Bull. Fac. Agr., Saga Univ. No. 83 : 37~42 (1998)

Genetic Analysis of Restriction Fragment Length Polymorphism on the Fatty Acid Synthesis in Soybean Mutants and Their Progenies: II. High oleic acid mutants with two microsomal ω-6 fatty acid desaturase cDNAs as probes

Takehito KINOSHITA, Shaikh Mizanur RAHMAN, Toyoaki ANAI and Yutaka TAKAGI
(Laboratory of Plant Breeding)
Received September 7, 1998

Summary

Mutant lines of soybean [*Glycine max* (L.) Merr.] , M11 and M23, were selected from X-ray treated Bay cultivar. While the oleic acid content of Bay was about 27.8% of total fatty acid, the contents of this fatty acid in these mutants were about 30.8%, and 48.6%, respectively. The remarkable increases of oleic acid content in these mutants were expected to be the result of mutations at the *Ol* loci induced by the X-ray irradiation. In this study, we analyzed for mutations by restriction fragment length polymorphism (RFLP) analysis using a microsomal ω-6 fatty acid desaturase cDNA as a probe. This probe hybridized with one band (4.6 kbp) in Bay and M11, but was absent in M23. Forty plants grown from the embryonic part of F_2 seeds made from a cross between M23 and Bay were analyzed with the same method. The F_2 seeds were also analyzed by gas chromatography for their oleic acid content. The result of this study showed that the segregation ratio for intensities of the 4.6 kbp band fitted the expected 1 : 2 : 1 ratio (Bay : F1 : M23 ; $\chi^2=0.16$, $P>0.80$). Moreover, the intensities of this fragments were completely comparable with their oleic acid contents. These results suggest that the high oleic acid content in M23 line was caused by some nucleotide modification on the *Ol* locus encoding for an isozyme of microsomal ω-6 fatty acid desaturase.

Key words: *Glycine max*, *Ol* locus, RFLP analysis, oil quality, microsomal ω-6 fatty acid desaturase

Introduction

The unsaturated fatty acids are essential nutrition in human diets. However, the polyunsaturated fatty acids easily oxidize and form the undesirable odors and flavors[1]. The oxidization of polyunsaturated fatty acids is the most important problem to keep the dietary oil quality. In the soybean oil, linoleic acid is an abundant fatty acid that has two unsaturated bonds. The linoleic acid is produced from oleic acid by desaturation at ω-6 position with ω-6 fatty acid desaturase. Oleic acid is the most stable unsaturated fatty acid in soybean oil. To improve the soybean oil quality, research must be done to increase

the oleic acid content and decrease the linoleic and linolenic acid content. The mutant lines, M11 and M23 were established by X-ray treated soybean cultivar Bay. The oleic acid content of M11 and M23 were about 30.8% and 48.6%, respectively, compared with 27.8% of their original cultivar[2),3)]. Previously, we reported that these mutants were derived from the mutations at only one locus *Ol*, because no segregation of oleic acid content could be shown in the cross of M11 and M23[3),4)].

Recently, the molecular basis of fatty acid synthesis was clarified with biochemical and molecular biological analysis[5)]. One distinct pathway for the biosynthesis of polyunsaturated fatty acids exists in plastid and the other one exists in endoplasmic reticulum. Seed storage oil is mainly synthesized in later pathway[5)]. The cDNA encoding microsomal ω-6 fatty acid desaturase was first isolated from *Arabidopsis thaliana* as *FAD2* gene[6)]. On the other hand, two clones encoding microsomal ω-6 fatty acid desaturase were isolated from soybean cDNA library and the analysis of their expression was reported[7)].

In this study, we tried to determine the molecular basis of soybean mutants harboring *Ol* locus for high oleic acid content using hybridization technique with two microsomal ω-6 fatty acid desaturase cDNA (*FAD2-1 and FAD2-2*) as probes.

Materials and Methods

Plant materials

The soybean lines used in this study were M11 and M23 mutants, and their original cultivar Bay. The F_1 and F_2 seeds of the cross M23 with Bay, and parental seeds were harvested in the field at Saga University in 1997. The half of dry seeds were cut off and subjected to aualyze the fatty acid composition, and the remaining part of dry seeds with embryo were planted in the field for DNA preparation.

Fatty acid analysis

Fatty acid composition was determined by gas chromatography, as described earlier[8)].

Probe preparation

A cDNA of microsomal ω-6 fatty acid desaturase was prepared from total RNA of developed soybean (Bay cultivar) seeds by RT-PCR method as described earlier[9)] except for PCR reactions. Two pairs of oligonu-

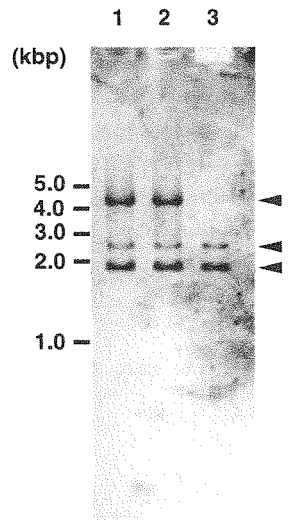

Fig. 1 Hybridization patterns of soybean genotypes with a microsomal ω-6 fatty acid desaturase probe. Total DNA was digested with *Eco*RI and molecular weights were given in kbp for the 1kbp DNA ladder (NEW ENGLAND Biolabs). Two μg of Bay (lane 1), M11 (lane 2) and M23 (lane 3) were subjected for southern-blot analysis. Three bands (1.9, 2.5 and 4.6 kbp) hybridized with the probe indicate with arrow heads.

cleotides for *FAD2-1* (ER-ω-6-1-F, 5'-atgggtctagcaaaggaaac-3' ; ER-ω-6-1-R, 5'-accatgatcgcaacaagctg-3') and for *FAD2-2* (ER-ω-6-2-F, 5'-cagattgtgtgttgatgggggc-3'; ER-ω-6-2-R, 5'-caaaaccgctatgcaactgtct-3') were designed to amplify the coding region of microsomal ω-6 fatty acid desaturase cDNAs[7]. The PCR reactions, subcloning, sequencing and labeling procedures were previously described[10].

Southern-blot analysis

The DNA extraction, quantification, and southern-blot analysis were carried out with same conditions as described earlier[10].

Results and Discussion

Soybean genotypes M11 and M23 were isolated as high oleic acid mutants from the X-ray treated population of Bay cultivar. Each of these mutants harbors a mutation at a same Ol locus. The oleic acid contents of these mutants were about 30.8% and 48.6%, and the linoleic acid contents were about 46.9% and 29.5%, respectively[2),3),4)]. The original cultivar Bay contains about 27.8% of oleic acid and 51.7% of linoleic acid. It may reasonable to expect that their mutations inhibit to produce linoleic acid from oleic acid, because the oleic acid contents were clearly increased and the linoleic acid contents were clearly decreased in these mutants than that of Bay. Microsomal ω-6 fatty acid desaturase is an essential enzyme to convert oleic acid to linoleic acid in microsome, and was first isolated from *Arabidopsis thaliana* as *FAD2* gene[6]. Two clones (*FAD2-1* and *FAD2-2*) were also isolated from soybean cDNA library by heterologous hybridization method[7].

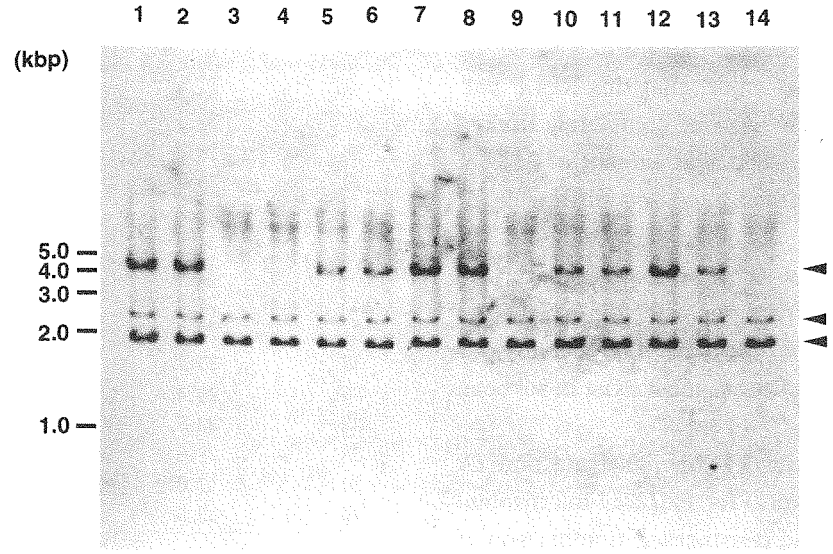

Fig. 2 Hybridization patterns of Bay, M23 and their progenies with same probe. Bay (lanes 1 and 2), M23 (lanes 3 and 4), F_1 (lanes 5 and 6) and F_2 plants (lanes 7 to 14) were analyzed with same as Figure 1. Each F_2 plant was completely comparable with No. 1 to 8 plant of Table 1.

With *FAD2-1* as a probe, the line M23 lacked 4.6 kbp band, whereas approximately 1.9, 2.5 and 4.6 kbp bands were observed in Bay and M11 (Fig. 1). In contrast, there was no difference between these lines with *FAD2-2* as a probe (data not shown). The differential RFLP pattern of *FAD2-1* gene could suggest that the high oleic acid phenotype of M23 line was caused by some modification on the nucleotide sequence of this gene. The modification could be a partially deleted at the coding region of this gene and the product of this gene could be inactive. It may support that the phenotype of M23 was more strict than that of M11. The mutation of M11 could be occured due to partially decrease of enzymatic activity.

The intensities of 4.6 kbp band in F2 plants were divided into three types, Bay, F1 and M23 (lanes 7-14, Fig. 2). The oleic acid content in F2 seeds was also devided into three groups. Segregation for the intensity of band and oleic acid contents were found to be completely associated and fitted the expected 1:2:1 ratio (Table 1). These results support that this RFLP pattern of *FAD2-1* was completely linked with the high oleic acid content of M23.

On the other hand, no difference of RFLP could be detected with *FAD2-2* probe between these mutants and Bay in this study. From this evidence, at least an unknown gene encoding microsomal ω-6 fatty acid desaturase must exist in soybean genome.

In this study, we demonstrate that *Ol* locus is structural for *FAD2-1* microsomal ω-6 fatty acid desaturase. Comparison of their mutation points at *Ol* locus may lead to a better understanding for enzymatic function of microsomal ω-6 fatty acid desaturase. Furthermore, *FAD2-2* cDNA can be used as an excellent tool to search another locus for

Table 1. Distribution of oleic acid content in F_2 seeds and intensity of 4.6 kbp band hybridized with a microsomal ω-6 fatty acid desaturase probe in F_2 plants of cross between Bay and M23.

Number of individual	Oleic acid content in F_2 seed	Band intensity in F_2 plant
1	29.3	++
2	28.6	++
3	42.1	−
4	36.1	+
5	33.7	+
6	27.8	++
7	32.2	+
8	44.5	−
9	43.5	−
10	36.7	+
11	35.7	+
12	45.7	−
13	34.2	+
14	30.8	++
15	36.7	+
16	37.0	+
17	43.3	−
18	36.6	+
19	41.4	−
20	41.2	−
21	44.8	−
22	29.0	++
23	32.2	+
24	37.0	+
25	35.9	+
26	34.2	+
27	32.1	+
28	28.5	++
29	33.7	+
30	29.5	++
31	34.0	+
32	32.5	+
33	34.2	+
34	27.2	++
35	33.7	+
36	43.4	−
37	33.3	+
38	28.6	++
39	42.0	−
40	33.5	+

high oleic acid in mutants, and also the transgenic soybean containing high oleic acid can be developed using anti-sense RNA technique.

References

1) Neff, W. E., Selke, E., Mounts, T. L., Rinsch, W., Frankel, E. N. and Zietoun, M. A. M. 1992. Effect of triacylglycerol composition and structures on oxidative stability of oils from selected soybean germplasm. *J. Am. Oil Chem. Soc.* **69** : 111-118.

2) Rahman, S. M., Takagi, Y., Kubota, K., Miyamoto, K. and Kawakita, T. 1994. High oleic acid mutant in soybean induced by X-ray irradiation. *Biosci. Biotech. Biochem.* **58** : 1070-1072.

3) Rahman, S. M., Takagi, Y. and Kinoshita, T. 1996. Genetic control of high oleic acid content in the seed oil of two soybean mutants. *Crop Sci.* **36** : 1125-1128.

4) Takagi, Y. and Rahman, SM 1996. Inheritance of high oleic acid content in the seed oil of soybean mutant M23. *Theor. Appl. Genet.* **92** : 179-182.

5) Browse, J. and Somerville, C. 1991. Glycerolipid synthesis: biochemistry and regulation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **42** : 467-506.

6) Okuley, J., Lightner, J., Feldman, K., Yadav, N. and Browse, J. 1994. The *Arabidopsis FAD2* gene encodes the enzyme that is essential for polyunsaturated lipid synthesis. *Plant Cell* **6** : 147-158.

7) Heppard, E. P., Kinney, A. J., Stecca, K. L. and Miao, G-H. 1996. Developmental and growth temperature regulation of two different microsomal ω-6 desaturase genes in soybean. *Plant Physiol.* **110** : 311-319.

8) Takagi, Y., Hossain, A. B. M. M., Yanagita, T. and Kusaba, S. 1989. High linolenic acid mutant in soybean induced by X-ray irradiation. *Jpn. J. Breed.* **39** : 403-409.

9) Anai, T., Miyata, M., Kosemura, S., Yamamura, S., Tsuge, T., Matsui, M., Uchida, H. and Hasegawa, K. 1997. Comparison of *abp1* primary sequences from monocotyledonous and dicotyledonous species. *J. Plant Physiol.* **151** : 446-449.

10) Yamashita, Y., Kinoshita, T., Rhaman, S. M., Okada, K., Anai, T. and Takagi, Y. 1998. Genetic analysis of restriction fragment length polymorphism on the fatty acid synthesis in soybean mutants and their progenies.: I. Low linoleic acid mutants with an microsomal ω-3 fatty acid desaturase cDNA as a probe. *Bull. Fac. Agr. Saga Univ.* **83** : 31-36

ダイズ脂肪酸生合成突然変異体とその子孫を用いた制限酵素断片長多型の遺伝分析 II．小胞体型ω-6脂肪酸不飽和化酵素cDNAをプローブとした高オレイン酸突然変異体の解析

木下　剛仁・S. M. ラーマン・穴井　豊昭・高木　　胖
(生物工学講座)

平成10年9月7日　受理

摘　　要

ダイズ［*Glycine max*(L.)Merr.］高オレイン系統J 38，M11及びM23は，X線照射された品種Bayより分離された突然変異体である．親品種であるBayではオレイン酸含量が総脂肪酸の約27.8%であったのに対して，これらの突然変異体では各々約44.5%，30.8%及び48.6%であった．この著しいオレイン酸含量の増加は，X線照射時に*Ol*遺伝子座において引き起こされた突然変異によるものであろうと考えられた．そこで，本研究において，我々は2種類の小胞体型ω-6脂肪酸不飽和化酵素のcDNA（*FAD2-1*および*FAD2-2*）をプローブとしたRFLP解析を行い，これらの突然変異について検出を試みた．その結果，*FAD2-1*をプローブとした場合にのみ，M23系統において他の系統には認められる1本のバンドが欠失することを見出した．さらに，M23及びBayを交配して得られた40系統のF_2集団についても同様の解析を行った．また，これらの種子のオレイン酸含量についてもガスクロマトグラフィーを用いた解析を行ったところ，これらのバンドの濃度は明確に1：2：1の比に分離し(Bay：F_1：M23型；$\chi^2=0.16$，$P>0.80$)，更に，これらのバンドの濃度とオレイン酸含量との間には完全な相関が認められた．以上の結果より，M23における高オレイン酸含量は小胞体型ω-6脂肪酸不飽和化酵素遺伝子のアイソザイムの一つをコードする*Ol*遺伝子座での何等かの塩基配列の変化に起因する事が明らかとなった．