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Seed Protein Quality of Soybean Mutants

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콩 돌연변이 계통의 단백질 특성

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ABSTRACT : The sulfur amino acid composition in soybean(*Glycine max* L.) seeds may be an essential characteristic of new cultivars for some animal diets. Variation in seed storage protein among genotypes might make it possible to improve the quality of seed protein by genetically altering seed storage protein composition through plant breeding. This study was carried out to determine if mutant strains have potential for improving seed protein quality in soybean. Ten mutant strains had a distinct characteristic of seed storage protein subunits. Among the mutant strains, the sulfur amino acid compositions(methionine plus cystein) of Keburi(P.I.417016), Keburi(P.I.506817), and P.I.54608-1 were relatively higher than those of the others and were 1.9, 2.1, and 1.8%, repectively, which might be due to low levels of α, α', and β subunits of 7S protein. Therefore, it is concluded that the mutant strains, Keburi(P.I.417016), Keburi(P.I.506817), and P.I.54608-1 appear to be potential materials for a breeding program for improving sulfur amino acid composition, and the others also seem to be possible breeding materials for other uses.

Key word : Seed protein, Mutant, Soybean

Soybean(*Glycine max* L.) seed protein is a major source of edible plant protein among major crop plants. Most of the protein is storage protein which is characterized by having no enzymatic activity. It functions as a nutritional source for seedling development. The seed storage protein in soybean consists of four subunits, 2S, 7S, 11S, and 15S, where "S(Svedberg unit)" is defined as the velocity of a sedimenting molecule per unit of gravitational field. Among them, 7S(β-conglycinin) and 11S(glycinin) subunits are the two major storage proteins and account for about 70% of the total seed protein. In general, they contain high levels of amide amino acids, glutamine and asparagine, and low levels of sulfur amino acids, methionine and cystein. Since some animal diets, which have soy protein as the main protein source, require supplementary methionine, much of the interest in soybean seed storage protein derives from a desire to change the amino acid composition

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with the goal of ultimately improving the protein quality. This study was carried out to determine if mutant strains have potential as breeding materials for improving seed protein quality in soybean.

7S Protein and its mutant strains

The 7S protein consists of four subunits, α', α, β and γ. In soybean, the three major subunits, α', α, and β, aggregate into trimers in six possible combinations, α', $\beta\beta$, $\alpha\beta\beta$, $\alpha\alpha'\beta$ $\alpha\alpha\beta$, $\alpha\alpha\alpha'$, and $\alpha\alpha\alpha$(thanh et al. 1978) to make up a family of 7S protein.

Several cultivars which lack or are low in a certain subunit of 7S protein have been found. Kitamura et al.(1984) found that absence of α' subunit in the cultivar Keburi(P.I.200485) was controlled by a single recessive allele. Later, Ladin et al. (1984) concluded that lack of the α'-subunit in the cultivar Keburi was due to a deletion mutation of the allele. Tsukada et al. (1986) found that low levels of the α and β subunits in the cultivar Mo-shi-dou(Gong 503) were controlled by the independent single α -low and β-low recessive alleles, respectively. Davies et al. (1985) found that P.I.88302-1 and Kuro-Daizu(P.I.81041-1) exhibited a double phenotype of the 7S subunit, and that P.I. 54608-1 revealed α-shifted and α'-shifted phenotypes of the 7S subunit. They also showed that P.I.90567-1 had double α and double β phenotypes of the 7S subunit in electrophoretic patterns from SDS-PAGE. Those double and shifted phenotypes of the 7S subunit might be another forms of α', α, and β, which might occur naturally by deletion or insertion of DNA sequences.

Ladin et al. (1984) found that there were four isomeric forms in each of α', α, and β subunits on 2-dimensional gel electrophoresis. This may indicate that each isomer is encoded by a gene at different loci or a different allele at the same locus. If there are genetic polymorphisms of the isomers, this suggests the possibility of genetically altering the amino acid composition of soy protein, by using genotypes which are absent or low some isomers as breeding lines.

11S protein and its mutant strains

The 11S protein consists of several acidic and basic polypeptides(Derbyshire et al. 1976). Moreira et al. (1981) indicated that there were six different acidic and five different basic polypeptides on the basis of differences in NH_2-terminal amino acid sequences. Staswick et al. (1983) found that acidic and basic polypeptides were associated in a cultivar with six A and five B polypeptides as follows : $A_{1a}B_2$, $A_{1b}B_{1b}$, A_2B_{1a}, A_3B_4, A_4B_3, and A_5B_3.

Tumer et al. (1982) suggested that each basic polypeptide was linked to a specific acidic polypeptide via disulfide bonding to form A-B subunit pairs, and that the specific pairings occurred because the acidic and basic components in each pairing were synthesized from the same mRNA as a single precursor molecule that was subsequently cleaved to form the A and B components. Recently, Nielsin et al. (1989) determined the structure, organization, and predominant glycinin subunits and diverged into two subfamilies that designated as Group I(Gy_1, Gy_2, and Gy_3) and Group II(Gy_4, and Gy_5). Each of the glycinin genes contained four exons and three introns. The five glycinin genes, which are named Gy_1, Gy_2, Gy_3, Gy_4, and Gy_5, coded for the polypeptides, $A_{1a}B_2$, A_2B_{1a}, $A_{1b}B_{1b}$, $A_4A_5B_3$, and A_3B_4, respectively. Cho et al. (1989) showed that the five glycinin genes were distributed among four independently

segregating genetic loci within the genome. Gy_1, and Gy_2 are tandomly linked at one locus, while the other three at each of the other three loci. Gy_4 is characterized by two alleles, dominant and recessive.

Several cultivars with 11S variants were found. Staswick et al. (1983) indicated that the cultivar Raiden lacked an $A_4A_5B_3$ subunit. A new polypeptide, which is called A_6, was also found in the cultivar. Ogawa et al. (1989) found that the cultivars, Oodate No. 1 and Suzuyutaka, lacked an $A_4A_5B_3$ subunit in the 11S protein. Fontes et al. (1984) that the cultivar Kura lacked the A_3 subunit in the 11S protein. Cho et al. (1989) found that the cultivar Forrest lacked the $A_{1b}B_{1b}$ subunit in the 11S protein.

Mori et al. (1981) found a difference in 11S proteins between genotypes by isoelectric focusing. He also classified them into 5 groups according to the number of acidic and basic subunits, which ranged from 6 to 7(acidic subunits) and from 3 to 8(basic subunits), respectively. The difference in the number of isomers among genotypes may indicate the possibility of altering the amino acid composition of soy protein to improve the quality of the soybean protein.

Material and Methods

Materials and Procedures

Ten Mutant strains were collected from the USDA-ARS soybean germplasm(Urbana, Illinois). They were characterized as follows : Raiden(P.I.360844) was absent in the $A_5A_4B_3$ subunit of glycinin ; Keburi(P.I.200485) and Keburi(P.I.506817) were absent in the α' subunit and low in the α and β subunits of β conglycinin ; Keburi(P.I.417016) was absent in the $A_5A_4B_3$ subunit of glycinin and low in the β subunits of β conglycinin ; P.I.54608-1, Kuro-Daizu(P.I.81041-1), and Mo-shi-dou(P.I. 461509) were low in the α', α, and β subunits of conglycinin ; P.I.90567-1 was low in the α' and β subunits of β conglycinin ; Forrest was absent in the $A_{1b}B_{1b}$ subunit of conglycinin, which is not clear in this study ; Kura was absent in the A_3B_4 subunit of conglycinin, which is not clear in this study ; P.I.54607-1 revealed α- and α' -shifted phenotypes of the 7S subunit.

This study was conducted in the greenhouse of the USDA-ARS, Raleigh, NC 27607. Seeds of the ten mutant strains were germinated for 2 days and then were transplanted in 254 mm pots. Thirty plants, 3 plants in a pot from each of 10 mutant strains, were planted with 2 replications and the experiment was repeated twice. Each plant was harvested in a separate bag.

Sample Preparation and Electrophoresis

A sample of ten seeds from each replicate was randomly chosen from each genotype and was ground in a coffee mill. Two grams of each ground sample were added to 4 ml of a sample buffer(0.05 M Tris-HCl with pH 8.0) including 2% sodium dodecyl sulfate(SDS), 0.1% 2-mercaptoethanol, and 5M urea(modified from Fontes et al. 1984). Seed storage proteins from each sample were extracted for 30 min and were centrifuged for 20 min to remove debris. The seed storage proteins were separated by SDS slab gel electrophoresis for 20 hours at a constant current of 6 mA per gel. A gradient polyacrylamide gel(12 to 15%) including 5 M urea with a stacking gel(5%) was used. After electrophoresis, a gel was stained in a staining solution(0.05% Coomassie blue, 10% acetic acid, and 40% ethanol), was

destained in a destaining solution(40% ethanol and 10% acetic acid), and was stored in a solution(10% ethanol and 7.5% acetic acid).

Analysis of Amino Acid Composition

A sample of seed storage protein after centrifuging was dialyzed over night and was hydrolysed in a mixed solution of 6N HCl and phenol(100:1) at 110℃ for 24 hours in a hydrolysis vial. After hydrolysis, a reducing solution(2:2:1 of ethanol, H_2O, TEA) was added and was lyophilized for 20 min. A derivatization reagent(7:1:1:1 of ethanol, TEA, H_2O, PITC) was added to each vial. Each vial was vortexed and was left at room temperature for 45 min or more. After derivatization, amino acid composition of diluted samples was measured by using HPLC.

Results and Discussion

Each mutant strain showed distinct characteristic of seed storage protein in the SDS-PAGE with urea(Fig. 1). All of the ten mutant strains showed a double β phenotype of 7S subunit and a double A_4 and acidic phenotypes of 11S subunit. Also, the cultivars, Mo-shi-dou and Kura, showed a α'-shifted phenotype of the 7S subunit and Mo-shi-dou revealed shifted A_4 and acidic phenotypes of the 11S subunit. Although the cultivar Kura lacked the A_3 subunit in the 11S protein (Fontes et al. 1984) and the cultivar Forrest lacked the $A_{1b}A_{1b}$ subunit in the 11S protein (Cho et al. 1989), their electrophoretic patterns were not clear in this study.

The sulfur amino acid compositions(meth-

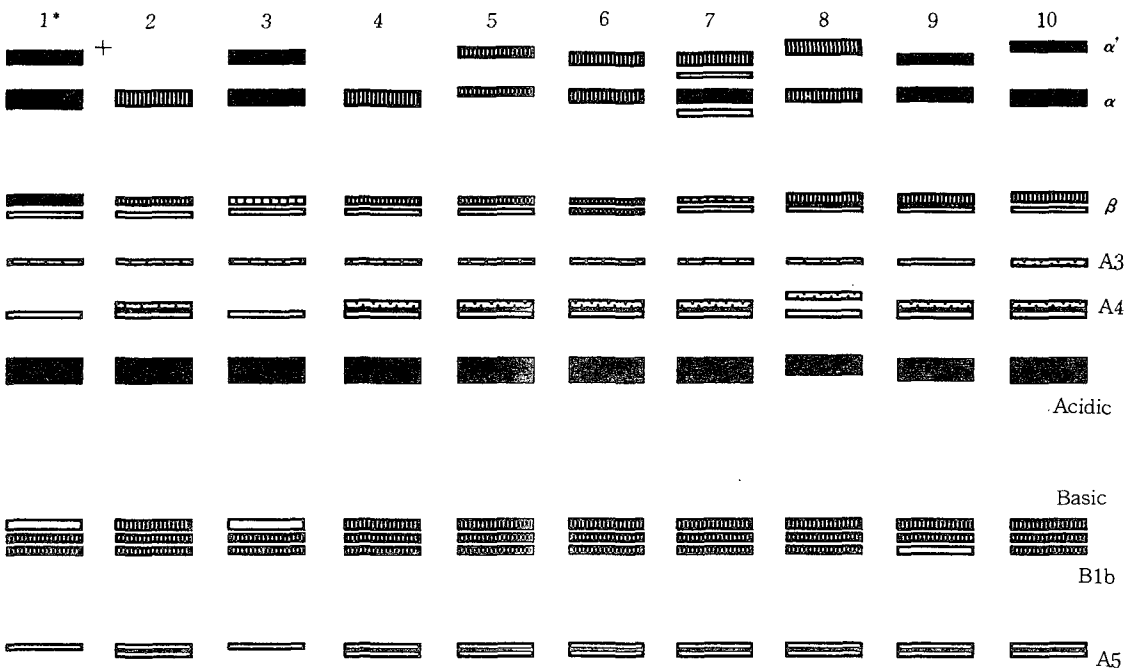

Fig. 1. SDS-PAGE electrophoretic pattern of soybean seed storage proteins.
*1. Raiden (P.I.360844) 2. Keburi (P.I.200485) 3. Keburi (P.I.417016) 4. Keburi (P.I. 506817) 5. P.I.54608-1 6. Kuro-Daizu (P.I.81041-1) 7. P.I.90567-1 8. Mo-shi-dou (P.I.461509)
+ Where the darker color intensity the more protein.
▬ > ▥ > ▤ > ▦ > ▭

ionine plus cystein) of the 10 mutant strains were shown(Tab. 1) Among the mutant strains, the three cultivars, Keburi(P.I. 417016), Keburi(P.I.506817), and P.I.54608-1, were relatively higher than those of the others and were 1.9, 2.1, and 1.8%, respectively. The high sulfur amino acid composition (1.9%) of Keburi(P.I.417016) may be due to low level of a β subunit. The highest sulfur amino acid composition(2.1%) of Keburi(P.I. 506817) may be due to absence of a α' subunit and low level of a β subunit. Although the electrophoretic pattern of Keburi(P.I.200485) was similar to that of Keburi(P.I.506817), its relatively lower sulfur amino acid composition(1.7%) than that of Keburi(P.I.506817) might be due to difference in a ratio of 7S and 11S subunits or others. The high sulfur amino acid composition(1.8%) of P.I.54608-1 may be due to low levels of α', α, and β subunits.

Table 1. Amino acid composition(%) in seed storage protein of soybean mutant strains

Kinds	1*	2	3	4	5	6	7	8	9	10
ASX	11.7	11.6	12.7	12.6	14.1	16.6	13.9	15.3	15.2	14.5
GLX	16.8	16.3	18.6	17.2	18.4	22.0	19.2	19.2	20.0	19.4
SER	6.2	6.4	7.3	6.5	7.0	6.6	6.3	6.9	6.5	6.5
GLY	7.8	8.6	8.8	9.2	8.7	6.8	7.1	7.3	7.1	7.1
HIS	2.5	2.7	2.3	2.7	2.4	1.6	2.4	2.1	1.7	1.9
ARG	6.2	6.2	5.1	5.2	5.2	5.3	5.9	5.3	5.4	5.5
THR	3.9	4.5	4.6	4.3	4.3	4.7	4.3	4.8	4.8	4.6
ALA	6.5	6.7	7.1	6.3	6.9	6.3	6.0	6.1	6.5	6.2
PRO	6.1	6.6	7.3	6.8	7.4	6.8	6.8	7.2	8.2	7.3
TYR	2.6	2.7	2.0	2.7	2.1	1.9	1.9	2.1	2.3	2.5
VAL	5.1	5.5	4.9	5.3	4.6	4.7	4.7	5.3	4.9	4.7
MET	1.2	1.4	1.7	1.7	1.5	1.1	1.3	1.2	1.1	1.2
CYS	0.5	0.3	0.2	0.4	0.3	0.3	0.4	0.2	0.2	0.4
ILE	4.5	4.1	3.9	4.3	3.9	3.4	3.9	3.5	3.4	3.8
LEU	8.0	7.3	6.8	7.6	6.7	5.8	7.0	6.4	6.1	6.7
PHE	4.4	3.8	3.5	4.3	3.2	2.9	3.7	3.3	3.5	3.6
LYS	5.9	5.4	3.2	3.0	3.2	3.3	4.3	3.6	3.0	3.9

*1. Raiden (P.I. 360844) 2. Keburi (P.I. 200485)
3. Keburi (P.I. 417016) 4. Keburi (P.I. 506817)
5. P.I. 54608-1 6. Kuro-Daizu (P.I. 81041-1)
7. P.I. 90567-1 8. Mo-shi-dou (P.I. 461509)
9. Forrest 10. Kura(SNA 66 III)

Although the electrophoretic patterns of Kuro-Daizu(P.I.81041-1) and relatively lower level of the acidic subunits in Mo-shi-dou(P.I. 461509).

These results suggest that the mutant strains, Keburi(P.I.417016), Keburi(P.I. 506817), and P.I.54608-1 appear to be potential materials for a breeding program for improving sulfur amino acid composition, and that the others also seem to be possible breeding materials for genetically altering amino acid composition for other uses.

In general, it is known that protein in soybean contains high levels of amide amino acids, glutamine and asparagine, and low levels of sulfur amino acids, methionine and cysteine. Since some animal diets, which have soy protein as the main protein source, require supplementary methionine, many studies have been devoted to increasing sulfur amino acids as a way to improve the quality of soybean seed protein. Millerd(1975), Staswick et al. (1983), and Coates et al. (1985) showed that there was wide variation in sulfur amino acids between 7S and 11S protein subunits in soybean. Nielsen et al. (1985) indicated that 7S and 11S proteins differ in levels of sulfur amino acids(1.8% for 11S and 0.6% for 7S). Ogawa et al. (1989) demonstrated that 7S-low lines were higher in sulfur amino acids than other normal cultivars. These studies suggest the possibility of genetically manipulating the nutritional quality of soy protein by altering the ratio of 7S and 11S protein subunits or by reducing 7S subunits. Wilson(1987) summarized approaches for increasing the sulfur amino acid composition of soy protein as follows : (1)elimination of the β subunit in 7S protein, (2)elimination of 7S proteins, (3) selection for a greater ratio of 11S to 7S subunits. This study also support Wilson's

approaches. Much interest in soybean seed storage protein quality has derived from a desire to improve the sulfur amino acid composition for some animal diets.

However, other uses of the protein may require other changes in amino acid composition. Such changes might be achieved in near future if the mutant strains are used in a breeding program for genetically improving seed protein quality of soybean.

적 요

콩단백질의 황 아미노산함량은 가축 영양학상 중요한 위치를 차지하기 때문에 신계통이 가져야만 할 필수조건일지도 모른다. 콩 계통간에 저장단백질의 유전적변이가 존재한다면 이는 기존의 육종방법을 통하여 콩의 종자단백질 구성성분을 유전적으로 변경하여 품질을 개량할 수 있는 가능성을 시사하고 있다. 본 연구는 여러 문헌에 보고된 콩종자 저장단백질의 돌연변이 계통들을 선별하여 콩단백질의 품질을 향상시키기 위한 육종 재료로서의 가능성을 평가하기 위하여 실행되었다. 수집된 돌연변이 계통들은 저장단백질의 또 다른 특성을 나타내었다. 그 돌연변이 계통들 중에서 Keburi(P.I.417016), Keburi(P.I.506817), P.I. 54608-1 등은 황 아미노산 함량이 상대적으로 다른 돌연변이 계통보다 높은 1.9, 2.1, 1.8%를 나타내었으며, 이는 7S 단백질인 α', α, β 단백질 함량이 상대적으로 낮기 때문인것으로 나타났다. 그러므로 그 돌연변이 계통들 중에서 Keburi(P.I. 417016), Keburi(P.I.506817), P.I.54608-1 등은 황 아미노산 함량을 향상시키기 위한 중요한 육종 재료로, 그 외 돌연변이 계통들은 다른 용도의 육종 재료로 이용할 수 있을 것으로 추측된다.

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