SOYBEAN (*GLYCINE MAX* L.) GERMPLASM SCREENING AND GEOGRAPHICAL DETERMINATION BASED ON TARGETED ISOFLAVONE METABOLOMICS

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Abstract. In this study, the combination of high-performance liquid chromatography (HPLC) based isoflavone profiling with multivariate analysis was used to screen specific soybean germplasms from Southwestern China, and simultaneously to investigate the influence of genotype, the geographical environment and the germplasm community size on isoflavone biosynthesis. A total of 144 soybean germplasms were evaluated for 12 isoflavones, which varied markedly due to genotypes and locations. Partial least squares analysis (PLS) results exhibited little variability among the 144 samples of four different locations. Five samples were separated from other genotypes based on the first PLS principal component that had high isoflavones. Daidzin, genistin, malonyldaidzin, malonylgenistin and acetylgenistin were the discriminating metabolites. Later the bidirectional orthogonal projection to latent structures-discriminant analysis (O2PLS-DA) was applied for geographical determination of five specific germplasms samples. Geographical environment influenced mainly acetylgenistin, acetyldaidzin, glycitein and malonylglycitin. Our results suggest that genetic factors play a vital role in the isoflavones biosynthesis of a major community, whereas the environmental factors can effectively regulate the isoflavones accumulation especially in the evaluation of small groups of soybean germplasms. These results demonstrate the use of the HPLC based metabolite profiling combined with multivariate analysis (PLS and O2PLS-DA) as a tool for the determination of specific germplasm, biomarker metabolite and geographical origin.
Keywords: *community size, environment, latitude, multivariate analysis, metabolite*

Introduction

Soybean (*Glycine max* L.) is a highly consumable legume crop on the basis of its nutritional value in the world. Soybean is regarded as a functional food with a variety of beneficial composition, especially in China. Soybeans contain several phytochemicals such as isoflavones, tocopherols, saponins and functional protein that promote and maintain human health (Sugano, 2005). Among all, isoflavones are presented in large amount and have gained more attention globally. Soybean isoflavones are an important secondary plant metabolite, which have variety of physiological activities. Having similar structure of endo-estrogen, isoflavones can exhibit weak estrogen-like activities so as to regulate the hormonal level (Wu et al., 2009). It is reported that isoflavones can reduce the occurrence of breast and prostate cancer, risk of cardiovascular diseases and relieve the osteoporosis and menopausal symptoms (Clubbs and Bomser, 2007; Howes et al.,

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2006; Wu et al., 2009). The major isoflavones can be divided into four groups: malonyl glycosides (malonyldaidzin, malonylgenistin, and malonylglycitin), aglycones (daidzein, genistein, and glycitein), acetyl glycosides (acetyldaidzin, acetylgenistin, and acetylglycitin), and glycosides (daidzin, genistin, and glycitin) (Kim et al., 2014). Each isoflavone has different physiological activities. For example, isoflavone aglycones have stronger biological activities and are absorbed faster, and in greater amounts by the body than corresponding glucosides (Izumi et al., 2000; Setchell et al., 2001). Moreover, daidzein and genistein exhibit high antioxidant activities (Devi et al., 2009; Heim et al., 2002), while genistein has high estrogenic potential, and daidzein has lower estrogenic effects (Kuiper et al., 1998).

Due to benefits described above, much effort has been spent to improve the nutritional quality of soybean by transgenic engineering approaches in recent years. For example, soybean genotype has been engineered to contain lower or higher levels of isoflavones (Lee et al., 2008; Paucar et al., 2010). However, phytochemicals are affected by genotype, weather and geographical sowing location, and they can be varied among soybean germplasms and within germplasm when planted in various geographical regions (Eldridge and Kwolek, 1983). Wang and Murphy observed that isoflavone contents varied across the year, and across growing locations within the same year for single soybean germplasms (Wang and Murphy, 1994). Similar studies confirm the results of previous research by studying multi cultivars over multi years and environment (Hoeck et al., 2000; Lee et al., 2003). However, the effects of germplasm community size on isoflavone biosynthesis are still need to be investigated.

China is regarded as the place of origin of soybean with the history of more than 5,000 years. China has built the largest repository where until 2000, 25,144 soybean germplasms have been collected. Southwestern China was identified as core origin, and most germplasms were provided by Yunnan, Sichuan (including Chongqing) and Guizhou provinces (Xinan et al., 1998). It shows the importance of soybean growth in this region, which provides abundant basic material for the selection and breeding of high quality soybean germplasms.

Although the soybean has been a major crop in Southwest of China, but few studies screened specific soybean germplasm, and investigated the correlation of isoflavone with genotype and geographical environment by metabolomics method previously. Therefore, it was significant to evaluate soybean germplasm from South-western China by using metabolomics method.

In the new era of metabolomics, metabolite profiling combined with chemometrics has become a useful tool for determining phenotypic variations, assessing food quality and identifying metabolic networks in biological systems that can lead to direct breeding strategies (Kim et al., 2014; Park et al., 2013). Thus, in the present study, the combination of HPLC based metabolic profiling with multivariate analysis (PLS and O2PLS-DA) was used to screen the specific isoflavone germplasms, and to investigate the influence of genotype, geographical environment and germplasm community size on isoflavone biosynthesis.

Materials and methods

Plant materials and chemicals

In this study, 144 soybean germplasms were collected from Southwest China in 2014. These germplasms include 102 from Sichuan province, 25 from Chongqing, 15

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from Guizhou province and 2 from Yunnan province (*Table S1* in the *Appendix*). Geographical locations and detailed information of these soybean germplasms are shown in *Figure 1* and *Table S1*, respectively. HPLC grade acetonitrile was provided by Thermo Fisher scientific inc. (NYSE: TMO). Six of the isoflavone standards (daidzein, gentstein, glycitein, dadzin, genistin and glyctin) were purchased from Weikeqi Biological Technology Co., Ltd. (China). Six external isoflavone standards (malonyldaidzin, malonylgenistin, malonylglycitin, acetyldaidzin, acetylgenistin and acetylglycitin) were purchased from Wako Pure Chemical Industries, Ltd. Japan.

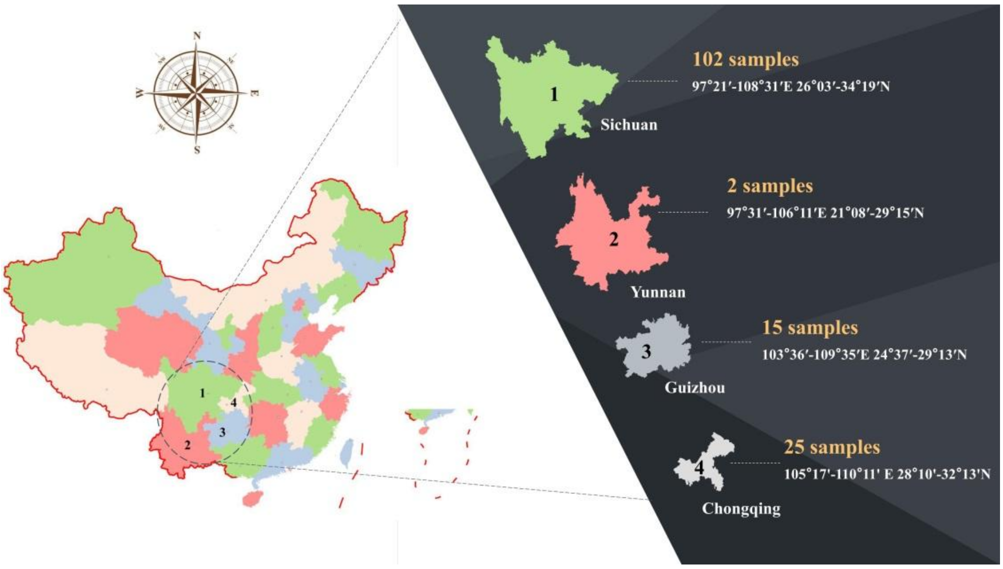

Figure 1. *Geographical locations of soybean germplasms from Southwest China*

Sample preparation

Soybean isoflavones were extracted using a protocol adapted from Liu et al. (2016) with small modifications. All soybeans samples were ground with cyclone miller (Cyclotec 1093 Foss, Denmark), screened through a mesh (size 250 µm) and heated at 40 °C for 24 h. Three extraction replications from soybeans were prepared for each sample. Approximately 200 mg of soya flour was dissolved into 5 mL of a pre chilled MeOH/H_2O (80/20 v: v). The mixture was extracted with ultrasonic for 3 h at 40 °C, and then centrifuged at 11,000 g for 10 min. About 1.5 mL supernatant was filtered through a 0.22 µm organic membrane filter before injecting in a sample bottle. The samples were stored at -20 °C before subjected to HPLC analysis.

Quantification

Agilent 1260 series high performance liquid chromatography (HPLC) system equipped with a mass spectrometric detector (Agilent Quadrupole LC/MS 6120) was used to identified and quantified twelve isoflavones. Three time quantitative HPLC analysis was performed on YMC-Pack ODS-AQ column (4.6 i.d. × 250 mm, RP-18, 5 µm). Previously described chromatographic conditions were used with small modifications (Liu et al., 2016). Linear gradient mobile phase was used, which includes 100% acetonitrile (solution A) and 0.1% acetic acid aqueous (solution B). The linear gradients were as follows: 0 min 15% A, 0~30 min 20% A， 30~60 min 40% A， 60~70

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min 40% A. The flow rate, column temperature and injection volume were 0.8 ml/min, 30 °C and 5 μL, respectively. The UV detector was set to 260 nm. Selected ion monitoring (SIM) mode was used in the mass spectral acquisition with scan range 100−700 amu. The following conditions were used for mass spectra: desolvation pressure, 35 psig; desolvation temperature, 350 °C; electrospray ionization (ESI), positive ion mode and capillary voltage, 3.8 kV. Nitrogen was used in the ion source and as the collision gas, and the desolvation gas flow rate was 10 L min^{-1}.

Each of the twelve isoflavones was identified by comparing the sample retention times with those of the isoflavone standards. However, the isoflavone concentrations were calculated in absolute term via linear regression.

Statistical analysis

Metabolomics analysis combined with chemometrics was used to investigate the quantification data acquired from HPLC. Partial least squares analysis (PLS) was performed to screen the specific soybean germplasms, and to evaluate the among-groups and within-groups variabilities of multivariate data. Score plot and loading plot were provided by the SIMCA-P 13.0 software (Umetrics, MKS Instruments Inc., Umea, Sweden), the former visualized the variation of all samples by observing the clustering or scattering of the point in score plot and the latter explained which variables contribute most to this difference. Specific soybean germplasms were subjected to O2PLS-DA to evaluate the relationships in terms of similarity or difference among them and predict the geographical origin. Furthermore, score-loading correspondence identified the biomarker metabolites which distinguished the samples from different regions.

Results and discussion

Variation of isoflavone in soybean germplasms

Twelve isoflavones including malonylglycosides (malonyldaidzin, malonylgenistin, and malonylglycitin), aglycones (daidzein, genistein, and glycitein), acetyl glycosides (acetyldaidzin, acetylgenistin, and acetylglycitin), and glycosides (daidzin, genistin, and glycitin) were detected in the 144 soybean germplasms (*Fig. 2*), and then analyzed by SPSS 20.0 to obtain the mean value ± standard error. The total isoflavone levels identified in 144 soybean germplasms are shown in *Table S2* (see *Appendix*). The total isoflavone contents varied significantly among all germplasms due to different genotypes and locations. As shown in *Table S2*, the total isoflavone contents of soybean germplasms varied from 0.612 $mg.g^{-1}$ to 7.635 $mg.g^{-1}$. Among the identified isoflavones, glycosides and malonylglycosides derivatives were observed predominant.

However, in Northern China the total isoflavone contents in cultivated and wild soybean germplasms ranged from 1.462-6.115 $mg.g^{-1}$ and 3.896-7.440 $mg.g^{-1}$, respectively (Cui et al., 2013). The reason for that could be the environmental conditions of Southwest China (Sichuan, Chongqing, Guizhou and Yunnan) are relatively cool and wet compared to Northern China. Therefore, high isoflavone accumulation was shown in this study, because earlier studies in warm and dry environments have shown low isoflavone accumulation (Lozovaya et al., 2005). A similar study has confirmed the change in soy isoflavone content with subtle changes in environmental factors (Caldwell et al., 2005).

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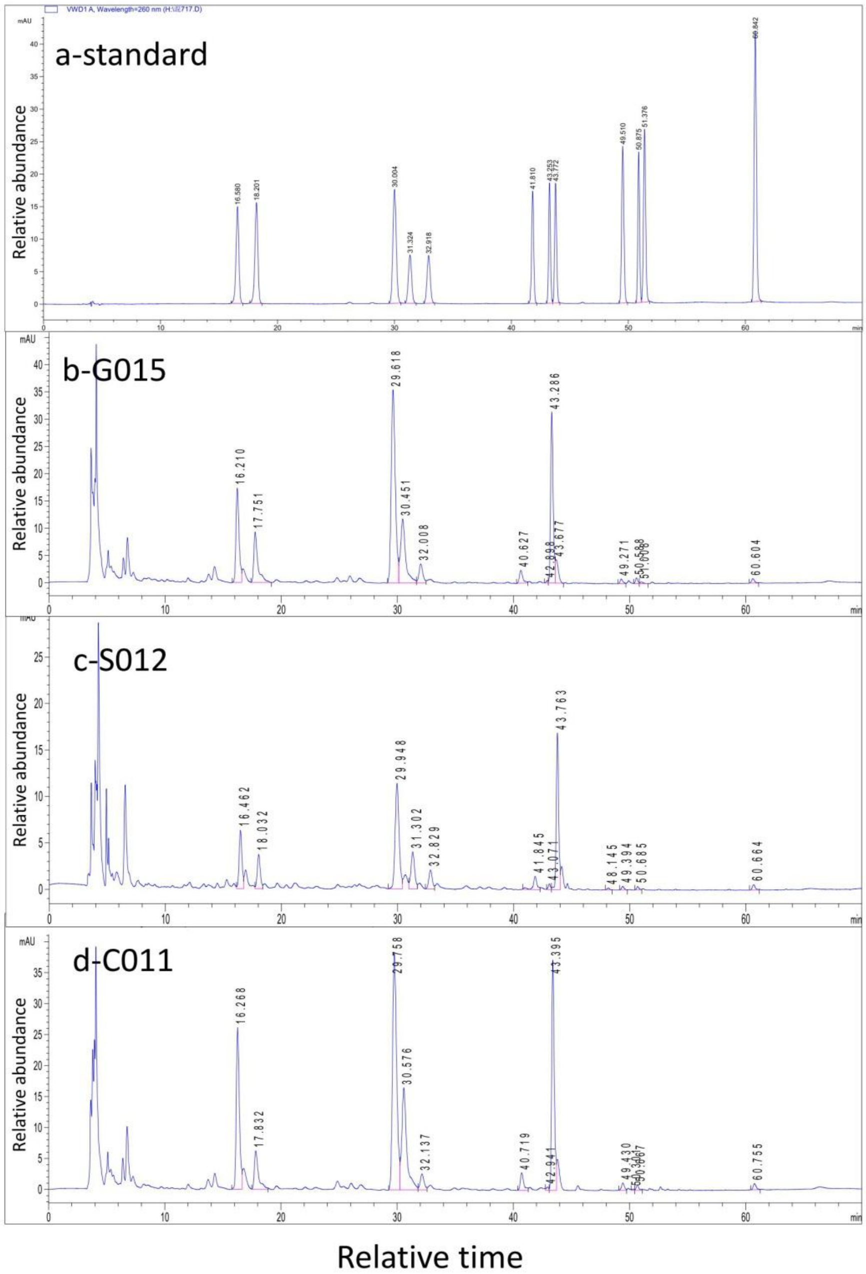

Figure 2. *Total ion chromatograms (TICs) of HPLC analysis of soy isoflavone standards and soybean samples. a, standard chromatogram; b, soybean germplasm "G015" (high MIS, high MIP); c, soybean germplasm "S012" (high MIS, low MIP); d, soybean germplasm "C011" (low MIS, low MIP); Peak assignment: peak 1-daidzin (DG); 2-glycitin (GLG); 3-genistin (GEG); 4-malonyldaidzin (MD); 5-malonylglycitin (MGL); 6-acetyldaidzin (AD); 7-acetylglycitin (AGL); 8-malonylgenistin (MG); 9-daidzein (DE); 10-acetylgeni.stin (AG); 11-glycitein (GLE); 12-genistein (GE); X axis names is Relative time; Y axis names is Relative abundance*

Particularly in recent years, more researches about genotype × environment interaction, especially about the secondary metabolism of plants to investigate the correlation with soybean isoflavones of different varieties and regions. Ge et al. (2011) measured the

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isoflavone of 100 Chinese soybean varieties of different origin, and indicated that the soybean grown in southern China had higher isoflavone content than in northern China. Similar study by Sun et al. (2004) measured the isoflavone content of 249 Chinese soybean varieties, and indicated the great variation among varieties and locations. Moreover, the total isoflavones of wild soybean were noted higher than cultivated soybean (Chune et al., 2010). Undoubtedly, genotype × environment interaction decides the most of the plant secondary metabolism, such as soy isoflavone biosynthesis.

In addition, comparative analysis of total isoflavone contents in soybean genotypes from four different locations showed higher isoflavone contents in samples grown in Chongqing (*Table S2*). This is because, Chongqing is located at a high longitude area, has a subtropical humid climate that promotes isoflavones accumulation in soybean seed. This is consistent with previous study that high longitudes are favourable for isoflavone biosynthesis (Wu et al., 2017).

Screening of germplasm by PLS-DA

HPLC-based isoflavone profiling was conducted to assess the variations in isoflavone contents of various soybean germplasms. Data regarding the 12 isoflavones identified by HPLC analysis were subjected to PLS-DA to identify the differences among soybean germplasms and four different regions of origin (*Fig. 3*).

The PLS-DA score plot showed the profile differences of samples, which are different in genotypes and source of regions (*Fig. 3A*). The corresponding loadings scatter plots of the isoflavone profiling is presented in *Figure 3B*. PLS-DA score plot demonstrated a little variability among samples of four regions of origin. However, five samples (C002, G010, G011, G013 and S075) clearly stood out from other genotypes in PLS component 1 (*Fig. 3A*). Previous studies have indicated the effects of genetic variation on isoflavone biosynthesis (Eldridge and Kwolek, 1983; Wang and Murphy, 1994). In our study, this could confirm that genotype plays an important role in the synthesis of isoflavones. However, PLS could not distinguish the influence of geographical environment.

The corresponding loading plot is used to identify those compounds that can exhibit maximum variability within a population (Kim et al., 2013). The five specific isoflavone germplasms were screened-out from all samples, resulted from their higher scores in component 1 of the PLS-DA. Daidzin, genistin, malonyldaidzin, malonylgenistin and acetylgenistin led to major contribution in component 1, for which the eigenvectors were 0.40, 0.42, 0.39, 0.40 and 0.36, respectively. The loading plot showed that isoflavone contents were higher in these five samples than others. Moreover, daidzin, genistin, malonyldaidzin, malonylgenistin and acetylgenistin were the discriminating metabolites which distinguished the specific isoflavone germplasms from the others. The isoflavone contents were controlled by genotype which contributed to the variation of specific germplasms in component 1 of the PLS-DA. The result is similar to Kim et al. (2014), however more studies confirmed the influence of the both the genotype and the environment on the isoflavones synthesis (Lee et al., 2003; Murphy et al., 2009). Several studies have shown the individual and total isoflavone contents of genotypes at different locations. Hoeck et al. (2000) found the significant differences among locations in one or more years for total and individual isoflavone contents. However, the consistency of the ranking among genotypes for the contents of individual and total isoflavones seems to depend on the magnitude of the differences in their inherent genetic potential for the traits (Zhang et al., 2014; Carter et al., 2018).

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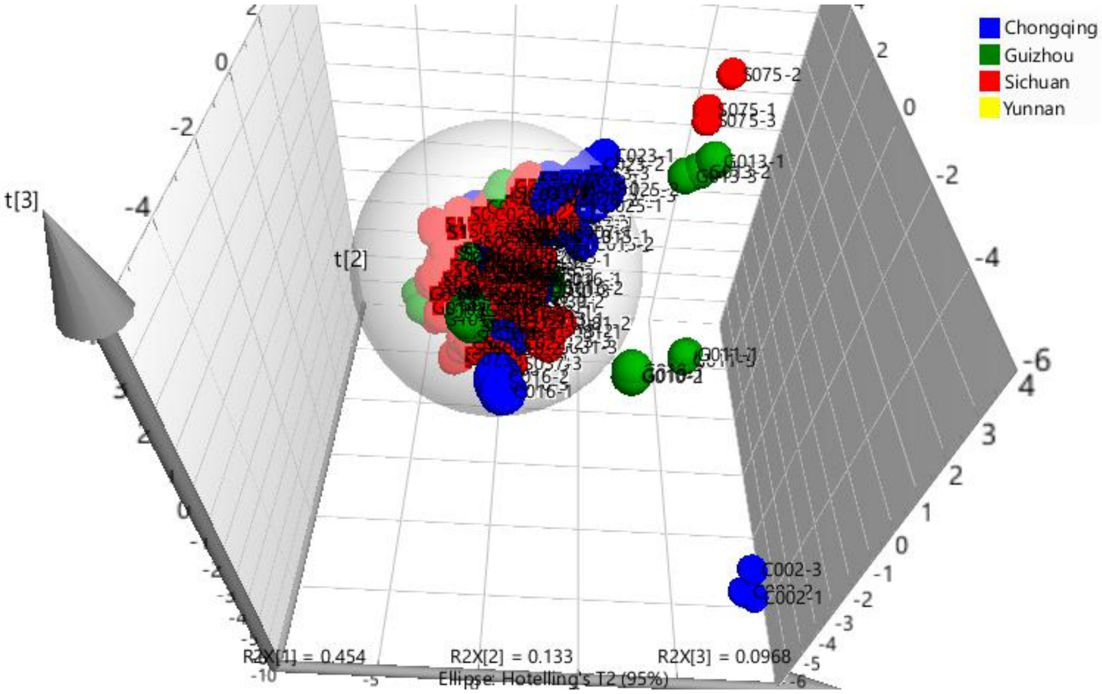

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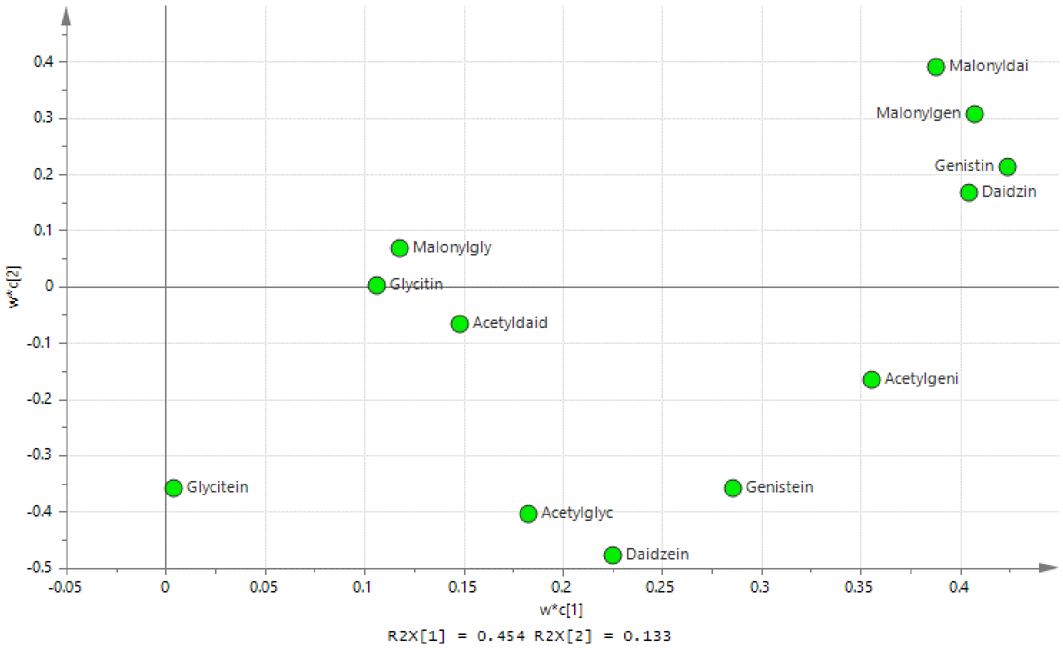

B

Figure 3. *PLS-DA score plots (A) and corresponding loadings scatter plots (B) of 144 geographical different soybean seeds*

To further elaborate the differences among 12 isoflavones in different samples, a heat map was created (*Fig. 4*). Different colors indicate the relative concentrations of corresponding metabolites in each sample. Red color indicates higher isoflavone level. The results clearly show that the content of daidzin, genistin, malonyldaidzin, malonylgenistin and acetylgenistin were higher in five specific germplasms (C002,

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G010, G011, G013 and S075) than others. These five specific germplasms had large amount of total isoflavone contents. Moreover, it also shows the daidzin, genistin, malonyldaidzin, malonylgenistin and acetylgenistin as discriminating metabolites which distinguished the specific isoflavone samples from the others.

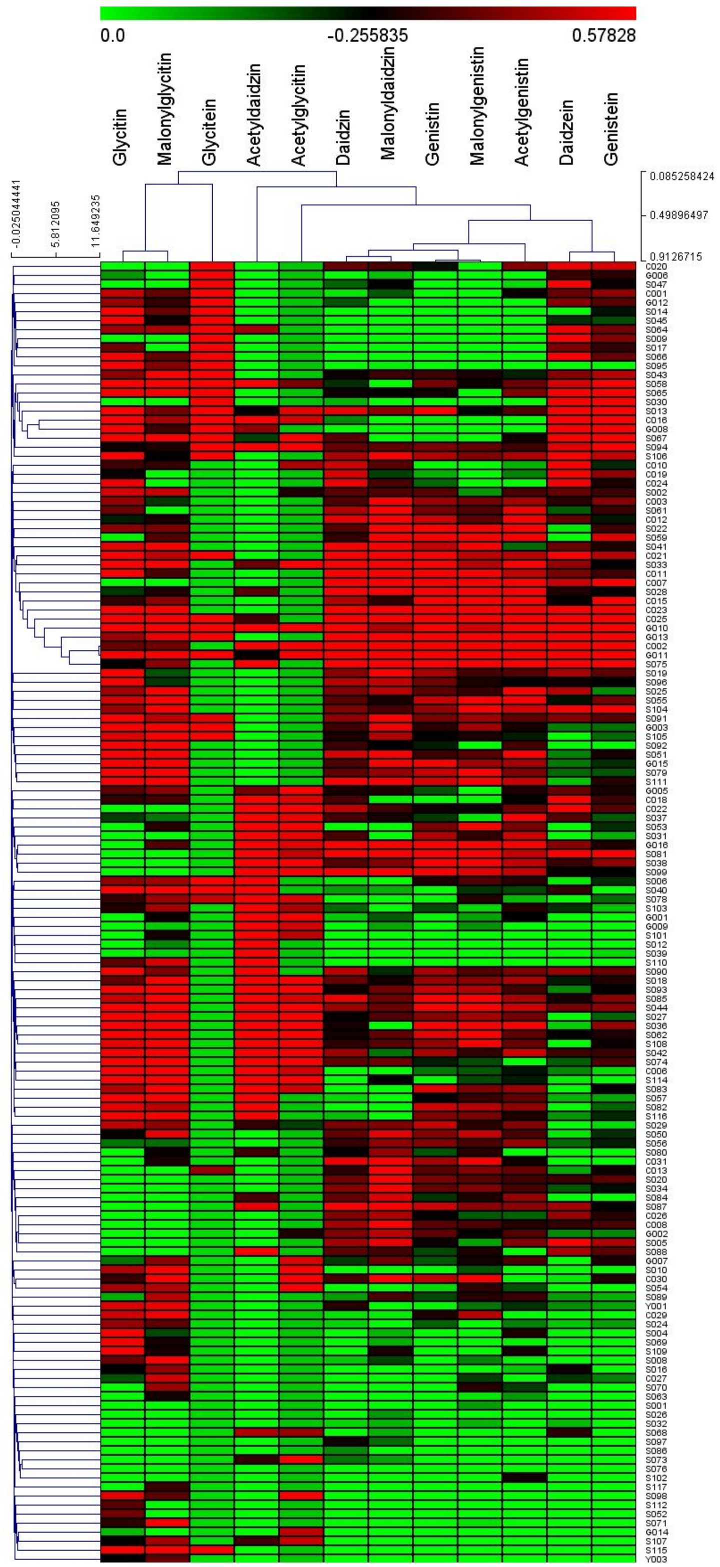

Figure 4. *Heat map and cluster analysis of 12 isoflavones in 144 soybean genotypes*

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Geographical determination

O2PLS-DA is a good multivariate projection method, which separates the structured noise in bidirectional way X and Y. O2PLS divides systematic variation into two parts (X and Y). One is regarded as the predictive part which is related to both X and Y (covarying), whereas the other is regarded as parallel part that is not related to orthogonal. As a result, O2PLS improves the interpretation of the predicted model and simplify it as well. It has been reported that O2PLS performed very well for geographical determination of different samples (Consonni et al., 2010). We therefore applied O2PLS-DA for geographical determination of five specific germplasms (*Fig. 5*).

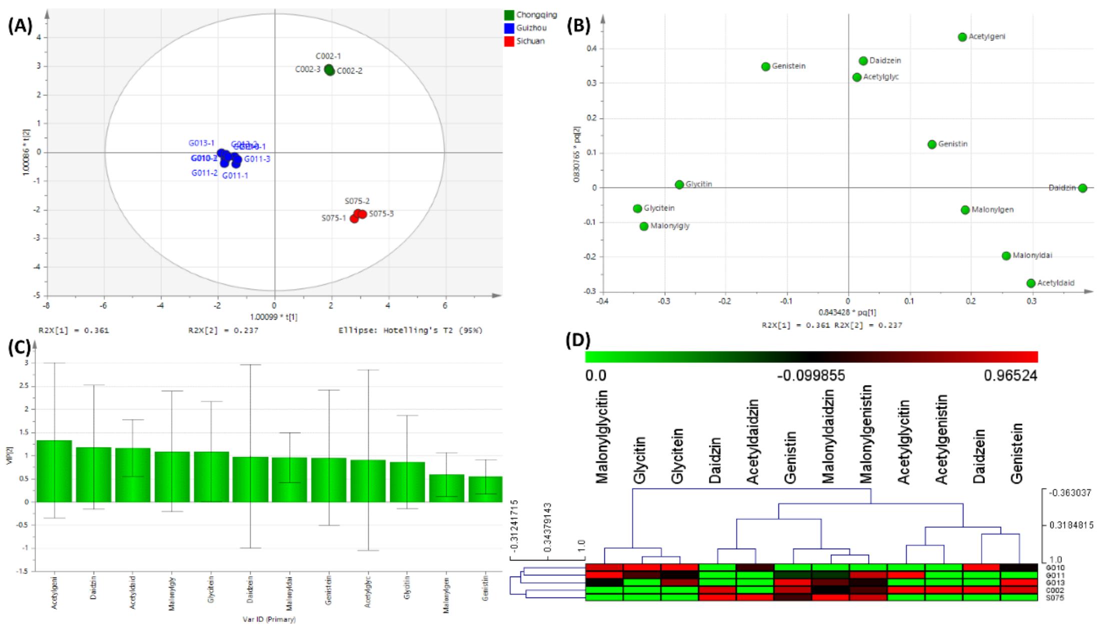

Figure 5. *O2PLS-DA score plots (A) and corresponding loadings scatter plots (B), important features identification (C), and heat map of cluster analysis (D) of 5 geographical different soybean seeds from Chongqing, Guizhou and Sichuan*

These five specific germplasm samples came from clustering and scattering in score scatter plot (*Fig. 5*). The parameters of cross-validation plot, i.e., R2(X) = 0.995, R2(Y) = 0.995, and Q2 = 0.989, indicated a valid model. All samples which had different geographical origins showed a clear separation into three groups: Chongqing, Guizhou and Sichuan (*Fig. 5A*). The different geographical environment led to the sample variations. The score scatter plot indicated that the samples grown in Guizhou were clearly separated from others. This variation was mainly attributed to glycitin, glycitein, and malonylglycitin, of which the corresponding loading scatter plot was negative and contributed mostly to the first O2PLS principal component (*Fig. 5B*). Combining with the VIP value (*Fig. 5C*), glycitein and malonylglycitin with VIP more than 1.0 were determined as biomarker metabolites for soybean seed from Guizhou. In addition, samples from Chongqing (component 2 dimension) and Sichuan (component 2 dimension) were distinguished based on the second O2PLS principal component. The corresponding loading scatter plot was positive for daidzein, acetylglycitin and

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acetylgenistin which contributed most to second O2PLS principal component. As a result, samples from Chongqing were separated from others. Combining the VIP values with the loading plot, the acetylgenistin with VIP more than 1.0 was determined as a biomarker metabolite to distinguish samples of Chongqing from others. However, as the loading plots showed that the acetyldaidzin had a negative loading value and contributed most to second O2PLS principal component. In addition, the VIP value of acetyldaidzin was more than 1.0. This confirmed that acetyldaidzin was a biomarker metabolite that distinguished samples of Sichuan from others.

The results suggest that the content of glycitein and malonylglycitin were higher in samples from Guizhou while the content of acetylgenistin and acetyldaidzin were higher in samples from Chongqing and Sichuan, respectively. Moreover, the comparative analysis of total isoflavone contents in five samples from different locations showed higher isoflavone contents in samples grown in Sichuan province followed by Chongqing (*Table S3* in the *Appendix*). This could be due to the high latitude of both Sichuan and Chongqing areas with favorable environment to promote soybean isoflavone accumulation (*Fig. 1*). It is consistent with earlier study that the geographical latitude of the genotype location plays an important role in separating soybean genotypes with different chemical profiles (Wu et al., 2017). A similar study was conducted by Zhang et al. (2007) in Northern China also confirmed the positive correlation between latitude and soybean isoflavone.

In addition, specific isoflavones were screened from all samples because their genotypes expressed higher isoflavone contents than the others. Therefore, the difference in regions led to the difference of isoflavone contents, which distinguished the five specific samples. It confirmed that geographical environment also influence the isoflavones synthesis besides genotypes. However, the geographical environment could distinguish small community size of specific samples.

A heat map was created to further explain the geographical determination of soybean genotypes based on the targeted isoflavone metabolomics, and to interpret the influence of geographical environment on isoflavone contents (*Fig. 5D*). Samples from different regions clustered different groups in the heat map. However, they were not completely divided into three groups on the base of regions. Samples variability could be distinguished by difference of 12 isoflavones presented in the heat map. As results show, the content of glycitein and malonylglycitin were higher in samples from Guizhou while the content of acetylgenistin and acetyldaidzin were found higher in samples from Chongqing and Sichuan, respectively. The results are the same as the conclusion drawn from O2PLS-DA. It confirmed that glycitein, malonylglycitin, acetylgenistin and acetyldaidzin were biomarker metabolites that could interpret the influence of geographical environment to the isoflavone contents.

Conclusion

In conclusion, the synthesis of isoflavone was affected by both the genotype and the geographical environment. When the community size of germplasm samples was large, the isoflavone contents were mainly subjected to the effect of genotypes which distinguished samples from the others. The genotype mainly influenced the content of daidzin, genistin, malonyldaidzin, malonylgenistin and acetylgenistin. These metabolites differentiated the specific isoflavone germplasms from others. However, the geographical environment also played an important role in distinguishing the small

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groups of soybean germplasms. Different geographical environments had different influence on the synthesis of isoflavones. The geographical environment affected the acetylgenistin, acetyldaidzin, glycitein and malonylglycitin, which were considered as biomarker metabolites. In addition, these distinguished specific samples from three different regions could be used for future breeding programs.

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APPENDIX

Table S1. A. *Detailed information of soybean germplasms. Schedule soybean germplasm resources details -1*

Code	Name	Origin	Code	Name	Origin	Code	Name	Origin
*G001	E119	Liu Hongchang collected from Guizhou	*S013	E237-5	Jiuzhaigou County, Sichuan Province	*S041	GYQC	Qingchuan County, Sichuan Province
*G002	E193	Dafang County, Guizhou Province	*S014	E238	Jiuzhaigou County, Sichuan Province	*S042	E233	Wangcang County, Sichuan Province
*G003	E194	Dafang County, Guizhou Province	*S016	E247	Wenchuan County, Sichuan Province	*S043	E316	Leshan City, Sichuan Province
*G005	E157	Daozhen County, Guizhou Province	*S017	E246-1	Wenchuan County, Sichuan Province	*S044	E133	Danling County, Sichuan Province
*G006	E159	Daozhen County, Guizhou Province	*S018	E248	Wenchuan County, Sichuan Province	*S045	E129	Leshan City, Sichuan Province
*G007	E160	Daozhen County, Guizhou Province	*S019	E327	Bazhong City, Sichuan Province	*S047	E294	Yanyuan County, Sichuan Province
*G008	E41	Kaige County, Guizhou Province	*S020	E331	Dujiangyan County, Sichuan Province	*S050	A12-3	Luzhou City, Sichuan Province
G009	14033	Guizhou Oil Research Institute	*S022	E5	Chengdu city, Sichuan Province	*S051	LLZ	Luzhou City, Sichuan Province
*G010	E276	Qinglong County, Guizhou Province	*S024	E278-2	Xinjin county, Sichuan Province	*S052	14030	Pengshan County, Sichuan Province
*G011	E180	Songtao County, Guizhou Province	*S025	E244-2	Xuanhan County, Sichuan Province	*S053	E333	Renshou County, Sichuan Province
*G012	E171	Zunyi City, Guizhou Province	*S026	E183	Zhongjiang County, Sichuan Province	*S054	E334	Renshou County, Sichuan Province
*G013	E252	Zunyi City, Guizhou Province	*S027	E184	Zhongjiang County, Sichuan Province	*S055	E188	Renshou County, Sichuan Province
*G014	E25	Zunyi City, Guizhou Province	*S028	E337	Zhongjiang County, Sichuan Province	*S056	E189	Renshou County, Sichuan Province
*G015	E172	Zunyi City, Guizhou Province	*S029	E338	Zhongjiang County, Sichuan Province	*S057	E347	An County, Sichuan Province
*G016	E173	Nanbai County, Guizhou Province	*S030	E339	Zhongjiang County, Sichuan Province	*S058	A17	Jiangyou County, Sichuan Province
S001	14063	Sichuan Agricultural University	*S031	A13	Zhongjiang County, Sichuan Province	*S059	E199-2	Pingwu County, Sichuan Province
S002	E197	Sichuan Agricultural University	*S032	14067	Deyang City, Sichuan Province	*S061	E205-2	Pingwu County, Sichuan Province
S004	14031	Sichuan Agricultural University	*S033	A45	Guang'an City, Sichuan Province	*S062	A36	Langzhong County, Sichuan Province
S005	E210	Sichuan Agricultural University	*S034	E178	Linshui County, Sichuan Province	*S063	14008	Langzhong County, Sichuan Province
S006	E209	Sichuan Agricultural University	*S036	A40	Yuechi County, Sichuan Province	S064	E318	Nanchong Institute of Agricultural Sciences
S008	14072	Sichuan Agricultural University	*S037	E288-2	Guangyuan City, Sichuan Province	S065	E319	Nanchong Institute of Agricultural Sciences
S009	E282	Sichuan Agricultural University	*S038	E317	Changxi County, Sichuan Province	S066	E320	Nanchong Institute of Agricultural Sciences
S010	14045	Sichuan Agricultural University	*S039	E312	Qingchuan County, Sichuan Province	S067	E324	Nanchong Institute of Agricultural Sciences
*S012	E340	Sichuan Province	*S040	E313	Qingchuan County, Sichuan Province	S068	14038	Nanchong Institute of Agricultural Sciences

Note: The code with * are landraces, and the code without * are cultivars

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Table S1. B. *Detailed information of soybean germplasms. Schedule soybean germplasm resources details -2*

Code	Name	Origin	Code	Name	Origin	Code	Name	Origin
S069	14050	Nanchong Institute of Agricultural Sciences	*S095	14091	Yibin City, Sichuan Province	*C002	E346	Chongqing
S070	14047	Nanchong Institute of Agricultural Sciences	*S096	E207-1	Yibin City, Sichuan Province	*C003	E242	Chongqing
S071	14060	Nanchong Institute of Agricultural Sciences	*S097	E207-2	Yibin City, Sichuan Province	*C006	14069	Dazu County, Chongqing
S073	14024	Nanchong Institute of Agricultural Sciences	*S098	14029	Anyue County, Sichuan Province	*C007	E175-1	Dianjiang County, Chongqing
S074	C103	Nanchong Institute of Agricultural Sciences	*S099	A18	Lezhi County, Sichuan Province	*C008	E175-2	Dianjiang County, Chongqing
S075	E352-3	Yingshan County, Sichuan Province	S101	14019	Zigong Institute of Agricultural Sciences	*C010	E176	Dianjiang County, Chongqing
*S076	14090	Neijiang City, Sichuan Province	S102	14023	Zigong Institute of Agricultural Sciences	*C011	E176-1	Dianjiang County, Chongqing
*S078	14020	Zizhong County, Sichuan Province	S103	E203	Zigong Institute of Agricultural Sciences	*C012	E176-3	Dianjiang County, Chongqing
*S079	YCQ-1	Weiyuan County, Sichuan Province	S104	E204	Zigong Institute of Agricultural Sciences	*C013	E176-4	Dianjiang County, Chongqing
*S080	YCQ-2	Weiyuan County, Sichuan Province	S105	14048	Zigong Institute of Agricultural Sciences	*C015	E66	Dianjiang County, Chongqing
*S081	E345	Shuangliu County, Sichuan Province	S106	E204-1	Zigong Institute of Agricultural Sciences	*C016	E241	Jiangjin District, Chongqing City
*S082	E281	Suining City, Sichuan Province	S107	14042	Zigong Institute of Agricultural Sciences	*C018	E145	Qujiang District, Chongqing City
*S083	E281-1	Suining City, Sichuan Province	S108	14052	Zigong Institute of Agricultural Sciences	*C019	14001	Qujiang District, Chongqing City
*S084	A25-1	Suining City, Sichuan Province	S109	14014	Zigong Institute of Agricultural Sciences	*C020	E148	Qujiang District, Chongqing City
*S085	A25-2	Suining City, Sichuan Province	S110	14056	Zigong Institute of Agricultural Sciences	*C021	E150	Qujiang District, Chongqing City
*S086	14037	Shehong County, Sichuan Province	S111	14028	Zigong Institute of Agricultural Sciences	*C022	E144	Qujiang District, Chongqing City
*S087	E326	Ya'an City, Sichuan Province	S112	14058	Zigong Institute of Agricultural Sciences	*C023	E164	Qujiang District, Chongqing City
*S088	E343	Ya'an City, Sichuan Province	S114	14071	Zigong Institute of Agricultural Sciences	*C024	E149	Qujiang District, Chongqing City
*S089	E285	Ya'an City, Sichuan Province	S115	14049	Zigong Institute of Agricultural Sciences	*C025	E151	Qujiang District, Chongqing City
*S090	E161	Ya'an City, Sichuan Province	S116	14068	Zigong Institute of Agricultural Sciences	*C026	E243	Rongchang County, Chongqing City
*S091	E162	Ya'an City, Sichuan Province	S117	14070	Zigong Institute of Agricultural Sciences	*C027	14025	Tongnan County, Chongqing City
*S092	E163	Ya'an City, Sichuan Province	*Y001	14161	Yunnan Province	*C029	14035	Tongnan County, Chongqing City
*S093	E165	Ya'an City, Sichuan Province	*Y003	14003	Chuxiong City, Yunnan Province	*C030	Y-1	Yunyang County, Chongqing City
*S094	E212	Ya'an City, Sichuan Province	*C001	E274	Chongqing	*C031	Y-2	Yunyang County, Chongqing City

Note: The code with * are landraces, and the code without * are cultivars

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Table S2. *The total isoflavone levels identified in 144 soybean germplasms*

Name	DG	GLG	GEG	MD	MGL	AD	AGL	MG	DE	AG	GLE	GE.	Total
	Daidzin	Glycitin	Genistin	Malonyldaidzin	Malonylglycitin	Acetyldaidzin	Acetylglycitin	Malonylgenistin	Daidzein	Acetylgenistin	Glycitein	Genistein	
C001	0.330±0.017	0.184±0.004	0.423±0.005	0.286±0.005	0.114±0.002	0.000±0.000	0.000±0.000	0.248±0.005	0.029±0.000	0.011±0.000	0.043±0.003	0.026±0.000	1.695±0,004
C002	1.823±0.059	0.165±0.011	2.253±0.091	0.939±0.035	0.116±0.003	0.044±0.001	0.158±0.015	1.288±0.062	0.119±0.001	0.064±0.001	0.000±0.000	0.077±0.002	7.044±0.144
C003	0.467±0.010	0.174±0.004	0.767±0.016	0.491±0.006	0.097±0.001	0.000±0.000	0.000±0.000	0.439±0.005	0.025±0.000	0.014±0.000	0.000±0.000	0.026±0.000	2.500±0.038
C006	0.287±0.008	0.252±0.006	0.527±0.013	0.191±0.003	0.169±0.003	0.046±0.003	0.020±0.001	0.323±0.008	0.020±0.000	0.010±0.001	0.000±0.000	0.019±0.000	1.864±0.042
C007	0.932±0.036	0.051±0.003	1.255±0.031	0.562±0.030	0.036±0.001	0.000±0.000	0.000±0.000	0.751±0.035	0.035±0.002	0.016±0.001	0.000±0.000	0.035±0.002	3.674±0.134
C008	0.544±0.015	0.037±0.001	0.684±0.020	0.529±0.013	0.000±0.000	0.000±0.000	0.000±0.000	0.407±0.012	0.026±0.001	0.011±0.001	0.000±0.000	0.024±0.000	2.262±0.062
C010	0.436±0.015	0.101±0.006	0.683±0.023	0.506±0.028	0.072±0.007	0.000±0.000	0.000±0.000	0.450±0.019	0.019±0.000	0.013±0.000	0.019±0.010	0.022±0.000	2.320±0.095
C011	0.587±0.006	0.157±0.002	0.477±0.004	0.362±0.022	0.107±0.005	0.000±0.000	0.017±0.000	0.246±0.004	0.049±0.002	0.009±0.001	0.000±0.000	0.021±0.000	2.032±0.037
C012	0.729±0.021	0.217±0.009	1.036±0.023	0.708±0.021	0.110±0.006	0.000±0.000	0.000±0.000	0.569±0.019	0.030±0.000	0.014±0.000	0.000±0.000	0.024±0.000	3.437±0.099
C013	0.604±0.002	0.141±0.001	0.822±0.005	0.540±0.007	0.103±0.001	0.000±0.000	0.000±0.000	0.417±0.002	0.029±0.002	0.016±0.000	0.000±0.000	0.021±0.000	2.693±0.012
C015	0.559±0.001	0.154±0.002	1.803±0.030	0.334±0.001	0.119±0.006	0.000±0.000	0.000±0.000	1.145±0.009	0.023±0.001	0.027±0.000	0.000±0.000	0.041±0.001	4.206±0.031
C016	0.357±0.002	0.214±0.002	0.482±0.004	0.188±0.002	0.123±0.002	0.040±0.001	0.019±0.000	0.242±0.003	0.093±0.002	0.008±0.000	0.157±0.004	0.051±0.001	1.975±0.015
C018	0.461±0.001	0.153±0.001	0.469±0.007	0.255±0.007	0.109±0.006	0.039±0.001	0.021±0.001	0.249±0.006	0.051±0.005	0.010±0.000	0.000±0.000	0.023±0.000	1.841±0.022
C019	0.592±0.030	0.146±0.020	0.504±0.006	0.291±0.040	0.058±0.029	0.000±0.000	0.000±0.000	0.26055±0.000	0.041±0.001	0.010±0.002	0.000±0.000	0.026±0.000	1.928±0.019
C020	0.488±0.022	0.126±0.005	0.566±0.017	0.353±0.012	0.070±0.003	0.000±0.000	0.000±0.000	0.244±0.005	0.037±0.001	0.013±0.000	0.035±0.001	0.029±0.000	1.959±0.065
C021	0.862±0.024	0.196±0.006	0.986±0.020	0.692±0.016	0.127±0.003	0.000±0.000	0.000±0.000	0.496±0.007	0.049±0.001	0.014±0.000	0.040±0.001	0.028±0.000	3.489±0.074
C022	0.572±0.024	0.116±0.003	0.589±0.023	0.348±0.014	0.079±0.002	0.054±0.004	0.023±0.001	0.355±0.015	0.047±0.002	0.013±0.001	0.000±0.000	0.024±0.001	2.219±0.089
C023	1.121±0.034	0.241±0.009	1.253±0.041	0.723±0.025	0.176±0.008	0.000±0.000	0.000±0.000	0.832±0.032	0.054±0.002	0.016±0.001	0.000±0.000	0.030±0.001	4.445±0.152
C024	0.611±0.010	0.193±0.006	0.532±0.005	0.354±0.005	0.080±0.003	0.000±0.000	0.000±0.000	0.259±0.002	0.037±0.000	0.008±0.000	0.000±0.000	0.022±0.000	2.096±0.029
C025	1.15±0.049	0.294±0.011	1.441±0.056	0.509±0.002	0.151±0.001	0.020±0.001	0.000±0.000	0.683±0.009	0.060±0.002	0.020±0.000	0.050±0.002	0.038±0.000	4.421±0.112
C026	0.514±0.003	0.059±0.002	0.577±0.001	0.420±0.003	0.000±0.000	0.000±0.000	0.000±0.000	0.322±0.005	0.032±0.001	0.010±0.000	0.000±0.000	0.023±0.000	1.956±0.007
C027	0.199±0.012	0.138±0.005	0.373±0.017	0.219±0.012	0.129±0.013	0.000±0.000	0.000±0.000	0.328±0.009	0.022±0.001	0.006±0.000	0.000±0.000	0.020±0.001	1.434±0.065
C029	0.332±0.036	0.186±0.006	0.610±0.044	0.256±0.016	0.134±0.006	0.000±0.000	0.000±0.000	0.490±0.051	0.010±0.005	0.009±0.003	0.000±0.000	0.018±0.000	2.047±0.021
C030	0.460±0.009	0.155±0.019	0.808±0.021	0.483±0.042	0.147±0.012	0.000±0.000	0.082±0.002	0.855±0.016	0.018±0.001	0.008±0.003	0.000±0.000	0.022±0.000	3.038±0.114
C031	0.612±0.022	0.098±0.003	0.776±0.024	0.557±0.017	0.103±0.002	0.000±0.000	0.000±0.000	0.716±0.024	0.018±0.000	0.011±0.000	0.000±0.000	0.018±0.000	2.910±0.015

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Name	DG	GLG	GEG	MD	MGL	AD	AGL	MG	DE	AG	GLE	GE.	Total
	Daidzin	Glycitin	Genistin	Malonyldaidzin	Malonylglycitin	Acetyldaidzin	Acetylglycitin	Malonylgenistin	Daidzein	Acetylgenistin	Glycitein	Genistein	
G001	0.339±0.037	0.126±0.004	0.479±0.007	0.285±0.022	0.101±0.002	0.040±0.000	0.022±0.000	0.304±0.003	0.016±0.000	0.010±0.000	0.000±0.000	0.018±0.000	1.740±0.053
G002	0.515±0.028	0.042±0.001	0.694±0.039	0.428±0.024	0.000±0.000	0.000±0.000	0.000±0.000	0.351±0.017	0.020±0.001	0.012±0.001	0.000±0.000	0.020±0.001	2.088±0.107
G003	0.455±0.006	0.219±0.003	0.623±0.008	0.464±0.008	0.149±0.006	0.000±0.000	0.000±0.000	0.473±0.007	0.020±0.000	0.011±0.000	0.038±0.001	0.020±0.000	2.472±0.028
G005	0.418±0.004	0.162±0.002	0.540±0.003	0.326±0.002	0.119±0.002	0.024±0.007	0.024±0.001	0.273±0.010	0.029±0.001	0.011±0.000	0.000±0.000	0.022±0.000	1.949±0.008
G006	0.280±0.013	0.133±0.005	0.414±0.014	0.265±0.010	0.080±0.004	0.000±0.000	0.000±0.000	0.223±0.008	0.026±0.001	0.008±0.000	0.030±0.002	0.023±0.000	1.482±0.056
G007	0.379±0.011	0.136±0.003	0.538±0.013	0.377±0.011	0.120±0.003	0.000±0.000	0.021±0.000	0.360±0.008	0.012±0.000	0.012±0.000	0.000±0.000	0.022±0.000	1.976±0.038
G008	0.273±0.002	0.227±0.003	0.421±0.002	0.128±0.002	0.108±0.001	0.027±0.001	0.000±0.000	0.195±0.005	0.068±0.003	0.008±0.000	0.141±0.005	0.044±0.000	1.641±0.003
G009	0.310±0.002	0.113±0.001	0.412±0.000	0.271±0.003	0.090±0.002	0.044±0.000	0.020±0.000	0.309±0.003	0.012±0.000	0.008±0.000	0.000±0.000	0.016±0.000	1.604±0.008
G010	1.163±0.008	0.282±0.004	1.316±0.013	0.419±0.015	0.150±0.004	0.073±0.001	0.025±0.000	0.509±0.009	0.111±0.000	0.021±0.000	0.088±0.002	0.060±0.000	4.216±0.009
G011	0.925±0.085	0.147±0.012	1.422±0.128	0.676±0.059	0.118±0.009	0.136±0.013	0.031±0.002	1.032±0.096	0.029±0.002	0.016±0.001	0.000±0.000	0.034±0.001	4.566±0.407
G012	0.371±0.006	0.175±0.002	0.437±0.002	0.232±0.022	0.106±0.002	0.000±0.000	0.000±0.000	0.190±0.008	0.031±0.001	0.009±0.000	0.044±0.002	0.024±0.000	1.620±0.032
G013	0.875±0.017	0.175±0.003	2.353±0.047	1.092±0.024	0.133±0.001	0.000±0.000	0.000±0.000	1.210±0.027	0.058±0.001	0.026±0.001	0.055±0.006	0.084±0.001	6.062±0.127
G014	0.081±0.001	0.131±0.003	0.142±0.004	0.073±0.003	0.083±0.001	0.000±0.000	0.018±0.001	0.062±0.002	0.005±0.005	0.002±0.000	0.000±0.000	0.015±0.000	0.612±0.018
G015	0.467±0.018	0.303±0.010	0.924±0.040	0.510±0.019	0.148±0.003	0.000±0.000	0.000±0.000	0.472±0.017	0.020±0.000	0.014±0.001	0.000±0.000	0.021±0.000	2.880±0.106
G016	0.822±0.005	0.122±0.001	0.987±0.008	0.538±0.009	0.111±0.002	0.097±0.001	0.021±0.000	0.625±0.010	0.031±0.001	0.018±0.001	0.000±0.000	0.022±0.000	3.395±0.013
S001	0.181±0.004	0.095±0.004	0.302±0.004	0.231±0.003	0.087±0.003	0.000±0.000	0.000±0.000	0.306±0.006	0.012±0.001	0.009±0.000	0.000±0.000	0.016±0.001	1.239±0.013
S002	0.479±0.020	0.188±0.003	0.682±0.005	0.357±0.016	0.128±0.006	0.000±0.000	0.000±0.000	0.307±0.003	0.029±0.000	0.012±0.001	0.000±0.000	0.023±0.001	2.218±0.012
S004	0.323±0.001	0.206±0.002	0.472±0.002	0.267±0.001	0.096±0.000	0.000±0.000	0.000±0.000	0.246±0.011	0.018±0.000	0.011±0.000	0.000±0.000	0.016±0.000	1.656±0.013
S005	0.546±0.006	0.057±0.000	0.588±0.004	0.460±0.008	0.000±0.000	0.000±0.000	0.000±0.000	0.305±0.004	0.049±0.002	0.013±0.000	0.000±0.000	0.027±0.001	2.045±0.022
S006	0.331±0.007	0.174±0.006	0.611±0.012	0.227±0.002	0.123±0.003	0.061±0.002	0.000±0.000	0.408±0.003	0.018±0.000	0.011±0.000	0.039±0.001	0.021±0.000	2.023±0.032
S008	0.340±0.005	0.165±0.002	0.472±0.008	0.290±0.002	0.143±0.004	0.000±0.000	0.000±0.000	0.312±0.003	0.013±0.000	0.009±0.001	0.000±0.000	0.018±0.000	1.761±0.025
S009	0.299±0.009	0.100±0.002	0.289±0.005	0.132±0.003	0.060±0.001	0.000±0.000	0.000±0.000	0.118±0.001	0.047±0.001	0.006±0.000	0.053±0.003	0.026±0.000	1.131±0.018
S010	0.283±0.004	0.180±0.002	0.450±0.006	0.211±0.004	0.137±0.002	0.000±0.000	0.029±0.002	0.3234±0.007	0.015±0.001	0.008±0.000	0.000±0.000	0.018±0.000	1.656±0.025
S012	0.150±0.001	0.113±0.002	0.343±0.002	0.132±0.004	0.093±0.009	0.037±0.002	0.000±0.000	0.244±0.007	0.013±0.000	0.007±0.000	0.000±0.000	0.018±0.000	1.150±0.024
S013	0.610±0.013	0.204±0.006	0.917±0.020	0.398±0.037	0.116±0.002	0.013±0.007	0.021±0.001	0.343±0.008	0.061±0.002	0.012±0.000	0.048±0.001	0.058±0.001	2.801±0.036
S014	0.255±0.005	0.229±0.001	0.432±0.008	0.110±0.001	0.114±0.001	0.000±0.000	0.000±0.000	0.182±0.002	0.017±0.000	0.009±0.000	0.047±0.001	0.021±0.000	1.418±0.017

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	Daidzin	Glycitin	Genistin	Malonyldaidzin	Malonylglycitin	Acetyldaidzin	Acetylglycitin	Malonylgenistin	Daidzein	Acetylgenistin	Glycitein	Genistein	
S016	0.287±0.020	0.144±0.001	0.404±0.003	0.182±0.002	0.120±0.004	0.000±0.000	0.000±0.000	0.268±0.002	0.023±0.001	0.009±0.000	0.000±0.000	0.018±0.000	1.455±0.018
S017	0.226±0.007	0.166±0.005	0.240±0.006	0.123±0.002	0.073±0.002	0.000±0.000	0.000±0.000	0.099±0.002	0.030±0.001	0.006±0.000	0.062±0.005	0.023±0.000	1.047±0.028
S018	0.492±0.012	0.165±0.003	0.761±0.016	0.316±0.004	0.147±0.003	0.063±0.002	0.025±0.001	0.453±0.007	0.022±0.000	0.014±0.000	0.000±0.000	0.022±0.000	2.479±0.046
S019	0.521±0.013	0.213±0.004	0.778±0.017	0.469±0.007	0.095±0.001	0.000±0.000	0.000±0.000	0.401±0.005	0.033±0.000	0.012±0.001	0.000±0.000	0.025±0.000	2.548±0.043
S020	0.495±0.021	0.087±0.003	0.726±0.016	0.474±0.010	0.055±0.001	0.000±0.000	0.000±0.000	0.416±0.008	0.026±0.001	0.012±0.000	0.000±0.000	0.024±0.000	2.317±0.037
S022	0.474±0.009	0.172±0.003	1.019±0.016	0.492±0.030	0.117±0.003	0.000±0.000	0.000±0.000	0.539±0.010	0.018±0.000	0.016±0.000	0.000±0.000	0.023±0.000	2.870±0.068
S024	0.336±0.007	0.175±0.003	0.534±0.008	0.177±0.002	0.113±0.003	0.000±0.000	0.000±0.000	0.273±0.003	0.019±0.000	0.010±0.000	0.000±0.000	0.019±0.000	1.656±0.019
S025	0.517±0.015	0.177±0.005	0.689±0.017	0.428±0.009	0.141±0.003	0.000±0.000	0.000±0.000	0.387±0.008	0.033±0.002	0.018±0.000	0.000±0.000	0.020±0.001	2.411±0.057
S026	0.274±0.004	0.101±0.002	0.414±0.004	0.273±0.004	0.065±0.002	0.000±0.000	0.000±0.000	0.234±0.002	0.017±0.000	0.007±0.000	0.000±0.000	0.017±0.000	1.403±0.011
S027	0.402±0.035	0.225±0.005	0.740±0.027	0.358±0.031	0.186±0.003	0.061±0.003	0.024±0.001	0.476±0.026	0.015±0.000	0.013±0.000	0.000±0.000	0.020±0.000	2.520±0.055
S028	0.957±0.004	0.140±0.002	1.305±0.017	0.846±0.004	0.104±0.002	0.022±0.003	0.000±0.000	0.629±0.012	0.025±0.000	0.021±0.001	0.000±0.000	0.021±0.000	4.069±0.031
S029	0.509±0.034	0.184±0.002	0.823±0.016	0.390±0.009	0.120±0.002	0.018±0.000	0.000±0.000	0.379±0.003	0.014±0.000	0.013±0.001	0.000±0.000	0.019±0.000	2.471±0.044
S030	0.344±0.012	0.120±0.005	0.398±0.007	0.218±0.006	0.053±0.002	0.000±0.000	0.000±0.000	0.176±0.005	0.059±0.002	0.006±0.001	0.043±0.003	0.051±0.001	1.469±0.044
S031	0.464±0.011	0.081±0.001	0.790±0.019	0.248±0.004	0.064±0.001	0.057±0.001	0.021±0.000	0.384±0.008	0.017±0.003	0.015±0.001	0.000±0.000	0.019±0.000	2.160±0.044
S032	0.334±0.015	0.065±0.002	0.380±0.018	0.267±0.009	0.058±0.001	0.000±0.000	0.000±0.000	0.300±0.008	0.019±0.001	0.009±0.000	0.000±0.000	0.017±0.000	1.449±0.053
S033	1.015±0.019	0.230±0.007	0.966±0.015	0.703±0.009	0.163±0.004	0.023±0.000	0.024±0.001	0.490±0.008	0.032±0.001	0.017±0.001	0.000±0.000	0.021±0.000	3.684±0.064
S034	0.520±0.004	0.068±0.001	0.699±0.004	0.559±0.004	0.055±0.001	0.000±0.000	0.000±0.000	0.449±0.001	0.021±0.000	0.012±0.000	0.000±0.000	0.021±0.000	2.404±0.009
S036	0.412±0.002	0.220±0.004	1.099±0.004	0.236±0.003	0.165±0.002	0.073±0.001	0.024±0.001	0.555±0.008	0.019±0.001	0.018±0.00	0.000±0.000	0.027±0.000	2.848±0.011
S037	0.463±0.026	0.139±0.002	0.549±0.022	0.320±0.032	0.094±0.010	0.040±0.005	0.021±0.001	0.289±0.009	0.028±0.003	0.017±0.005	0.000±0.000	0.020±0.000	1.980±0.093
S038	0.473±0.007	0.057±0.001	1.029±0.018	0.389±0.038	0.045±0.001	0.071±0.002	0.023±0.001	0.603±0.044	0.026±0.000	0.014±0.000	0.000±0.000	0.026±0.000	2.759±0.039
S039	0.220±0.006	0.128±0.002	0.388±0.010	0.154±0.004	0.090±0.001	0.040±0.002	0.000±0.000	0.235±0.007	0.018±0.000	0.009±0.001	0.000±0.000	0.019±0.000	1.300±0.031
S040	0.350±0.005	0.212±0.002	0.475±0.007	0.269±0.003	0.164±0.001	0.050±0.001	0.000±0.000	0.332±0.005	0.026±0.001	0.010±0.000	0.042±0.001	0.018±0.000	1.946±0.024
S041	0.724±0.007	0.190±0.004	0.817±0.011	0.525±0.018	0.144±0.007	0.000±0.000	0.000±0.000	0.606±0.012	0.029±0.001	0.010±0.000	0.000±0.000	0.022±0.000	3.069±0.038
S042	0.541±0.007	0.257±0.004	0.782±0.015	0.280±0.007	0.158±0.003	0.054±0.000	0.022±0.000	0.385±0.004	0.026±0.001	0.015±0.000	0.000±0.000	0.023±0.000	2.543±0.033
S043	0.407±0.020	0.172±0.006	0.650±0.032	0.345±0.016	0.143±0.008	0.000±0.000	0.000±0.000	0.366±0.017	0.031±0.001	0.010±0.000	0.038±0.002	0.028±0.001	2.190±0.102
S044	0.536±0.008	0.205±0.009	0.882±0.015	0.374±0.017	0.158±0.001	0.068±0.000	0.024±0.000	0.608±0.004	0.030±0.000	0.015±0.000	0.000±0.000	0.026±0.000	2.927±0.027

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	Daidzin	Glycitin	Genistin	Malonyldaidzin	Malonylglycitin	Acetyldaidzin	Acetylglycitin	Malonylgenistin	Daidzein	Acetylgenistin	Glycitein	Genistein	
S045	0.303±0.002	0.224±0.003	0.317±0.007	0.197±0.002	0.101±0.002	0.000±0.000	0.000±0.000	0.157±0.001	0.022±0.000	0.008±0.000	0.041±0.000	0.021±0.000	1.391±0.007
S047	0.367±0.023	0.118±0.006	0.421±0.023	0.307±0.014	0.054±0.001	0.000±0.000	0.000±0.000	0.252±0.012	0.038±0.001	0.009±0.001	0.040±0.001	0.022±0.000	1.628±0.083
S050	0.487±0.005	0.145±0.004	0.740±0.019	0.454±0.043	0.132±0.005	0.000±0.000	0.000±0.000	0.503±0.011	0.018±0.000	0.012±0.000	0.000±0.000	0.021±0.000	2.512±0.034
S051	0.590±0.013	0.240±0.005	0.647±0.010	0.473±0.005	0.174±0.001	0.000±0.000	0.000±0.000	0.414±0.007	0.020±0.001	0.024±0.001	0.000±0.000	0.022±0.000	2.604±0.040
S052	0.133±0.016	0.166±0.006	0.283±0.007	0.162±0.004	0.084±0.002	0.000±0.000	0.000±0.000	0.187±0.007	0.009±0.000	0.007±0.000	0.000±0.000	0.015±0.000	1.047±0.015
S053	0.289±0.012	0.126±0.006	0.724±0.024	0.238±0.010	0.105±0.000	0.069±0.002	0.023±0.004	0.549±0.020	0.015±0.001	0.013±0.000	0.000±0.000	0.021±0.000	2.172±0.078
S054	0.197±0.004	0.177±0.007	0.489±0.010	0.161±0.005	0.126±0.002	0.000±0.000	0.047±0.001	0.384±0.007	0.013±0.002	0.010±0.001	0.000±0.000	0.017±0.000	1.620±0.026
S055	0.479±0.009	0.187±0.002	0.819±0.014	0.320±0.009	0.144±0.005	0.000±0.000	0.000±0.000	0.568±0.013	0.022±0.000	0.016±0.000	0.000±0.000	0.025±0.000	2.581±0.044
S056	0.444±0.007	0.136±0.003	0.741±0.015	0.400±0.004	0.094±0.001	0.000±0.000	0.000±0.000	0.405±0.006	0.021±0.000	0.014±0.000	0.000±0.000	0.021±0.000	2.275±0.030
S057	0.335±0.007	0.217±0.004	0.576±0.011	0.250±0.002	0.162±0.001	0.055±0.002	0.000±0.000	0.390±0.003	0.019±0.000	0.012±0.000	0.000±0.000	0.019±0.000	2.035±0.024
S058	0.382±0.006	0.260±0.005	0.719±0.008	0.193±0.003	0.143±0.001	0.054±0.001	0.013±0.007	0.357±0.005	0.035±0.000	0.013±0.000	0.078±0.005	0.031±0.000	2.278±0.017
S059	0.442±0.003	0.128±0.001	1.192±0.017	0.525±0.003	0.111±0.005	0.000±0.000	0.000±0.000	0.605±0.006	0.015±0.000	0.018±0.000	0.000±0.000	0.029±0.002	3.065±0.021
S061	0.556±0.011	0.166±0.004	0.718±0.014	0.510±0.010	0.088±0.001	0.000±0.000	0.000±0.000	0.406±0.014	0.021±0.000	0.015±0.000	0.000±0.000	0.023±0.000	2.504±0.041
S062	0.415±0.009	0.197±0.005	0.853±0.012	0.369±0.004	0.166±0.004	0.083±0.001	0.031±0.000	0.660±0.002	0.023±0.000	0.012±0.000	0.000±0.000	0.022±0.000	2.833±0.028
S063	0.284±0.010	0.110±0.003	0.396±0.010	0.205±0.006	0.102±0.003	0.000±0.000	0.000±0.000	0.288±0.010	0.015±0.000	0.010±0.000	0.000±0.000	0.016±0.000	1.426±0.040
S064	0.244±0.006	0.177±0.004	0.366±0.008	0.139±0.004	0.124±0.003	0.029±0.001	0.000±0.000	0.164±0.002	0.037±0.000	0.007±0.000	0.058±0.000	0.025±0.000	1.371±0.020
S065	0.409±0.014	0.222±0.013	0.596±0.014	0.308±0.002	0.147±0.007	0.000±0.000	0.000±0.000	0.262±0.005	0.051±0.001	0.013±0.001	0.058±0.002	0.038±0.002	2.104±0.051
S066	0.313±0.018	0.200±0.011	0.382±0.021	0.145±0.015	0.114±0.008	0.000±0.000	0.000±0.000	0.167±0.007	0.040±0.002	0.009±0.000	0.058±0.002	0.025±0.000	1.451±0.081
S067	0.479±0.006	0.213±0.002	0.482±0.003	0.241±0.002	0.138±0.001	0.011±0.000	0.040±0.000	0.262±0.002	0.070±0.001	0.011±0.000	0.080±0.002	0.033±0.000	2.06±0.015
S068	0.294±0.002	0.050±0.001	0.280±0.002	0.268±0.001	0.038±0.001	0.030±0.000	0.016±0.000	0.215±0.001	0.025±0.007	0.008±0.002	0.000±0.000	0.015±0.000	1.239±0.006
S069	0.319±0.010	0.199±0.005	0.441±0.012	0.268±0.007	0.102±0.002	0.000±0.000	0.000±0.000	0.244±0.005	0.011±0.000	0.009±0.000	0.000±0.000	0.017±0.000	1.610±0.041
S070	0.187±0.008	0.120±0.007	0.367±0.011	0.200±0.005	0.122±0.003	0.000±0.000	0.000±0.000	0.376±0.010	0.014±0.000	0.010±0.000	0.000±0.000	0.018±0.000	1.415±0.034
S071	0.136±0.012	0.153±0.005	0.264±0.016	0.169±0.010	0.141±0.009	0.000±0.000	0.000±0.000	0.290±0.017	0.009±0.005	0.007±0.000	0.000±0.000	0.015±0.000	1.185±0.073
S073	0.363±0.032	0.000±0.000	0.278±0.007	0.271±0.007	0.000±0.000	0.018±0.000	0.051±0.001	0.251±0.007	0.018±0.000	0.007±0.000	0.000±0.000	0.016±0.000	1.272±0.045
S074	0.485±0.034	0.254±0.008	0.560±0.018	0.376±0.023	0.173±0.006	0.043±0.001	0.026±0.001	0.326±0.020	0.021±0.001	0.008±0.002	0.000±0.000	0.024±0.000	2.295±0.088
S075	2.008±0.073	0.144±0.002	2.05±0.057	1.502±0.058	0.119±0.003	0.185±0.009	0.000±0.000	1.513±0.047	0.050±0.000	0.024±0.001	0.000±0.000	0.036±0.001	7.635±0.224

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Name	DG	GLG	GEG	MD	MGL	AD	AGL	MG	DE	AG	GLE	GE.	Total
	Daidzin	Glycitin	Genistin	Malonyldaidzin	Malonylglycitin	Acetyldaidzin	Acetylglycitin	Malonylgenistin	Daidzein	Acetylgenistin	Glycitein	Genistein	
S076	0.279±0.016	0.033±0.001	0.232±0.010	0.160±0.007	0.000±0.000	0.000±0.000	0.000±0.000	0.124±0.004	0.010±0.000	0.005±0.000	0.000±0.000	0.014±0.000	0.857±0.037
S078	0.204±0.005	0.154±0.004	0.471±0.011	0.190±0.006	0.122±0.004	0.048±0.001	0.020±0.000	0.374±0.010	0.016±0.000	0.009±0.000	0.023±0.012	0.020±0.000	1.653±0.051
S079	0.483±0.006	0.263±0.013	0.818±0.012	0.403±0.005	0.201±0.005	0.000±0.000	0.000±0.000	0.787±0.011	0.021±0.001	0.013±0.000	0.000±0.000	0.020±0.000	3.009±0.046
S080	0.400±0.014	0.093±0.004	0.539±0.019	0.404±0.016	0.101±0.002	0.019±0.000	0.000±0.000	0.376±0.016	0.013±0.001	0.008±0.000	0.000±0.000	0.017±0.000	1.970±0.071
S081	0.754±0.023	0.115±0.002	0.970±0.021	0.477±0.022	0.077±0.002	0.083±0.002	0.019±0.003	0.582±0.017	0.056±0.001	0.014±0.000	0.000±0.000	0.048±0.001	3.195±0.084
S082	0.312±0.009	0.191±0.005	0.830±0.019	0.193±0.004	0.127±0.003	0.070±0.003	0.000±0.000	0.492±0.011	0.015±0.000	0.013±0.001	0.000±0.000	0.020±0.000	2.263±0.054
S083	0.316±0.004	0.180±0.004	0.849±0.004	0.181±0.003	0.148±0.003	0.058±0.001	0.020±0.002	0.444±0.017	0.014±0.001	0.014±0.000	0.000±0.000	0.021±0.000	2.245±0.009
S084	0.489±0.006	0.036±0.000	0.551±0.008	0.448±0.005	0.061±0.030	0.020±0.000	0.000±0.000	0.368±0.007	0.015±0.000	0.014±0.000	0.000±0.000	0.016±0.000	2.018±0.007
S085	0.610±0.027	0.184±0.008	0.974±0.037	0.378±0.019	0.147±0.004	0.078±0.004	0.020±0.001	0.579±0.030	0.024±0.000	0.014±0.000	0.000±0.000	0.026±0.000	3.033±0.129
S086	0.356±0.007	0.012±0.002	0.348±0.000	0.140±0.066	0.065±0.000	0.000±0.000	0.000±0.000	0.256±0.002	0.017±0.001	0.007±0.000	0.000±0.000	0.017±0.000	1.217±0.059
S087	0.638±0.0008	0.047±0.001	0.826±0.016	0.533±0.003	0.052±0.001	0.036±0.015	0.000±0.000	0.456±0.025	0.033±0.001	0.013±0.000	0.000±0.000	0.021±0.000	2.655±0.018
S088	0.480±0.002	0.039±0.001	0.543±0.001	0.350±0.000	0.027±0.000	0.056±0.001	0.000±0.000	0.369±0.002	0.033±0.001	0.008±0.000	0.000±0.000	0.024±0.000	1.930±0.002
S089	0.351±0.013	0.132±0.004	0.545±0.020	0.354±0.022	0.126±0.002	0.000±0.000	0.000±0.000	0.377±0.007	0.020±0.000	0.012±0.000	0.000±0.000	0.019±0.000	1.934±0.059
S090	0.566±0.006	0.201±0.004	0.778±0.012	0.295±0.003	0.118±0.002	0.060±0.004	0.000±0.000	0.411±0.005	0.032±0.001	0.013±0.001	0.000±0.000	0.025±0.000	2.499±0.021
S091	0.529±0.015	0.198±0.014	0.721±0.022	0.547±0.016	0.126±0.007	0.000±0.000	0.000±0.000	0.471±0.017	0.031±0.000	0.012±0.000	0.026±0.013	0.024±0.000	2.684±0.092
S092	0.450±0.035	0.286±0.001	0.560±0.010	0.304±0.008	0.168±0.003	0.000±0.000	0.000±0.000	0.241±0.005	0.013±0.000	0.012±0.001	0.000±0.000	0.016±0.000	2.051±0.059
S093	0.408±0.005	0.221±0.003	0.819±0.004	0.378±0.040	0.167±0.001	0.059±0.002	0.024±0.001	0.487±0.026	0.020±0.000	0.012±0.000	0.000±0.000	0.021±0.000	2.616±0.030
S094	0.491±0.008	0.146±0.003	0.727±0.013	0.367±0.049	0.104±0.001	0.053±0.001	0.023±0.000	0.425±0.019	0.036±0.000	0.012±0.000	0.056±0.000	0.031±0.000	2.470±0.045
S095	0.271±0.006	0.215±0.004	0.403±0.006	0.255±0.003	0.119±0.001	0.000±0.000	0.000±0.000	0.266±0.002	0.009±0.005	0.008±0.000	0.032±0.001	0.018±0.000	1.596±0.020
S096	0.565±0.006	0.200±0.004	0.717±0.008	0.433±0.007	0.097±0.002	0.000±0.000	0.000±0.000	0.367±0.024	0.023±0.000	0.010±0.000	0.000±0.000	0.021±0.000	2.434±0.051
S097	0.412±0.027	0.051±0.001	0.400±0.009	0.282±0.003	0.041±0.001	0.000±0.000	0.000±0.000	0.193±0.008	0.015±0.000	0.008±0.000	0.000±0.000	0.016±0.000	1.419±0.015
S098	0.148±0.005	0.190±0.006	0.369±0.005	0.192±0.007	0.113±0.003	0.000±0.000	0.023±0.003	0.195±0.008	0.015±0.000	0.007±0.001	0.000±0.000	0.016±0.000	1.268±0.026
S099	0.658±0.006	0.036±0.000	0.890±0.008	0.584±0.013	0.054±0.001	0.052±0.002	0.024±0.001	0.524±0.013	0.023±0.000	0.014±0.000	0.000±0.000	0.021±0.000	2.879±0.020
S101	0.276±0.003	0.118±0.002	0.379±0.002	0.215±0.005	0.102±0.008	0.039±0.002	0.018±0.001	0.255±0.004	0.010±0.005	0.008±0.000	0.000±0.000	0.016±0.000	1.437±0.023
S102	0.228±0.018	0.033±0.001	0.213±0.014	0.190±0.010	0.039±0.001	0.000±0.000	0.000±0.000	0.125±0.007	0.009±0.004	0.011±0.000	0.000±0.000	0.014±0.000	0.862±0.046
S103	0.365±0.007	0.151±0.000	0.544±0.010	0.207±0.003	0.123±0.003	0.047±0.001	0.017±0.001	0.292±0.006	0.022±0.000	0.011±0.000	0.000±0.000	0.019±0.000	1.797±0.024

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	Daidzin	Glycitin	Genistin	Malonyldaidzin	Malonylglycitin	Acetyldaidzin	Acetylglycitin	Malonylgenistin	Daidzein	Acetylgenistin	Glycitein	Genistein	
S104	0.552±0.009	0.217±0.003	0.794±0.018	0.508±0.008	0.100±0.002	0.000±0.000	0.000±0.000	0.447±0.010	0.052±0.000	0.014±0.000	0.045±0.001	0.028±0.000	2.757±0.048
S105	0.420±0.024	0.189±0.002	0.576±0.006	0.421±0.006	0.159±0.008	0.000±0.000	0.000±0.000	0.368±0.005	0.017±0.000	0.010±0.000	0.035±0.001	0.020±0.000	2.215±0.040
S106	0.512±0.014	0.175±0.005	0.758±0.026	0.353±0.003	0.136±0.007	0.000±0.000	0.000±0.000	0.542±0.008	0.043±0.001	0.015±0.000	0.000±0.000	0.031±0.000	2.564±0.045
S107	0.092±0.002	0.144±0.003	0.182±0.001	0.096±0.002	0.123±0.004	0.019±0.000	0.019±0.001	0.122±0.002	0.013±0.000	0.006±0.000	0.000±0.000	0.014±0.000	0.830±0.011
S108	0.437±0.020	0.192±0.008	0.759±0.035	0.364±0.011	0.190±0.005	0.080±0.002	0.022±0.002	0.606±0.018	0.018±0.000	0.013±0.000	0.000±0.000	0.022±0.000	2.703±0.090
S109	0.312±0.004	0.207±0.003	0.404±0.007	0.321±0.010	0.103±0.004	0.000±0.000	0.000±0.000	0.250±0.008	0.013±0.000	0.011±0.000	0.000±0.000	0.017±0.000	1.638±0.034
S110	0.194±0.013	0.163±0.020	0.373±0.037	0.144±0.007	0.130±0.016	0.038±0.001	0.000±0.000	0.236±0.023	0.012±0.001	0.007±0.000	0.000±0.000	0.016±0.000	1.313±0.046
S111	0.667±0.010	0.216±0.011	0.837±0.005	0.487±0.009	0.166±0.005	0.000±0.000	0.000±0.000	0.724±0.011	0.019±0.000	0.015±0.001	0.000±0.000	0.023±0.000	3.154±0.034
S112	0.253±0.004	0.161±0.004	0.305±0.005	0.244±0.003	0.086±0.001	0.000±0.000	0.000±0.000	0.199±0.003	0.013±0.000	0.007±0.000	0.000±0.000	0.014±0.000	1.283±0.017
S114	0.314±0.005	0.213±0.004	0.392±0.004	0.308±0.020	0.210±0.016	0.044±0.003	0.023±0.001	0.326±0.004	0.018±0.000	0.010±0.001	0.000±0.000	0.016±0.000	1.875±0.021
S115	0.144±0.002	0.220±0.005	0.220±0.000	0.131±0,005	0.174±0.007	0.000±0.000	0.000±0.000	0.143±0.002	0.000±0.000	0.006±0.001	0.037±0.002	0.015±0.000	1.090±0.009
S116	0.342±0.013	0.209±0.005	0.733±0.023	0.214±0.007	0.144±0.008	0.059±0.004	0.000±0.000	0.441±0.017	0.020±0.001	0.011±0.001	0.000±0.000	0.021±0.000	2.194±0.073
S117	0.212±0.018	0.110±0.003	0.301±0.011	0.216±0.011	0.108±0.006	0.000±0.000	0.000±0.000	0.259±0.010	0.016±0.001	0.007±0.000	0.000±0.000	0.016±0.000	1.245±0.054
Y001	0.430±0.028	0.189±0.003	0.478±0.003	0.245±0.004	0.133±0.002	0.000±0.000	0.000±0.000	0.315±0.004	0.020±0.000	0.010±0.000	0.000±0.000	0.019±0.000	1.839±0.035
Y003	0.322±0.006	0.144±0.002	0.338±0.004	0.237±0.004	0.113±0.002	0.000±0.000	0.000±0.000	0.210±0.000	0.018±0.000	0.007±0.000	0.000±0.000	0.018±0.000	1.407±0.014

Table S3. *The total isoflavone contents of five specific germplasm samples from different locations. Chongqing (C002), Sichuan (S075) and Guizhou (G010, G011 and G013)*

Name	Daidzin	Glycitin	Genistin	Malonyldaidzin	Malonylglycitin	Acetyldaidzin	Acetylglycitin	Malonylgenistin	Daidzein	Acetylgenistin	Glycitein	Genistein	Total
C002	1.823±0.059	0.165±0.011	2.253±0.091	0.939±0.035	0.116±0.003	0.044±0.001	0.158±0.015	1.288±0.062	0.119±0.001	0.064±0.001	0.000±0.000	0.077±0.002	7.044±0.144
G010	1.163±0.008	0.282±0.004	1.316±0.013	0.419±0.015	0.150±0.004	0.073±0.001	0.025±0.000	0.509±0.009	0.111±0.000	0.021±0.000	0.088±0.002	0.060±0.000	4.216±0.009
G011	0.925±0.085	0.147±0.012	1.422±0.128	0.676±0.059	0.118±0.009	0.136±0.013	0.031±0.002	1.032±0.096	0.029±0.002	0.016±0.001	0.000±0.000	0.034±0.001	4.566±0.407
G013	0.875±0.017	0.175±0.003	2.353±0.047	1.092±0.024	0.133±0.001	0.000±0.000	0.000±0.000	1.210±0.027	0.058±0.001	0.026±0.001	0.055±0.006	0.084±0.001	6.062±0.127
S075	2.008±0.073	0.144±0.002	2.05±0.057	1.502±0.058	0.119±0.003	0.185±0.009	0.000±0.000	1.513±0.047	0.050±0.000	0.024±0.001	0.000±0.000	0.036±0.001	7.635±0.224

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