Dominant Inheritance of a Trait Lacking β -conglycinin Detected in a Wild Soybean Line

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Summary

The QT2 line, which lacks β -conglycinin in the seed storage protein, was detected from our wild soybean germplasm collection. Although induced mutant lines lacking β -conglycinin were reported to be non viable in previous studies, the QT2 line grew normally and produced successive generations. To distinguish it from the non viable β -conglycinin-lacking line, this phenotype was referred to as the "QT2-type". In these studies, we investigated the mode of inheritance of the QT2-type and the genetic relationships between the QT2-type and some other soybean storage proteins. In spite of the fact that β -conglycinin was composed of three major subunits, genetic analysis of the QT2 line indicated that the QT2-type was controlled by one single dominant gene. The new genetic symbol of *Scg* was assigned to the QT2 type in this report. Furthermore, the mode of inheritance of the QT2-type was independent of that of the α' -subunit of β -conglycinin and seed lipoygenases. The QT2-type hybrids, even without the presence of seed lipoygenases, did not show any physiological abnormalities. These results indicate that the QT2 line is a suitable gene source to breed soybean varieties lacking β -conglycinin.

Key Words : *Glycine max*, soybean, *Glycine soja*, 7S globulin, seed storage protein, lipoygenase, dominant gene.

Introduction

Soybean contains about 40 % protein in the seed and is one of the major sources of vegetable protein. However, due to the low content of sulfur-containing amino acids, such as cysteine and methionine, soybean protein is not the most nutritious protein.

Soybean storage protein consists of four major components, 2S, 7S, 11S and 15S. Especially, about 70 % of the total protein consists of two major storage proteins, 7S globulin (β -conglycinin) and 11S globulin (glycinin) (Derbyshire *et al.* 1976). There are chemical and physical differences between β -conglycinin and glycinin. β -conglycinin is a glycoprotein composed of three major subunits called α -, α' - and β -subunits (Thanh and Shibasaki, 1976), and glycinin is an oligomeric protein composed of 6 acidic and basic subunits

(Badley *et al.* 1975).

Glycinin is a better protein source than β -conglycinin in soybean food, because of its higher content of sulfur-containing amino acids and gel-forming ability (Koshiyama 1968, Fukushima 1991). In addition, Oga-*wa et al.* (1989) reported that there was a highly negative correlation between the contents of β -conglycinin and glycinin and they suggested that it is possible to breed soybean lines with a high 11S content without causing a reduction in the total protein content.

By screening genetic resources, some variations in the subunits of β -conglycinin have already been detected. Kitamura and Kaizuma (1981) identified two mutant soybean lines, one lacking the α' -subunit (Keburi) and the other containing half amounts of both α - and β -subunits (Mo-shi-do). Furthermore, Tsukada *et al.* (1986) identified other mutant lines including a line with a β' -subunit. However, neither a line lacking an α -subunit nor a line lacking the β -subunit has been detected and it anticipated that a mutant line lacking these subunits could be induced by mutation.

Kaizuma *et al.* (1989) induced a mutant line which lacked the α -subunit and β -subunit by gamma-ray irradiation. Kitagawa *et al.* (1991) also induced a mutant line containing remarkably low levels of the α -subunit and β -subunit by irradiation. However, these mutant lines were not viable and had to be maintained on heterozygous plants. Therefore, these lines could not be used as breeding materials.

Recently, Takahashi *et al.* (1994) has succeeded in inducing a mutant line which lacked the α -subunit from Karikei 434 (lacking α' -subunit and containing a low level of α -subunit and β -subunit) by gamma-ray irradiation. This mutant line contained remarkably low level of β -conglycinin and went through two generations without physiological abnormalities. However, due to the absence of a line that lacks the β -subunit, we could not develop a soybean line lacking all the subunits of β -conglycinin.

In 1994, when we investigated our wild soybean collections from Amakusa Island, we identified a wild soybean line lacking all the subunits of β -conglycinin (Hajika *et al.* 1996). This line was viable and matured to produce seeds which showed the same trait lacking all the subunits of β -conglycinin. Therefore, we concluded that the trait lacking β -conglycinin was truly inherited trait and the line was designated as QT2. Since the wild soybean can be easily crossed with soybean cultivars, the QT2 line is expected to be a gene source for the

breeding of a soybean line lacking β -conglycinin.

In this study, we report the mode of inheritance of the phenotype of QT2(QT2-type) and the genetic relationships between the QT2-type and other storage proteins of soybean.

Materials and Methods

1. Plant materials and Crossing tests

Two varieties and two lines were used for the genetic analysis. Fukuyutaka and Toyoshirome are normal-type soybean varieties with β -conglycinin subunits and lipoxigenases. Tohoku 101 is a line lacking the α' -subunit, and Kyukou 693 is a line lacking all three seed lipoxigenases.

To study the mode of inheritance of the QT2-type, the following two crosses were made: 1) Fukuyutaka $\times$ QT2 line, 2) Toyoshirome $\times$ QT2 line. Further, to study the mode of the genetic relationships between the QT2-type and other storage proteins, such as α' -subunit and seed lipoxigenases, the following two crosses were made: 3) Tohoku 101 $\times$ QT2 line, and 4) Kyukou 693 $\times$ QT2 line.

These crosses were made in the spring of 1995, and the F_2 seeds were obtained by selfing of F_1 plants grown in the field. Furthermore, the F_1 plants derived from the Fukuyutaka $\times$ QT2 line were backcrossed to Fukuyutaka and B_1F_1 seeds were obtained. Some of the B_1F_1 seeds which showed the QT2-type were planted to produce B_1F_2 seeds.

2. Analysis of phenotypes

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted on each single seed to determine the presence or absence of subunits of β -conglycinin according to the method of Laemmli (1970). Furthermore, improved SDS-PAGE (Kitamura, 1984) was used for the analysis of the lipoxigenase isozymes.

Results and Discussion

1. Inheritance of QT2-type

Three and twelve F_1 seeds were obtained from the Fukuyutaka $\times$ QT2 line and the Toyoshirome $\times$ QT2 line, respectively, and all these F_1 seeds showed the QT2-type (Table 1). The analysis of 673 F_2 seeds from these F_1 plants revealed only two phenotypes, the QT2-type and normal-type. The observed segregation frequencies obviously fitted well to the 3 : 1 (QT2-type : normal-type) ratio, suggesting that the QT2-type is controlled by a single dominant gene. Furthermore, the B_1F_1 and B_1F_2 seeds derived from backcrosses to Fukuyutaka showed a 1:1 ratio and 3:1 ratio, respectively (Table 2). From these results we concluded that the QT2-type is controlled by one single dominant gene. Therefore, we assigned the symbol, *Scg* (suppressor of β -conglycinin) to this gene.

The fact that the QT2-type is controlled by a single dominant gene is very interesting, because no dominant gene has been reported to suppress the presence of a storage protein in soybeans. The traits lacking the soybean storage proteins, such as α -less (Takahashi *et al.* 1996) and α' -less (Kitamura *et al.* 1984), were controlled by a recessive gene. This fact suggests that *Scg* does not result from simple deleterious mutations of the structural genes encoding each β -conglycinin subunit but a mutation of a regulatory gene may be involved in the synthesis and degradation of β -conglycinin. If *Scg* is a single dominant gene, the following hypotheses may be proposed: 1) *Scg* encodes a specific breakdown enzyme of β -conglycinin, 2) *Scg* is a suppressor gene which product interacts with the promoters of the β -conglycinin genes and down-regulates their gene expression, and 3) *Scg* results from the mutation of a gene which is involved in targeting of β -conglycinin subunits from ER (endoplasmic reticulum) to the protein body.

Later, during the backcrossing to Fukuyutaka, we obtained B_5F_2 seeds lacking β -conglycinin and this line also did not show any physiological abnormalities. These results indicate that the QT2 line is definitely a suitable gene source for breeding soybean varieties lacking β -conglycinin.

2. Genetic relationships between QT2-type and pres-

Table 1. Observed and expected segregation of F_1 and F_2 seeds derived from the crosses between soybeans and QT2 line

Cross combination	Phenotype		Chi-square value (3:1)	Probability
	QT2-type	normal type		
(F ₁)				
Fukuyutaka × QT2 line	12	0	—	—
Toyoshirome × QT2 line	3	0	—	—
(F ₂)				
Fukuyutaka × QT2 line	220(234.75) ¹⁾	93(78.25)	3.71	> 0.05
Toyoshirome × QT2 line	267(268.5)	91(89.5)	0.03	> 0.80

¹⁾ Values in parenthesis are expected frequencies.

Table 2. Observed and expected segregation of B_1F_1 and B_1F_2 seeds derived from a backcross to Fukuyutaka

Cross combination	Phenotype		Chi-square value (3:1)	Probability
	QT2-type	normal-type		
B_1F_1	5	5		
$B_1F_2^{1)}$				
NO.1	19	4		
NO.2	21	3		
NO.3	22	7		
NO.4	29	18		
NO.5	28	9		
Total of NO.1-NO.5	119(120) ²⁾	41(40)	0.03	> 0.8

¹⁾ NO.1-NO.5 are the progeny of B_1F_1 seeds which showed the QT2 type.²⁾ Values in Parenthesis are expected frequencies.**Table 3.** Observed and expected segregation of F_2 seeds derived from crosses between Tohoku 101 (lacking α') and QT2 line

Phenotype	Seed number	Chi-square value (12:3:1)	Probability
QT2-type	593(615.8) ¹⁾	0.84	3.47 > 0.1
Normal	173(153.9)	2.36	
Locking α'	55(51.3)	0.27	

¹⁾ Values in parenthesis are expected frequencies.**Table 4.** Observed and expected segregation of F_2 seeds derived from crosses between Kyukou 693 line and QT2 line

7S	Phenotype			Seed number	Chi-square value 3:9:9:27:1:3:3:9	Probability
	L-1	L-2	L-3			
—	—	—	—	16(15.0)	0.06	4.97 > 0.5
—	+	+	—	38(45.1)	1.13	
—	—	—	+	46(45.1)	0.02	
—	+	+	+	131(135.4)	0.14	
+	—	—	—	5(5.0)	0.00	
+	+	+	—	22(15.0)	3.21	
+	—	—	+	14(15.0)	0.07	
+	+	+	+	49(45.1)	0.33	

¹⁾ Values in parenthesis are expected frequencies.

ence of α' -subunit

The results of the analysis of F_2 seeds derived from the cross Tohoku 101 $\times$ QT2 line are shown in Table 3. The trait lacking α' -subunit is controlled by a single recessive gene *cgy1*. The segregation ratio fitted to the theoretical ratio of 12 : 3 : 1 (QT2-type : normal-type : α' -subunit lacking-type) assuming that *Scg* is independent of *cgy1*.

3. Genetic relationships between QT2-type and presence of lipoxigenases.

The results of the analysis of F_2 seeds derived from the cross of Kyukou 693 $\times$ QT2 line are shown in Table 4. The soybean lipoxigenases consisted of three isozymes, designated as L-1, L-2 and L-3 (Axelrod *et al.* 1981), and although trait lacking each isozyme is controlled by a single recessive gene, *lx1*, *lx2* and *lx3*, respectively (Hildebrand and Hymowitz 1982, Davies and Nielsen 1986, Kitamura *et al.* 1985), there was a tight linkage between the *lx1* and *lx2* loci and these two isozymes behaved like a single gene (Hajika *et al.* 1992). The chi-square test of the F_2 segregation ratio from the cross of

Kyukou 693 $\times$ QT2 line showed a good agreement with the expected ratio, suggesting that QT2-type is inherited independently from the presence of these lipoxigenase isozymes. Moreover, the F₂ seeds lacking both the β -conglycinin and all lipoxigenase isozymes produced progeny without physiological abnormalities.

These results indicate that it is possible to breed a soybean line lacking both the β -conglycinin and all lipoxigenase isozymes at the same time. In other words, it is possible to breed soybean lines with a high 11S protein (glycinin) content and lacking all the lipoxigenase isozymes, resulting in a wider utilization of soybean protein.

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