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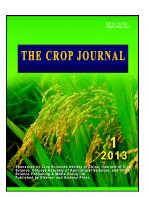
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Identification of novel QTL associated with soybean isoflavone content

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Abstract: Soybean isoflavones are essential secondary metabolites synthesized in the phenylpropanoid pathway and benefit human health. In the present study, high-resolution QTL mapping for isoflavone components was performed using specific-locus amplified fragment sequencing (SLAF-seq) with a recombinant inbred line (RIL) population (F_{5:7}) derived from a cross between two cultivated soybean varieties, Luheidou 2 (LHD2) and Nanhuizao (NHZ). Using a high-density genetic map comprising 3541 SLAF markers and the isoflavone contents of soybean seeds in the 200 lines in four environments, 24 stable QTL were identified for isoflavone components, explaining 4.2%–21.2% of phenotypic variation. Of these QTL, four novel stable QTL (*qG8*, *qMD19*, *qMG18*, and *qTIF19*) were identified for genistin, malonyldaidzin, malonylgenistin, and total isoflavones, respectively. Gene annotation revealed three genes involved in isoflavone biosynthesis (*Gm4CL*, *GmIFR*, and *GmCHR*) and 13 *MYB-like* genes within genomic regions corresponding to stable QTL intervals, suggesting candidate genes underlying these loci. Nine epistatic QTL were identified for isoflavone components, explaining 4.7–15.6% of phenotypic variation. These results will facilitate understanding the genetic bases of isoflavone accumulation in soybean seeds. The stable QTL and tightly linked SLAF markers may be used for marker-assisted selection in soybean breeding programs.

Keywords: Soybean (*Glycine max* L. Merrill); QTL mapping; Isoflavones; Specific-locus amplified fragment sequencing (SLAF-seq)

1 Introduction

Soybean (*Glycine max* L. Merrill) is one of the most important oilseed crops in the world. It provides the world's supply of vegetable protein and oil. Soybean also produces biologically active substances with potential benefit for human health, including isoflavones, soyasaponin, and lunasin [1–3].

Isoflavones belong to a group of secondary metabolites derived from the phenylpropanoid pathway, and are mainly produced in legumes. As precursors of major phytoalexin glyceollins, isoflavones play important roles in plant—microbe interaction [4, 5]. Isoflavones also function as signal molecules in soybean nodulation [6, 7]. Isoflavones have attracted increasing attention in recent years owing to their potential benefits for human health. As biologically active substances, isoflavones reduce the risk of menopausal symptoms, breast cancer, osteoporosis, dementia, and cardiovascular diseases [8–12]. In view of their important roles, studies of the biosynthesis and accumulation of isoflavones in soybean seeds have been performed [13–16]. The ultimate goal of these studies is to clarify the genetic basis of isoflavone accumulation and to develop soybean cultivars with desired isoflavone contents. Given that soybean isoflavone contents are typical quantitative traits influenced by both genetic and environmental factors, identification of stable QTL for isoflavone components across environments will facilitate

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