Journal of Life Science 2008 Vol. 18. No. 12. 1665~1670 ©JLS / ISSN 1225-9918

Analysis of Quantitative Trait Loci (QTLs) for Unsaturated Fatty Acid Contents in Soybean Seed Using Recombinant Inbred Lines

Hyeun-Kyeung Kim, Moo-Hyeog Im[1] and Myoung-Gun Choung[2]*

Major of Horticulture Bioscience, Collage of Natural Resource & Life Science, Pusan National University, Milyang 627-706, Korea
[1]*Dept. of Food Safety Evaluation, Korea Food & Drug Administration, Seoul 122-704, Korea*
[2]*Dept. of Herbal Medicine Resource, Kangwon National University, Samcheok 245-711, Korea*

Received November 28, 2008 / Accepted December 2, 2008

Soybean oil is an important source of vegetable oil for human food and nonfood applications and accounts for approximately 22% of the world's total edible oil production. Improvement of the quality and quantity of soybean seed oil constituents is one of the most important objectives in soybean breeding. The objective of this study was to identify quantitative trait loci (QTLs) that control oleic, linoleic, and linolenic acid contents in soybean. The 117 $F_{2:10}$ recombinant inbred lines (RIL) developed from a cross of 'Keunolkong' and 'Shinpaldalkong' were used. Narrow-sense heritability estimates based on a plot mean on seed weight, protein and oil content were 0.85, 0.82 and 0.81, respectively. Eight independent QTLs for oleic acid content were identified from linkage group (LG) A2, C1, D2, F, G, L, and O. Seven QTLs for linoleic acid content were located on LG D1b, E, H, I and L. Oil content was related with five QTLs located on LG C1, H, J, K, and L. Oleic, linoleic, and linolenic acid have two common QTLs on LG C1 and L. Thus, we identified major loci improving soybean oil quality.

Key words : Soybean, unsaturated fatty acid, QTL, SSR marker

Introduction

Soybean oil is an important source of vegetable oil for human food and nonfood applications and accounts for approximately 22% of the world's total edible oil production [5,10]. Soybean oil consists mainly of palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acid (wilson, 1991). Conventional soybean cultivars oil with an average of 15.0% saturated fatty acid (palmitic and stearic acid) and 85% unsaturated fatty acid (oleic, linoleic and linloenic acid). The fatty acid composion of soybean oil completely determines its nutritional value [7], storage compatibility [15], industrial properties [12] and potential food applications [14,19].

On the other hand, plamitic acid and lonolenic acid was known as representative fatty acid that deteriorate quality of smell, taste etc.. among soybean's fatty acid. Specially, reduction of palmitic acid content of soybean oil would lower the total saturated fatty acid content and improve the oil quality for human consumption. Also, may reduce fatty acid's regeneration by reducing linolenic acid content in soybean oil. Like this, can make soybean oil by fatty acid of good quality by doing. Therefore, the manipulation of soybean oil quality by altering fatty acid composition is an important breeding objective in the world [18,21].

Previously, we reported that many mutant allele contribute to alter the fatty acid content in soybean oil [17]. Recent advances in molecular marker technology, especially the development of SSR markers in soybean and an integrated soybean genetic mapping and dissection of qualitative and quantitative traits in soybean [16]. Brummer et al. [2] mapped the *fan* allele controlling reduced linolenic acid from C1640 on LG-B2. With mapping population formed from *Glycine max* × Glycine soja Siebold & Zucc., Diers and Shoemaker [3] mapped QTL conditioning five major fatty acids mainly on two linkage groups of the USDA/ISU map using RFLP marker. The primary objective of the present research was to improve the breeding efficiency of unsaturated fatty acid quality in soybean. This study was conducted to identify simple sequence repeats (SSR) markers associated with QTLs and marker-assisted selection (MAS) for oleic, linoleic, and linolenic acid contents in soybean.

*Corresponding author
Tel : +82-33-570-6491, Fax : +82-33-570-6499
E-mail : cmg7004@kangwon.ac.kr

Materials and Methods

Plant materials and field evaluation

The well-characterized soybean cultivars, 'Keunolkong'

and 'Shinpaldalkong' were used as mapping parents. 'Keunolkong' is the pure-line derived from a local variety selected in Korea. It possesses early maturity, large seed size, high protein content, and low oil content. 'Shinpaldalkong' is the typical cultivars released from systemic breeding programs through the deliberate crossings, 'Will'× ('Elf'× SS74185). 'Shinpaldalkong' showed the late maturity, small seed size, low protein content, and high oil content.

The RIL populations were obtained from the crosses of 'Keunolkong' and 'Shinpaldalkong'. The cross 'Keunolkong' ×'Shinpaldalkong' (K/S) generated 117 F_{10} RILs derived from individual F_2 plants by single seed descent (SSD). The F_{10} seeds of each line were planted in a randomized complete-block design with two replications at NICS, RDA, Milyang, Korea in 2001. Each entry was planted in a 1.5 m long paired-row plot with two seeds per hill. Spacing was 60 cm between rows and 10 cm between plants. Seed weight was determined by weighting 100 seeds per plot.

Determination of fatty acids

In order to determine the composition of soybean fatty acids, solvent extract oil was used. About 50 mg of oils was placed in a screw-capped vial, and 5 ml of methylation solution (H_2SO_4 : MeOH : toluene = 1 ml : 20 ml : 10 ml) was added. The sealed vial was heated on a water bath (100°C) for 60 min, and allowed to cool. After 5 ml of water and 5 ml of diethyl ether were added and shaken. The mixture was separated two layer, and the upper layer (diethyl ether) was taken by Pasteur pipette. The diethyl ether layer was dried using anhydrous sodium sulfate for 5 min. Then the 1 μl of diethyl ether solution was directly injected on to the GLC. A DS 6200 (DONAM Instruments Inc., Korea) gas chromatography with a flame ionization detector (FID) and 0.32 mm i.d. × 25 m HP-FFAP capillary column was used. The oven temperature was raised from 140°C (2 min holding) to 200°C at a constant rate of 8°C per min, and then held 10 minutes. The injector and detector port temperature were kept at 230 and 250°C, respectively. The carrier gas was nitrogen at a flow rate of 0.5 ml per min, and the split ratio at the injector port was 50:1.

DNA isolation and analysis

Genomic DNA was isolated from fresh leaves following the procedure described by Keim et al. [8]. A total of 199 soybean SSR markers (http://soybase.agron.iastate.edu/ssr.htm) were used to screen for polymorphisms between mapping parents. The primer pairs showing parental polymorphisms were used for SSR genotyping in RIL progenies. The PCR reaction was performed in a total volume of 10 μl containing 25 ng of template DNA, 0.15 M of each forward and reverse primers, 200 M of each dNTP, 2 mM $MgCl_2$, 0.1% Triton X-100, 1× reaction buffer (10 mM Tris-HCl pH 8.5, 100 mM KCl) and 0.5 U of *Taq* DNA polymerase (BioBasic Taq Polymerase, Applied Bio Basic, Canada). Template DNA was initially denaturated at 94°C for 2 min, followed by 40 cycles of PCR amplification using the following conditions; denaturation at 94°C for 25 sec, annealing at 47°C for 25 sec and extension at 68°C for 60 sec on a 96-Well GeneAmp PCR system 9700 (Applied Biosystems, USA).

Map construction and statistical analysis

Means of traits, correlation, and analysis of variance were determined by SAS program (SAS, 2002). Narrow-sense heritability was calculated on a per-plot basis, using estimates of the variance components [4]. Based on the segregation data subsets for SSRs and morphological markers, we constructed a linkage map with MapManager QT version 2.8 software [11]. Recombination fractions were converted to map distances by applying the Haldane map function [6].

The association between marker and QTL was tested according to the interval mapping methods of Whittaker et al. [20], using MapManager QT and single-factor ANOVA (SF-ANOVA). For each SSR and morphological marker, the class means for seed weight, protein and oil concentration were compared for significance ($p<0.05$) using an *F*-test from the Type Ⅲ mean squares, as obtained from the General Linear Model (GLM) procedure of SAS.

If SF-ANOVA identified two or more linked markers associated with the seed weight, protein and oil content, multiple regression analysis was conducted by including all the significant markers on that linkage group in the model (SLG-Regr). All significant markers from the SLG-Regr analysis were then combined into a multiple-linkage group regression (MLG-Regr) at $p<0.05$ to determine the combination of independent markers those were explaining the greatest amount of phenotypic variation in a given trait. The probability level of 0.05 was selected to enhance our ability to detect QTLs associated with seed weight, protein and oil concentration. Finally, the coefficient of determination (R^2) obtained from MLG-Regr was used to provide an estimate of the percentage phenotypic variation explained by the markers.

Table 1. Descriptive statistics for oil, oleic acid, linoleic acid and linolenic acid contents in 117 RIL population of 'Keunolkong' × 'Shinpaldalkong'

Traits	Parents		RIL population		$h^{2\dagger}$
	Keunolkong (mean±SD)	Shinpaldalkong (mean±SD)	Range	Mean±SD	
Oil content	17.32±0.37	20.10±0.80	16.82~24.56	20.60±1.48	0.71
Oleic acid	27.45±0.58	24.36±1.75	20.94~41.19	27.68±3.73	0.85
Linoleic acid	51.31±0.91	53.99±0.93	39.69~46.76	50.94±3.14	0.82
Linolenic acid	7.67±0.17	7.71±0.14	4.49~9.13	6.84±0.73	0.81

† Heritability on a per-plot basis.

Results and Discussion

Oleic, linoleic, and linolenic acid contents

Variation of oil content [9], oleic, linoleic, and linolenic acid contents in the $F_{2:10}$ RIL population is presented in Table 1 and Fig. 1. 'Keounolkong' shows low oil and linolenic acid content, and high oleic and linoleic acid contents while 'Shinpaldalkong' is characterized with high oil and linolenic acid contents, and low oleic and linolenic acid contents. There was significant difference (p<0.05) among the RIL for each traits in the population. Transgressive segregation for unsaturated fatty acid was found a few lines being significantly greater or lower than high and low parents (Fig. 1).

Oleic acid content ranged from 20.94 to 41.19% and linoleic acid content ranged from 39.69 to 46.76%, and linolenic acid content ranged 4.49 to 29.13%. Narrow-sense heritability of oleic, linoleic, and linolenic acid contents were 0.85, 0.82 and 0.81, respectively (Table 1). There was a strongly negative correlation between oil and linolenic acid content (r=-0.413, p<0.001), oleic acid and linoleic acid content (r=-0.913, p<0.001), and oleic acid and linolenic acid contene (r=-698, p<0.001). On the other hand, linoleic acid content was strongly positively correlated with linolenic acid content (r=0.559, p<0.001) (Table 2).

The heritability observed in our population for indicated that selection response would be reasonable for achieving genetic gain.

Construction of linkage map based on RIL

Based on the RIL F_{10} generation, a genetic linkage map with 108 SSR markers and two morphological markers (total 110) was constructed. The map covered a distanced 1,890 cM of the soybean genome using the Haldane function, comprising 19 linkage groups. On average, this map revealed a marker density of 1.0 per17.2 cM. The order of most of

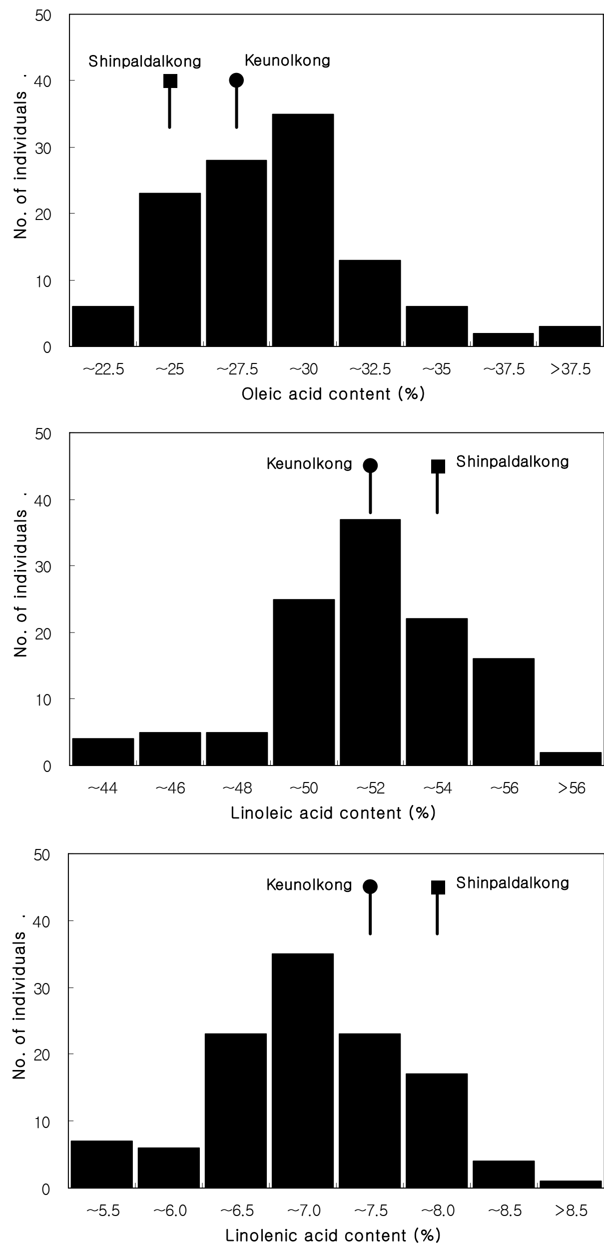

Fig. 1. Frequency distribution of oleic acid, linoleic acid and linolenic acid contents in 117 RILs of 'Keunolkong' × 'Shinpaldalkong'.

Table 2. Correlation coefficients among oil, oleic acid, linoleic acid and linolenic acid contents in 117 RILs of 'Keunolkong' × 'Shinpaldalkong

	Oil content	Oleic acid	Linoleic acid
Oleic acid	-0.055^{ns}		
Linoleic acid	0.134^{ns}	-0.963***	
Linolenic acid	-0.413***	-0.698***	0.559***

*** Significant at 0.001 probability level.
ns, Not significant (p>0.05).

the markers was in agreement with the public soybean molecular linkage map [16].

QTL analysis for seed traits

The SF-ANOVA analysis identified fifteen markers (data not show) as potentially associated with oleic acid content. MLG-Regr analysis showed that eight QTL markers on LG A2, C1, D2, F, G, L, and O were significantly associated with oleic acid content (Table 3). Individual QTLs explained relatively phenotypic variation (4.54~11.98%) though they accounted for total phenotypic variations of 53.19% for oleic acid content. One major QTL, satt190 on LG C1, explained 11.98% of the phenotypic variation.

The SF-ANOVA revealed that thirteen markers (data not show) were detected as potentially associated with linolwic acid content. MLG-Regr analysis identified seven QTLs on LG A1, C1, D1b, D2, F, and G (Table 3). Individual QTLs explained relatively phenotypic variation (4.27~10.08%) though they accounted for total phenotypic variations of 45.41% for linoleic acid content

Based on the SF-ANOVA analysis in th RIL population, twenty-four markers (data not show) were found significantly (p<0.05) associated with linolenic acid content. Five QTLs were identified on LG C1, H, J, K and L. In the MLG-Regr analysis, those QTLs explained 40.37% of total phenotypic variation of the linolenic acid content. One major QTL, satt294 on LG C1, explained 16.44% of the phenotypic variation (Table 3).

A lot of QTLs have been reported in soybeans. But, QTL regarding fatty acid is less relatively. QTLs for oleic acid content were reportedly located in linkage groups A1, A2, and E. QTLs of linolenic acid content were reported in linkage groups A1, B1, E, K, and L. QTLs for linolenic acid content were reported in linkage groups E, K, and L [1,3,13].

Table 3. Marker distributions and QTLs associated with oleic acid, linoleic acid and linolenic acid contents in 117 RIL of 'Keunolkong' × 'Shinpaldalkong

Traits	LG	Markers	SF-ANOVA†		Alleic means		MLG-Regra	
			P	R^2 (%)	K/K‡	S/S‡	P	R^2 (%)
Oleic acid	A2	satt177	0.049	3.49	27.28	28.67	0.012	4.87
	C1	satt190	0.006	6.88	28.75	26.75	0.001	11.98
	D2	satt458	0.044	3.65	27.09	28.5	0.014	5.33
	F	satt334	0.034	3.91	26.9	28.37	0.007	6.02
	G	satt324	0.006	6.7	28.55	26.59	0.003	9.2
	G	satt472	0.033	4.24	27.1	28.64	0.008	4.54
	L	satt495	0.002	7.86	26.54	28.63	0.01	6.3
	O	satt592	0.044	3.65	28.22	26.78	0.008	4.94
Linoleic acid	A2	satt177	0.0444	3.62	51.29	50.09	0.0233	4.47
	C1	satt190	0.0205	4.96	50.19	51.63	0.0037	10.08
	D1b	satt216	0.0149	5.13	50.30	51.73	0.0042	7.93
	D2	satt226	0.0400	3.74	51.55	50.33	0.0187	4.27
	F	satt334	0.0047	6.87	51.80	50.15	0.0051	8.53
	G	satt324	0.0076	6.25	50.23	51.83	0.0186	4.54
	G	satt472	0.0277	4.53	51.44	50.08	0.0139	5.59
Linolenic acid	C1	satt294	0.013	5.62	6.65	6.99	3E-04	16.44
	H	satt279	9E-04	9.99	6.58	7.06	0.028	4.43
	J	satt285	3E-04	11.23	7.09	6.61	0.013	6.34
	K	satt137	0.009	5.86	7	6.64	0.006	8.62
	L	satt513	0.032	3.99	6.68	6.97	0.03	4.54

† SF-ANOVA: single-factor analysis of variance, MLG-Regr: multiple regression with all significant markers from the SLG-Regr model.
‡ K/K: Keunolkong, S/S: Shinpaldalkong.

For both unsaturated fatty acid contents, we observed that different allele effects depended on the chromosomal background. For example, the 'Keunolkong'- derived marker allele on LG C1 increased the contents of oleic and linoleic acid. On the other side, reverse effect was seen with the linolenic acid content. This result is exactly agreed with the result that the linolenic acid and oleic acid contents are negatively correlated with each other. And, the linolenic acid and linoleic acid contents are positively correlated with each other. The both those contents are controlled in the same direction under the same genes. Nevertheless, we detected no tentative QTLs in LG C1 that were related to oleic, linoleic, and linolenic acid contents. Therefore, further study is needed to confirm whether those QTLs are real or that, instead, environmental factors affected those results. Finally, we hope that the QTLs identified from this study and recent germplasm line that we have relased will benefit breeders in accumulation favorable alleles for improvement in soybeaqn seed quality.

Acknowledgement

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2006-331-F00002).

References

1. Brummer, E. C., A. D. Nickell, J. R. Wilcox and R. C. Shoemaker. 1995. Mapping the Fan locus controlling linolenic acid content in soybean oil. *J. Hered.* **86,** 245-247.
2. Brummer, E. C., G. L. Graef, J. Orf, J. R. Wilcox and R. C. Shoemaker. 1997. Mapping QTL for seed protein and oil content eight soybean populations. *Crop Sci.* **37,** 370-378.
3. Diers, B. W. and R. C. Shoemaker. 1992. Restriction fragment length polymorphism analysis of soybean fatty acid content. *J. Am. Oil. Chem.* **69,** 1242-1244.
4. Frey, K. J. and T. Horner. 1957 Heritability in standard units. *Agron. J.* **49,** 59-62.
5. Glaudements, H. D., M. M. J. Timmermans and H. Rijkse. 1998. The world of edible oils. pp. 1-5, Robobank International Marketing, Utrecht, the Netherlands.
6. Haldane, J. B. S. 1919. The combination of linkage values and the calculation of distances between the loci of linked factors. *J. Genet.* **8,** 299-309.
7. Hu, F. B., M. J. Stampfer, J. E. Manson, E. Rimm, G. A. Colditz, B. A. Rosner, C. H. Hennekens and W. C. Willett. 1997. Dietary fat intake and the risk of coronary heart disease in women. *N. Eng. J. Med.* **337,** 1491-1499.
8. Keim, P., T. C. Olson and R. C. Shoemaker. 1988. A rapid protocol for isolating soybean DNA. *Soybean Genet. Newsl.* **15,** 150-154.
9. Kim, H. K., S. T. Kang, M. G Chung, C. S. Jung, K. W. Oh, I. Y. Baek and B. G. Son. 2006. Simple sequence repeat markers linked to quantitative trait loci controlling seed weight, protein and oil contents in soybean. *J. Life Sci.* **16,** 949-954.
10. Li, Z., R. F. Wilson, W. E. Rayford and H. R. Boerma. 2002. Molecular mapping genes conditioning reduced palmitic acid content. *Crop Sci.* **42,** 373-378.
11. Mainly, K. F. and J. M. Olson. 1999. Overview of QTL mapping software and introduction to Map Manager QT. *Mamm. Genome* **10,** 327-334.
12. Miller, J. F., D. C. Zimmerman and B. A. Vick. 1987. Genetic control of high oleic acid content in sunflower oil. *Crop Sci.* **27,** 923-926.
13. Maughan, P. J., M. A. Saghai, G. R. Buss. 2000. Identification of quantitative trait loci controlling sucrose content in soybean (*Glycine max*). *Mol. Breed.* **6,** 105-111.
14. Rakow, G. and D. I. McGegor. 1973. Opportunities and problems in modification of levels of rapeseed C18 unsaturated fatty acid. *J. Am. Oil Chem. Soc.* **50,** 400-403.
15. Robertson, J. A. and J. K. Tomas. 1976. Chemical and microbial changes in dehulled confectionary sunflower kernels during storage under controlled conditions. *J. Milk Food Technol.* **39,** 18-23.
16. Song, Q. J., L. F. Marek, R. C. Shoemaker, K. G. Lark, V. C. Concibido, X. Delannay, J. E. Specht and P. B. Cregan. 2004. A new integrated genetic linkage map of the soybean. *Theor. Appl. Genet.* **109,** 122-128.
17. Tagagi, Y., S. M. Rahman and T. Anai. 1998. Construction of novel fatty acid composition in soybean oil by induced mutation. *Gamma Field Symposia* **37,** 17-28.
18. Topfer, R., N. Martini and J. Schell. 1995. Modification of plant lipid synthesis. *Science* **268,** 681-686.
19. Vles, R. O. and J. J. Gottrnbos. 1989. Nutritional characteristics and food use of vegetable oils. In "Oil crops of the World, Their Breeding and Utilization" Robbelen, G. et al. (eds.), McGraw-Hill, New York. pp. 63-86.
20. Whittaker, J. C., R. Thompson, D. M. Vissche. 1996. On the mapping of QTL by regression of phenotype on marker type. *Heredity* **77,** 23-32.
21. Wilson, R. F., J. W. Burton and C. A Brim. 1981. Progress in the selection for altered fatty acid composition in soybean. *Crop Sci.* **27,** 788-791.

초록 : 콩에서 microsatellite marker를 이용한 불포화지방산 함량의 양적형질 유전자좌의 분석

김현경 · 임무혁[1] · 정명근[2]*
(부산대학교 생명자원개발연구소, [1]식품의약품안전청 식품평가부, [2]강원대학교 생약자원개발학과)

콩의 oil은 식량유지 자원으로서 매우 중요한 부분을 차지하고 있으며, 전세계 식용유의 22%를 콩 oil이 차지하고 있으며 식품에서 매우 중요한 영양학적인 요소이다. 이중 불포화지방산은 지방산 중에서 종자 구성물질들은 poly-genetic 형질들로 되어있다. 본 시험은 큰올콩과×신팔달콩의 RIL 계통과 SSR marker를 이용하여 유전자지도를 작성하고, 이를 바탕으로 불포화지방산의 함량과 관련된 양적형질 유전자좌(QTLs)를 탐색하였다. Oleic acid 함량과 관련된 QTLs는 7개의 연관군에서 8개의 마커가 확인되었으며, linoleic acid는 5개의 연관군에서 7개의 마커가 확인되었다. 그리고 linolenic acid는 5개의 연관군에서 각각 하나씩의 마커가 확인되었다. 본 시험의 결과 불포화지방산에 공통적으로 나타난 QTL은 연관군 C1과 L이었다.