isoflavones are associated with many health benefits of soy consumption, and isoflavone levels are one of the most important output traits of soybean. Isoflavones are synthesized from the phenylpropanoid pathway. Isoflavone synthase (IFS) acts as the key metabolic entry point for the formation of all kinds of isoflavones. We have cloned, sequenced, and analyzed the *IFS1* and *IFS2* genomic regions from 33 Chinese soybean accessions including 16 *Glycine soja* and 17 *Glycine max*. The isoflavone levels in these accessions vary greatly, ranging from 536.6 μg/g to 5509.1 μg/g dry seed weight. High nucleotide diversity and low extent of linkage disequilibrium (LD) in these two genes provided sufficient genetic resolution for association analysis of polymorphisms in these genes and soybean seed isoflavone levels. As a result, three single nucleotide polymorphisms (SNPs) in *IFS1* gene and two SNPs in *IFS2* gene were found closely associated ($P < 0.05$) with all individual and total isoflavone levels in seeds, regardless of population structure. These results indicated that *IFS1* and *IFS2* gene both contributed to the levels of isoflavones in seeds. These polymorphisms may serve as important molecular markers for breeding.

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1. Introduction

Isoflavones are phenolic secondary metabolites found mostly in legumes. Epidemiological studies comparing populations in Asia, where soy consumption is high, and Western countries with relative low soy consumption, suggested soybean food may contribute to multiple health benefits [1]. Additional research has demonstrated that isoflavones in soybean are beneficial for decreasing certain cancers, osteoporosis, cardiovascular disease, and menopausal symptoms in animal models and some human trials [2–5]. Although the health benefits of isoflavones are generally accepted, they are not without controversy [6]. Some concerns persist about isoflavones fed to infants with formula [7] and the safety levels of isoflavones for adults [8]. Thus, metabolic engineering of isoflavonoid biosynthesis to either increase or decrease isoflavone levels in soybean seeds may have significant nutritional impact by controlling dietary isoflavone levels for the improvement of human health [9].

In soybean seeds, the three types of isoflavones, daidzein (Dai), genistein (Gen), and glycitein (Gly) are predominately occurred as glucosides or malonyl-glucosides [10,11]. Total seed isoflavone concentrations are the sum of these aglycones and conjugates. Total isoflavones fluctuate by crop years and planting locations, indicating a large environmental effect [12–14]. Other studies have shown large variations in isoflavone concentrations and compositions among soybean genotypes as well [15]. Together, both genetic and environmental factors make breeding isoflavone levels very difficult in soybean. Several quantitative trait loci (QTL) for individual and total isoflavone concentrations in soybean seeds have recently been discovered, demonstrating the complexity of isoflavone traits [16,17]. Additionally, it is not clear whether or how these isoflavone variations are affected by some critical structural enzymes. Since sequence variations are heretically stable and can have a major impact on how the organism develops and responds to the environment [18], these variations in the genes of the key isoflavone synthesis enzymes could have major impact on seed isoflavone levels.

In plants, isoflavones biosynthesis is a part of the phenylpropanoid pathway (Fig. 1). Isoflavone synthase (IFS) is the first committed enzyme in the isoflavone pathway and converts flavanone substrates to isoflavone products. The unique aryl migration reaction to create isoflavones is mediated by this enzyme which belongs to the CYP93C subfamily of cytochrome P450 monooxygenase and the encoding gene of which has been identified [19–21]. When IFS genes were silenced in soybean, isoflavone biosynthesis was blocked, and no isoflavone accumula-

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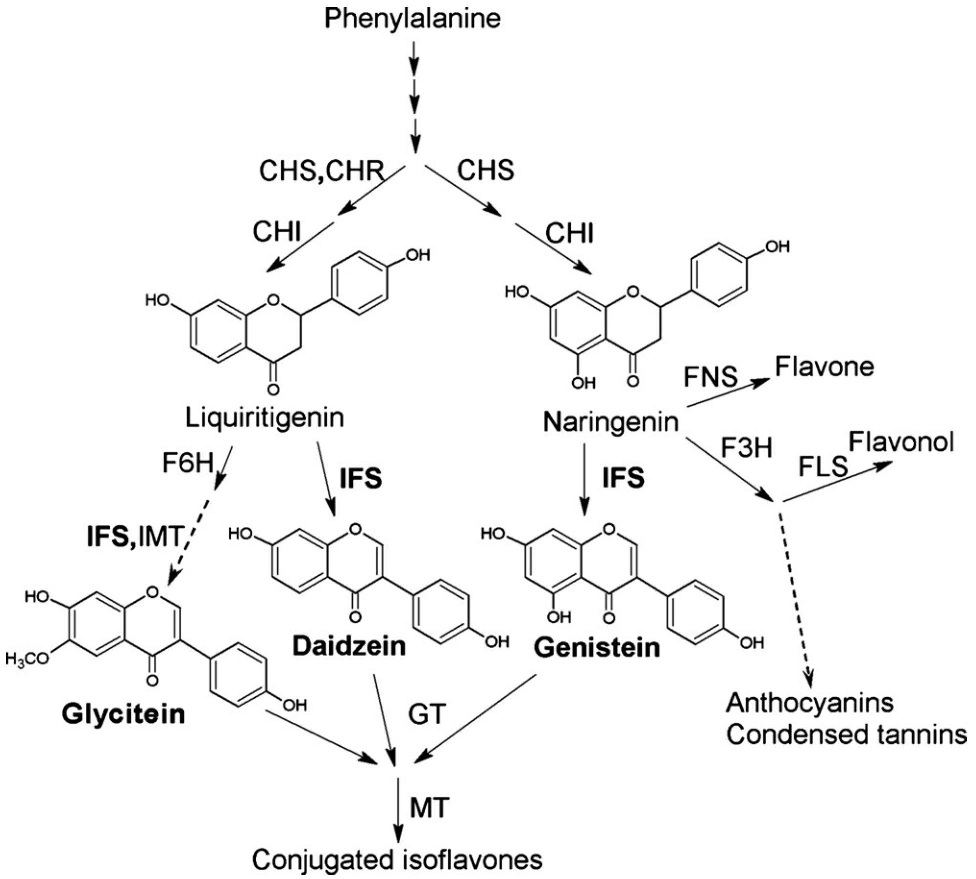

Fig. 1. Partial diagram of the isoflavonoid biosynthesis in soybean. CHI, chalcone isomerase; CHR, chalcone reductase; CHS, chalcone synthase; F3H, flavanone-3-hydroxylase; F6H, flavanone-6-hydroxylase; FLS, Flavonol synthase; FNS, Flavone synthase; GT, glucosyl-transferase; IFS, isoflavone synthase; IMT, isoflavone methyl-transferase; MT, malonyl-transferase. Dotted arrows represent multiple or uncertain steps.

tion was detected [22]. Clearly, IFS genes are essential for the biosynthesis and accumulation of isoflavones in plants. So far, two IFS genes, designated as *IFS1* and *IFS2*, have been reported in soybean [21]. Thus, in our study, we focused on the polymorphisms of both *IFS1* and *IFS2* genes to evaluate how these two genes affect the accumulation of soybean isoflavones.

Single nucleotide polymorphisms (SNPs), which include single base pair changes and small insertions/deletions (indels), can serve as molecular genetic markers. SNPs are abundant and relatively stable in the genome, and have been discovered within genes underlying observed traits [23]. To identify the causative SNPs that are associated with the soybean seed isoflavone levels in the *IFS1* and *IFS2* gene, we carried out a set of association analyses in this study. Association analysis, also known as association mapping, is a population-based survey used to identify trait-marker relationships based on linkage disequilibrium (LD) [24]. Association analysis has the potential to identify a single polymorphism within a gene that is responsible for the difference in phenotypes [24]. It was originally developed for mapping human disease genes [25,26] and has been used extensively in medical genetics [27]. The primary obstacle to successful association studies in plants is the nature of population structure [28]. The presence of subgroups with an unequal distribution of alleles within a population can result in non-functional, spurious associations [29]. To solve this problem, Pritchard et al. [30] developed methods that account for population structure by including a vector quantity for sub-population memberships derived from simple sequence repeat (SSR) in the association model. Thornsberry et al. [31] extended Pritchard's method for quantitative traits and applied it to evaluate maize flowering times. Since then, there have been increasing reports on association analysis in plants, such as in maize [32–34], *Arabidopsis* [35,36], and wheat [37]. These reports proved that association analysis could be a reliable method for finding causative SNPs.

In our analysis, we found many SNPs closely associated ($P < 0.05$) with either individual types of seed isoflavones or the total isoflavone concentrations at each of the two genes, regardless of population structures. More importantly, common sites, including three SNPs in *IFS1* gene and two SNPs in *IFS2* gene were found closely associated ($P < 0.05$) with all individual types and total seed isoflavone concentrations. These results indicated that both *IFS1* and *IFS2* gene played critical roles in the synthesis and accumulation of soybean isoflavones. And these SNPs will be valuable genetic markers for future breeding efforts in soybean.

2. Materials and methods

2.1. Plant materials

A collection of 33 Chinese soybean accessions consisting of 16 wild (*Glycine Soja*) and 17 cultivated (*Glycine Max*) accessions collected from latitude 19–49°N and longitude 106–131°E were included in the analysis (Table 1). This population of accessions was selected to sample not only all six ecological regions of soybean in China [38] but also soybeans with diverse seed isoflavone levels. Seeds of all accessions were obtained from Germplasm Storage of Chinese National Center for Soybean Improvement (Nanjing Agricultural University, Nanjing, China).

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