Emirates Journal of Food and Agriculture. 2016. 28(2): 85-92
doi: 10.9755/ejfa.2015-09-787
http://www.ejfa.me/

REGULAR ARTICLE

Exploring elite alleles for seed isoflavones concentration in soybean by association analysis

Cheng Wang, Yuan Guo, Jiafeng Hu, Jie Zhang, Liu Yang, Bei Guo, Jun Han, Hao Xie*

Beijing Key Laboratory of New Technology in Agricultural Application, College of Plant Science and Technology, Beijing University of Agriculture, Beijing 102206, China

ABSTRACT

Soybean isoflavones are valuable in certain medicines, cosmetics, foods and feeds. Selection for high-isoflavone content in seeds along with agronomic traits is a goal of many soybean breeders. In our study, with 2 tables association mapping is a useful alternative to linkage mapping for the detection of marker-phenotype associations. Association analysis studies can be used to test for associations between molecular markers and target phenotype. The main objective of this study is to identify simple sequence repeat (SSR) markers associated with the soybean quality traits of isoflavones content. The four quality traits were evaluated in 135 soybean cultivar accessions from China, and the 135 accessions were genotyped with 100 SSR markers, analysis of population structure revealed three subgroups in the population. A total of 31 marker-trait associations related to the four traits were identified. According to the results, the association analysis in this study can be an effective method for QTL mapping and can help breeders to develop new approach for improving the content of isoflavones in soybean.

Keywords: Isoflavone content; Association analysis; Soybean; SSR; Genotype

INTRODUCTION

Soybean (*Glycine max*) has had a long history as a domesticated plant, originated from the eleventh century BC in China (Stephen Barnes 2010) and become a popular crop plant in China and East Asia, where they have long been cultivated as an important nutritional component of diets and used in many foods, such as soybean oil, soybean sprout, paste, soymilk and tofu (Kim EH et al. 2006). Nowadays, soybean is gaining acceptance in many countries largely as one of the best vegetable protein and oil sources, owing to its beans contains about 40% protein and 20% oil. By 2012, annual world planting area had reached to 108.749 million hectares and production had risen to 267.999 million tons.

In recent decades, several studies have shown some components in soybean possess the health benefits. Regular consumption of soybean foods can reduce the incidence of breast, colon, and prostate cancers (Isanga J et al. 2008), prevent heart disease, osteoporosis (Messina M 2005) and lower plasma cholesterol (Hsu CS et al. 2001), and reduce menopausal symptoms (TY Tai et al. 2012). Isoflavone, a naturally occurring plant chemicals belonging to a category of polyphenols in soybean, were recognized most likely one of the components responsible for the health benefits of soybean and play a potential role in therapeutic or preventive effects on a range of hormone-dependent conditions (Messina M et al. 2006). These discoveries have resulted in the development and application of many functional foods and food supplements based on soybean isoflavones.

Isoflavones belong to a group of compounds that share a basic structure consisting of two benzyl rings joined by a three-carbon bridge. In soybean seed and soybean products, Isoflavones exist as aglycones (daidzein, genistein, and glycitein), 7-O-β-glucosides and two glucoside conjugate forms, acetylglucosides and malonylglucosides. Daidzein and genistein, the most abundant isoflavones found in soybeans, have chemical structures similar to estradiol (Knight DC and Eden JA 1996) and are both strong antioxidants and occupied 80% of antioxidant potential of soybean (Arora A et al. 2000; Hsu CS et al. 2001; Hwang J

***Corresponding author:**
Hao Xie, Beijing Key Laboratory of New Technology in Agricultural Application, College of Plant Science and Technology, Beijing University of Agriculture, Beijing 102206, China. Fax: +86 10 80799489, Tel: +86 10 80799489, E-mail: xiehao@126.com

Received: 26 September 2015; **Revised:** 14 January 2016; **Accepted:** 16 January 2016; **Published Online:** 24 January 2016

et al. 2001). They have efficiently controlled or inhibited the growth of human breast cancer cell lines in culture (Lee HP 1991). These biological characters have resulted in attracting more attention to soybean seed isoflavones and an increasing interest in changes the isoflavones concentrations of soybean practical varieties.

Many of studies shown soybean seed isoflavones concentrations have a great fluctuations, because many biotic and abiotic factors influence their synthesis and accumulation. For example, the isoflavones could be determined over a wide concentration range (0.8-1135.0 mg/kg for daidzein, 1.9-1442.5 mg/kg for genistein and 0.5-54.6 mg/kg for glycitein) (Beatrix Preinerstorfer et al. 2004). Lucimara et al reported that isoflavones concentrations were influenced by the cytoplasm and the nuclear genes of the maternal parent (Lucimara Chiari *et al* 2006). Nevertheless, in spite of genetic factors, the environment interactions was largely influence on isoflavones concentration in seeds, such as soybean variety, cultivation year, cultivation location, and temperature (Hoeck JA et al. 2000; Lee SJ et al. 2002; Mebrahtu T et al. 2004; Murphy SE et al. 2009; Juan Jose et al. 2009). Juan et al found farther that isoflavones accumulation in seeds was influenced by multiple interacting genetic loci (Beatrix Preinerstorfer and Gerhard Sontag 2004), meanwhile, when plants grew in variable environments, epistasis has been considered as an important source of genetic variation.

Although seed isoflavones were attributable to genetic and environment causes as well as g × e interaction, the heritability of variety was still a key factor causing less phenotypic change in diverse environments. Hence, the main target of the present study was to investigate the variations in isoflavones concentrations in soybean seeds with cropping year. These informations may suggest ways to realize the heritability of isoflavones concentrations in soybean variety, which is very important for soybean breeders aiming to cultivate varieties with high isoflavones concentrations.

MATERIALS AND METHODS

Plant materials

A total of 135 soybean (*Glycine max*) varieties from different regions for this experiment were cultivated at same location during 2012-2014 in Beijing (Table S1). The soil was a silt clay loam year by year. The planting arrangement was 3×1.5 m per plot, each plot consisted of three rows (3m long and 0.5m between rows) and the experiment completely randomized block design with three replicates. The fertilizers were applied prior to plowing at the recommended rates of 6.2, 4.6 and 5.0 kg per 666.67 m^2

Table S1: Soybean varieties in our study

135 soybean varieties	
S1	Zhonghuang4
S2	Zhonghuang5
S3	Zhonghuang7
S4	Zhonghuang10
S5	Zhonghuang18
S6	Zhongpin94
S7	Zhongpin6034
S8	Zhongpin661
S9	Zhongzuo96
S10	Zhongzuo97
S11	Zhongzuoyc17
S12	Zhongzuo92
S13	Zhongzuo98
S14	Zhongzuo99
S15	Jingfeng1
S16	Ludou4
S17	Ludou7
S18	Ludou10
S19	Yuejin4
S20	Yuejin5
S21	Hai94
S22	JinDou1
S23	Jindou3
S24	Jindou19
S25	Jindou20
S26	Jindou24
S27	Jindou25
S28	Jindou27
S29	Jindou47
S30	Jinda74
S31	Fendou53
S32	Fendou57
S33	Changzhi0201
S34	Yudou6
S35	Yudou8
S36	Yudou10
S37	Yudou16
S38	Yudou18
S39	Yudou19
S40	Yudou21
S41	Yaoheidou9011
S42	Zhengdou6
S43	Jichengdou1
S44	Jidou7
S45	Jidou12
S46	Datuziyandou
S47	Bahong1
S48	Dandou5
S49	Dabaibian
S50	Xiaobaiqi
S51	Dabaiqi
S52	Dahuangke
S53	Tuzidun
S54	Tiefeng23
S55	Tiefeng29

Contd...

Table S1: Soybean varieties in our study

135 soybean varieties	
S56	Kaiyu10
S57	Xiaojianke
S58	Bu76
S59	Andou3
S60	Longjiang2
S61	Suinong14
S62	Huanandajinhuang
S63	Hongfeng10
S64	Hefeng25
S65	Longjianghuang
S66	longjianghei
S67	Dongbeihei
S68	Zhechundou2
S69	Zhechundou3
S70	Zhechundou13
S71	Zhechundou14
S72	Zhechundou18
S73	Xudou3
S74	Xudou5
S75	Xudou7
S76	Xudou8
S77	Xudou9
S78	Huaidou3
S79	Xudou8901
S80	Xinxuan2
S81	Fengxianhonghuacao
S82	Aijiaozao
S83	Neimengdou1
S84	Neimengdou2
S85	Gansu3
S86	Nannong94
S87	Dagudi2
S88	Bao9201
S89	Qianjindou
S90	Xiafeng2
S91	Zhengdou5
S92	Hefeng35
S93	Silihuang
S94	Jianheili
S95	Heidou1948
S96	Kelong1
S97	Kefeng2
S98	Kefeng34
S99	Dongbeidahongdou
S100	Kehuang1
S101	Ji221
S102	Daqingdou
S103	Zhonghuang1
S104	Beinong103
S105	Kefeng6
S106	Kefeng14
S107	Yi1358
S108	Ludou9
S109	Ludou11
S110	Shandongsijiaodou

Contd...

Table S1: Soybean varieties in our study

135 soybean varieties	
S111	Jindou11
S112	Jindou23
S113	Jinda841
S114	Jinda125
S115	Fendou41
S116	Taiguzao
S117	Dandou4
S118	Dandou6
S119	Liufeng209
S120	Chidou
S121	Jilin37
S122	Gongjiao5610
S123	Bawangbian
S124	Suzao2
S125	Qingsu2
S126	Zhonglong1
S127	Tongsu823
S128	Nannong9610
S129	Chuxiu
S130	Qingrenwudou
S131	Fuxian1
S132	Zhe8018
S133	Taiwan292
S134	Tongsu1
S135	Qixing

for N, P_2O_5 and K_2O, respectively. Soybean seeds were sowed on June 15-16 every year and harvested after mature completely for each variety from each replicate at each crop year and each plot were harvested only middle row as seed samples, and then stored at freezer with under -18°C until analyzed for isoflavone concentration. Whole seed samples were analyzed the isoflavones and this analysis was undertaken at Beijing key laboratory of new technology in agricultural application, Beijing University of Agriculture.

Isoflavone extraction and quantification

Isoflavone concentrations were determined using HPLC as described by Vyn et al. (2002). Approximately 1g sample was mixed with 5ml methanol (100%) in 20ml plastic bottle and was used ultrasonic waves to make it dissolve quicker 1h, and then, static solution for 24h at room temperature. 1.5 ml methanol solution was extracted into centrifuge tube to separate 20 min at 14000 $r \cdot min^{-1}$ using a refrigerated centrifuge. The supernatant liquor was filtered through a 0.45μm nylon syringe filter paper (Whatmanno42) to HPLC analysis.

The HPLC system consisted of an Agilent 1200 liquid chromatography pump and an detector (Agilent Technologies Co. ltd). The column for analysis was a TC-C18 (250 mm×4.6 mm, 5 μm), and UV absorption was measured at 254nm. The mobile phases consisted of solvents A and B in the HPLC analysis. Solvent A was

40% methanol and solvent B was 0.1% glacial acetic acid (pH3.22).The injection time was 20 min with 10µl sample and solvent flow rate 1ml·min^{-1}. The following gradient of mobile phase was used: 0-20min isocratic at 40% A and 60% B, 20-25min linear gradient from 40 to 100% A and from 60 to 0% B, and then held during 25-40 min, 40-45 min linear gradient from 100 to 40% A and 0 to 60% B, 45-50minisocratic at 40% A and 60% B. Identification and quantification of each isoflavone component were based on available standards (Indofine Chemical Co., Somerville, NJ). Measurements are given as micrograms of isoflavone per gram of seeds plus/minus standard deviation or standard error, when corresponds (µg/g ± SD or SE).

SSR analyses

Total DNA of each seed were isolated from freeze-dried leaf tissue by CTAB method (Doyle and Doyle 1990) (Doyle JJ et al. 1990). SSR analysis was performed with the primers developed by Cregan et al. (1999). PCR was performed in 20µl containing 2µl genomic DNA (25 ng/µl), 1.5 µl $MgCl_2$ (25 mM), 0.3µl dNTP mixtures (10 mM), 2 µl 10 X PCR buffer, 2µl SSR primer (2 µM), 0.2 µl Taq polymerase (10 units/µl), 12 µl double-distilled water. The amplification temperature profiles were 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 47°C, 30s at 72°C, then 5 min at 72°C. After amplification, the PCR products were mixed with loading buffer (2.5 mg/ml bromophenol blue, 2.5 mg/ml diphenylamine blue, 10 mM EDTA, 95% (v/v) formamide), and denatured for 5 min at 94°C and then kept on ice for 5 min. The denatured PCR products were separated on 6% (w/v) denaturing polyacrylamide gel and visualized by silver straining (Trigizano RN et al. 1998).

Population structure

Population structure was estimated by STRUCTURE v2.3.2 (Pritchard et al. 2000) (Pritchard JK et al. 2000). The number of hypothetical subpopulations (K) was set from 2 to 9 with a burn-in period length of 50,000 iterations and a run of 500,000 replications of Markov Chain Monte Carlo (MCMC) after burn-in. Each K was duplicated five times. The admixture model of STRUCTURE allowed for population mixture and correlated allele frequencies. The most appropriate K value was evaluated by lnP(D) in the STRUCTURE output (Evanno et al. 2005). According to the most appropriate K value, the Q-matrix of five repeats was integrated by using the CLUMPP software (Jakobsson and Rosenberg 2007).

Association mapping

For marker-trait association, a structured association approach was implemented by a general linear model (GLM) in TASSEL 2.1 (Bradbury PJ et al. 2007). In order to correct for spurious associations, the Q-matrix was used in the model. The threshold (P value) for significant association between markers and traits was 0.001. The phenotypic variance explained (PVE) for each significantly associated locus was evaluated by R^2 values for the markers (Zhang J et al. 2011).

Statistical analysis

The soybeans were cultivated using a completely randomized design, which was replicated three times. The analysis of isoflavones by HPLC was repeated three times with each variety. Analyses of variance for all data were undertaken using the general linear model procedure and the SPASS17.0 software. The pooled mean values were separated on the basis of least significant differences at the 0.05 probability level.

RESULTS

Phenotypic analysis of isoflavone content

According to the result of isoflavone content, the mean content of total isoflavone (TI) in different soybean varieties was 1579.14 µg/g, ranging from 479.32 to 2721.44 µg/g, with a coefficient of variation(CV) of 38.66%. The mean content of daidzein(DZ) in different soybean varieties was 587.15 µg/g, ranging from 219.67 to 1346.96 µg/g, with a coefficient of variation(CV) of 45.11%. The mean content of genistein(GT) in different soybean varieties was 801.72 µg/g, ranging from 224.11 to 1620.08 µg/g, with a coefficient of variation(CV) of 41.84%, The mean content of glycitein(GC) in different soybean varieties was 190.27 µg/g, ranging from 3.87 to 122.45 µg/g, with a coefficient of variation(CV) of 15.9% (Table 1). The high values of CV indicated wide phenotypic variation among accessions, which was suitable for association analysis.

Allelic diversity and population structure

A total of 100 SSR markers were used to detect polymorphisms in all soybean varieties. A key issue for association analysis is estimation of population structure, which can result in spurious associations between phenotypes and markers. The Q-matrix from STRUCTURE can help to reduce the risk of false positives arising from population structure (Bradburyet al. 2007). One hundred SSR markers were selected to estimate the population structure. The average lnP(D) value for each K (from 1 to 8) is visualized in Fig S1 and the inflection point appeared at K = 3. According to lnP(D), Population was classified into three subpopulations, containing 65, 16 and 66 accessions, respectively (Fig. S2).

Content of isoflavone by association analysis

Candidate SSR markers for content of isoflavone, located indifferent regions, were used for association analysis in

Table 1: Conditional phenotypic values for isoflavone

Content	Mean±SD (μg/g)	CV (%)	Min. (μg/g)	Max. (μg/g)
Daidzein	587.15±29.36	45.1	219.7	1347.0
Genistein	801.72±40.09	41.8	224.1	1620.1
Glycitein	190.27±9.51	15.9	3.87	122.45
Isoflavone	1579.14±78.96	38.7	479.3	2721.4

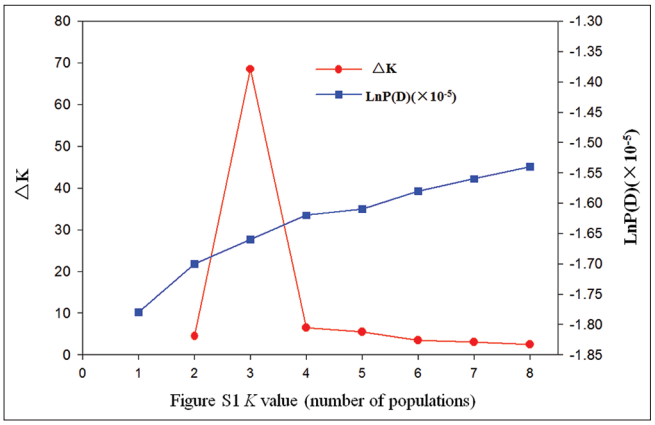

Fig S1. Values of ΔK with its modal value used to detect the true K of three subgroups (K=3).

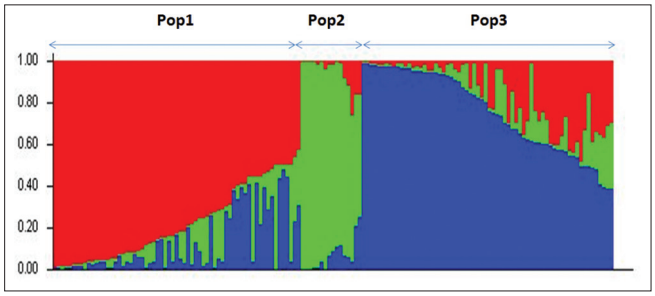

Fig S2. Population was classified into three subpopulations.

population. One hundred SSR markers which distributed in every chromosome were selected to estimate the association analysis for daidzein, genistein, glycitein and total content of isoflavone.We selected the models about GLM, found too many loci association with the correlative traits. Under the GLM model, twelve loci (Satt440、Satt592、Satt155、Satt376, Satt186, Satt384, Satt038, Satt621, Satt375, Satt 540, Satt241, Sat_120) significantly associated with DZ, located in I, O, A1, C2, D2, E, G, J, K, M, O and F, respectively. Of them, phenotypic variation explained is about 5% to 13% (Table 2). Fourteen loci (Satt687, Sat_337, Satt009, Satt629, Satt431, Satt187, Satt038, Satt288, Satt54 6, Satt621, Satt264, Sat_096, Satt 002, Satt114) significantly associated with GC, located in B2, C1, H, H, J, A2, G, G, D1b, J, K, D1b, D2 and F, respectively. Of thm, phenotypic variation explained is about 6% to 34% (Table 2). Twelve loci (Satt129, Sat_592, Satt376, Satt540, Satt546, Satt587, Satt375, Satt245, Satt323, Satt080, Satt516, Satt543) significantly associated with GT, located in D1a, O, C2, M, D1b, I, K, M, M, N, D1a and D2, respectively. Of them, phenotypic variation explained is about 4.2% to 12.9% (Table 2). eight loci (Satt592、Satt376, Satt546, Sat_218, Satt587, Satt375, Satt540, Satt543)significantly associated with TI, located in O, C2, D1b, H, I, K, M, and D2, respectively. Of them, phenotypic variation explained is about 4.1% to 10.6% (Table 2).

Table 2: The list of marker analysis for associated with isoflavone under GLM model

Traits	Primer	P value	R^2 (%)	Linkage group	Locus
DZ	Satt440	0.00026	5.0	I	112.7
	Satt592	0.00034	10.3	O	100.38
	Satt155	0.00030	13.4	A1	32.68
	Satt376	0.00025	9.8	C2	97.83
	Satt186	0.00027	4.9	D2	105.45
	Satt384	0.00036	5.8	E	19.3
	Satt038	0.00048	5.4	G	1.84
	Satt621	0.00032	6.0	J	53.68
	Satt375	0.00020	6.7	K	45.81
	Satt540	0.00013	8.6	M	53.54
	Satt241	0.00015	7.1	O	59.49
	Sat_120	0.00045	10.7	F	75.97
GC	Satt687	0.00001	18.5	B2	113.61
	Sat_337	0.00019	6.7	C1	32.1
	Sctt009	0.00023	7.6	H	38.89
	Satt629	0.00010	33.6	H	72.18
	Satt431	0.00010	10.5	J	78.57
	Satt187	0.00025	6.3	A2	54.92
	Satt038	0.00023	6.5	G	1.84
	Satt288	0.00035	10.1	G	76.77
	Satt546	0.00027	7.4	D1b	99.5
	Satt621	0.00310	6.0	J	53.68
	Satt264	0.00010	17.3	K	46.25
	Sat_096	0.00015	7.1	D1b	0
	Satt002	0.00010	18.3	D2	47.73
	Satt114	0.00024	10.8	F	63.69
GT	Satt129	0.00036	12.9	D1a	109.67
	Satt592	0.00027	9.9	O	100.38
	Satt376	0.00049	8.0	C2	97.83
	Satt540	0.00032	9.6	M	53.54
	Satt546	0.00026	6.9	D1b	99.5
	Satt587	0.00016	8.7	I	31.49
	Satt375	0.00011	7.0	K	45.81
	Satt245	0.00025	7.0	M	53.54
	Satt323	0.00049	6.0	M	60.05
	Satt080	0.00020	16.2	N	45.14
	Satt516	0.00045	7.1	D1a	55.68
	Satt543	0.00036	4.2	D2	88.02
TI	Satt592	0.00023	10.6	O	100.38
	Satt376	0.00015	10.3	C2	97.83
	Satt546	0.00037	9.8	D1b	87.2
	Sat_218	0.00026	7.2	H	99.5
	Satt587	0.00020	8.8	I	31.49
	Satt375	0.00040	8.6	K	45.81
	Satt540	0.00030	9.4	M	53.54
	Satt543	0.00045	4.1	D2	88.02

R^2 Indicates the degree of the locus of the phenotypic variance explained at P<0.05 level

DISCUSSION

Soybean seed isoflavones have many uses in foods, medicines, cosmetics, and animal farming (Brouns F 2002). Thus, the improvement of seed isoflavone content in soybean cultivar is increasingly focused by breeders. Fendou53 (2721.44 μg/g) was proved to have highest isoflavone content in all soybean varieties for three years. Choi et al. (1996) showed their results that total isoflavone content changed from 458 to 3309 μg/g across location within the same year to single or multiple soybean cultivars (Wang H and Murphy P 1994; Choi JS et al. 1996). In our study. The TI values see table 1 (Table 1). Meanwhile, seed isoflavones were attributable to genetic and environment causes as well as g × e interaction or year × location, these studies showed that for effective cultivar improvement, the main genotypic effects of total and individual isoflavone have the important influence.

The 100 SSR markers selected for association analysis, twelve loci associated with DZ, fourteen loci associated with DC, twelve loci associated with GT, and eight loci associated with TI were mapped onto eleven, eleven, ten and eight LGs, respectively. There association loci explained 4.1-10.6% of phenotypic variation for total isoflavone. Most of variation was <30%. In soybean seeds, the low level of phenotypic variation evaluated by association analysis was similar to the other studies (Njiti VK 1999; Meksem K et al. 2001; Kassem MA et al. 2004; Kassem MA et al. 2006; Primomo VS et al. 2005).

In this study, thirty-one SSR markers associated with DZ、GC、GT and TI, and in these markers, same marker (satt540 in LG M) had been detected in DZ、GT and TI at the same time. Wang yan et al. (2014) used 'Zhongdou27' (high isoflavone) × 'Jiunong20' (low isoflavone) to dentify eQTL underlying expression of four gene families encoding isoflavone synthetic enzymes involved in the phenylpropanoid pathway including the Satt540 marker. Primomo et al. (2005) detected Satt540 marker was from different isoflavone content in soybean seeds (Primomo VS et al. 2005). Zeng Guoliang et al (2009) also detected Satt540 marker was associated with GC、GT and TI. In our study, same marker (satt540 in LG M) had been detected in DZ、GT and TI. This suggests Satt540 was weakly influenced by genetic background and environment. Meanwhile, Satt540 was associated with certain foliar resistances such as to aphids and to white mold (Li Y *et al.* 2007; Guo XM et al. 2008). Furthermore, isoflavones in leaves that protectd soybeans from pests or pathogenic microbes may be transported to seed (Morris PF et al. 1991; Benhamou N et al. 1999). Therefore, Satt540 marker in this region could represent a major seed isoflavone content locus. SSR marker (Satt546 in LG D1b) was associated with GC、GT and TI, it was a creativity discover related to seed isoflavone content, in this region of soybean genome, a QTL for expression of gene family encoding isoflavone synthetic enzymes: *C4H* (cinnamate-4-hydroxylase) (Yan Wang et al. 2014).

The present study investigated a number of SSR marker associated with DZ、GC、GT and TI, and predicted some SSR markers related to the isoflavone contents in soybean seeds.

Although seed isoflavonoids display a broad range of variation, their synthesis and accumulation are affected by many biotic and abiotic factors. There are considerableadvances in these studies. In our study, the usability of these markers associated with isoflavones in soybean seeds could promote MAS in breeding programs, and improve an efficient method for developing soybean cultivars.

CONCLUSION

In summary, our findings suggested that 31 marker-trait associations related to the four traits were identified, including the SSR markers (Satt540 and Satt546) which have been previously reported. The results also suggested that the use of the SSR marker (Satt540 and Satt546) could probably improve an efficient method for developing high-isoflavone soybean cultivars.

ACKNOWLEDGEMENTS

This work was supported by the Beijing Natural Science Foundation (5122009); The National Natural Science Fund (31371648) and the "863" State High Tech Programs (2012AA101106).

Author contribution

Study Concepts: Cheng Wang, H. X.; Study Design: C. W.; Material Preparation: Y.G., J.F.H.; Literature Research: C. W., B. G.; Data analysis/Interpretation: C. W.; Statistical analysis: C. W., H. X., L. Y.; Manuscript Preparation: C. W., J. Z.; Manuscript definition of intellectual content: C. W.; Manuscript editing: C. W.; Manuscript Revision/Review: C. W., J. H.; Manuscript Final Version Approval: C. W.All authors read and approved the manuscript.

REFERENCES

Arora, A., S. Valcic, S. Cornejo, M. G. Nair, B. N. Timmermann and D. C. Liebler. 2000. Reactions of Genistein with alkylperoxyl radicals. Chem. Res. Toxicol. 13(7): 638-645.

Preinerstorfer, B. and G. Sontag. 2004. Determination of isoflavones in commercial soy products by HPLC and coulometric electrode array detection. Eur. Food Res. Technol. 219: 305-310.

Benhamou, N. and M. Nicole. 1999. Cell biology of plant immunization against microbial infection: The potential of induced resistance in controlling plant diseases. Plant Physiol. Biochem. 37(10): 703-719.

Bradbury, P. J., Z. W. Zhang, D. E. Kroon, T. M. Casstevens, Y. Ramdoss and E. S. Buckler. 2007. TASSEL: Software for association mapping of complex traits in diverse samples. Bioinformatics. 23: 2633-2635.

Brouns, F. 2002. Soya isoflavones: A new and promising ingredient for the health food sector. Food Res. Int. 35(2-3): 187-193.

Choi, J. S., T. W. Kwon and J. S. Kim. 1996. Isoflavone contents in some varieties of soybean. Korean Food Biotechnol. 5: 167-169.

Cregan, P. B., T. Jarvik, A. L. Bush, R. C. Shoemaker, K. G. Lark, A. L. Kahler, T. T. Van Toai, D. G. Lohnes, J. Chung and J. E. Specht. 1999. An integrated genetic linkage map of the soybean genome. Crop Sci. 39: 1464-1490.

Doyle, J. J. and J. L. Doyle. 1990. Isolation of plant DNA from fresh tissue. Focus. 12: 13-15.

Evanno, G., S. Regnaut and J. Goudet. 2005. Detecting the number of clusters of individuals using the software structure: Asimulation study. Ecol. Entomol. 14: 2611-2620.

Zeng, G., D. Li, Y. Han, W. Teng, J. Wang, L. Qiu and W. Li. 2009. Identification of QTL underlying isoflavone contents in soybean seeds among multiple environments. Theor. Appl. Genet. 118: 1455-1463.

Guo, X. M., D. H. Wang, S. G. Gordon, E. Helliwell, T. Smith, S. A. Berry, S. K. St Martin and A. E. Dorrance. 2008. Genetic mapping of QTLs underlying partial resistance to *Sclerotinia sclerotiorum i*n soybean PI 391589A and PI 391589B. Crop Sci. 48: 1129-1139.

Hsu, C. S., W. W. Shen and Y. M. Hsueh. 2001. Soybean isoflavones supplementation in postmenopausal women. Effects on plasma lipids, antioxidant enzyme activities and bone density. J. Reprod. Med. 46(3): 221-226.

Hwang, J., H. N. Hodis and A. Sevanian. 2001. Soy and alfalfa phytoestrogen extracts become potent low-density lipoprotein antioxidants in the presence of acerola cherry extract. J. Agric. Food Chem. 49(1): 308-314.

Hoeck, J. A., W. R. Fehr, P. A. Murphy, and G. A. Welke. 2000. Influence of genotype andenvironment on isoflavones concentrations of soybean. Crop Sci. 40: 48-51.

Jakobsson, M. and N. A. Rosenberg. 2007. CLUMPP: A cluster matching and permutation program for dealing with labels witching and multimodality in analysis of population structure. Bioinformatics. 23: 1801-1806.

Isanga, J. and G. N. Zhang. 2008. Soybean bioactive components and their implications to health - A review. Food Rev. Int. 24: 252-276.

Gutierrez-Gonzalez, J. J., X. Wu, J. Zhang, J. D. Lee, M. Ellersieck, J. G. Shannon and O. Yu. 2009. Genetic control of soybean seed isoflavones concentration: Importance of statistical model and epistasis in complex traits. Theor. Appl. Genet. 119: 1069-1083.

Kassem, M. A., K. Meksem, V. Njiti, W. J. Banz, T. A. Winters and A. J. Wood. 2004. Definition of soybean genomic regions that control seed phytoestrogen amounts. J. Biomed. Biotechnol. 2: 52-60.

Kassem, M. A., J. Shultz, K. Meksem, Y. Cho and A. J. Wood. 2006. An updated 'Essex' by 'Forrest' linkage map and first composite interval map of QTL underlying six soybean traits. Theor. Appl. Genet. 113: 1015-1026.

Kim, E. H., J. I. Chung, H. Y. Chi, J. A. Kim and I. M. Chung. 2006. Analysis of phenolic compounds and isoflavones in soybean seeds (*Glycine max* (L.) Merill) and sprouts grown under different conditions. Eur. Food Res. Technol. 222: 201-208.

Knight, D. C. and J. A. Eden. 1996. A review of the clinical effects of phytoestrogens. Obstet. Gynecol. 87: 897-904.

Lee, H. P., L. Gourley, S. W. Duffy, J. Esteve and N. E. Day. 1991. Dietary effects on breast cancer in Singapore. Lancet. 537: 1197-1200.

Lee, S. J., W. Yan and A. Chung. 2002. Effects of year, site, genotype and their interactions on various soybean isoflavones. Field Crops Res. 4150: 1-12.

Li, Y., C. B. Hill, S. R. Carlson, B. W. Diers and G. L. Hartman. 2007. Soybean aphid resistance genes in the soybean cultivars Dowling and Jackson map to linkage group M. Mol. Breeding. 19: 25-34.

Lucimara, C., L. K. Naoel and N. D. Piovesan. 2006. Inheritance of isoflavones concentrations in soybean seeds. Euphytica. 150: 141-147.

Mebrahtu, T., A. Mohamed, C. Y. Wang and T. Andebrhan. 2004. Analysis of isoflavones concentrations in vegetable soybeans. Plant Foods Hum. Nutr. 59: 55-61.

Meksem, K., V. Njiti, W. J. Banz, M. M. Kassem, D. Hyten, J. Yuang and T. A. Winters. 2001. Molecular markers of phytoestrogen content in soybeans. J. Biomed. Biotechnol. 1: 38-44.

Messina, M., O. Kucuk and J. W. Lampe. 2006. An overview of the health effects of isoflavones with an emphasis on prostate cancer risk and prostate-specific antigen levels. J. Aoac. Int. 89: 1121-1134.

Messina, M. 2005. Soyfoods and soybean isoflavones for promoting bone health. Agro Food Ind. Hi-Tech. 16: 30-32.

Morris, P. F., M. E. Savard, and E. W. B. Ward. 1991. Identification and accumulation of isoflavonoids and isoflavone glucosides in soybean leaves and hypocotyls in resistance responses to *Phytophthora megasperma* f.sp. glycinea. Physiol. Mol. Plant P. 39(3): 229-244.

Murphy, S. E., E. A. Lee, L. Woodrow, P. Seguin, J. Kumar, I. Rajcan and G. R. Ablett. 2009. Genotype environment interaction and stability for isoflavones concentration in soybean. Crop Sci. 49: 1313-1321.

Njiti, V. K., D. A. Meksem, W. J. Banz and T. A. Winters. 1999. Molecular markers of phytoestrogen content in soybeans. J. Med. Food. 2: 165-167.

Primomo, V. S., V. Poysa, G. R. Ablett, C. J. Jackson, M. Gijzen and I. Rajcan. 2005. Mapping QTL for individual and total isoflavone content in soybean seeds. Crop Sci. 45: 2454-2464.

Pritchard, J. K., M. Stephens, N. A. Rosenberg and P. Donnelly. 2000. Association mapping in structured populations. Hum. Genet. 67: 170-181.

Barnes, S. 2010. The biochemistry, chemistry and physiology of the isoflavones in soybeans and their food products. Lymphatic Res. Biol. 8(1): 89-98.

Trigizano, R. N. and Caetano-Anolles, G. 1998. Laboratory exercises on DNA amplification fingerprinting for evaluating the molecular diversity of horticultural species. Horttechnology. 8: 413-423.

Tai, T. Y., K. S. Tsai, S. T. Tu, J. S. Wu, C. I. Chang, C. L. Chen, N. S. Shaw, H. Y. Peng, S. Y. Wang and C. H. Wu. 2012. Effect of soy isoflavones on bone mineral density in postmenopausal Taiwanese women with bone loss: A 2-year randomized double-

blind placebo-controlled study. Osteoporos. Int. 23: 1571-1580.

Vyn, T. J., X. Yin, T. W. Bruulsema, C. C. Jackson, I. Rajcan and S. M. Brouder. 2002. Potassium fertilization effects on isoflavone concentrations in soybean (Glycine max (L.) Merr.). J. Agric. Food Chem. 50: 3501-3506.

Wang, Y., Y. Han, W. Teng, X. Zhao, Y. Li, L. Wu, D. Li and W. Li. 2014. Expression quantitative trait loci infer the regulation of isoflavone accumulation in soybean (Glycine max L. Merr.) seed. BMC Genomics. 15: 680.

Zhang, J., C. Hao, Q. Ren, X. Chang, G. Liu and R. Jing. 2011. Association mapping of dynamic developmental plant height incommon wheat. Planta. 234: 891-902.