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Induction of a Soybean [*Glycine max* (L.) Merrill] Line Lacking All Seed Lipoxygenase Isozymes

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Abstract
Lipoxygenases are the main factors responsible for the grassy-beany flavor of soybeans and soybean products. Three soybean lipoxygenases, L-1, L-2 and L-3 have been identified. Soybean lines lacking L-1 and L-3 or L-2 and L-3 have been bred. In this study, we successfully induced a line lacking L-1, L-2 and L-3 by gamma-ray irradiation. The soybean line lacking all three lipoxygenases did not show any abnormalities when grown under field conditions, suggesting that the lipoxygenases are not essential for normal growth. The development of soybean cultivars lacking seed lipoxygenases should eliminate the need for heat treatment of soybeans to inactivate these enzymes in the processing of soybean food products.

Discipline: Crop production
Additional key words: gamma-ray, flavor, mutant

Introduction

Lipoxygenase (linoleate:oxygen oxidoreductase, EC 1.13.11.12) is one of the enzymes which catalyze the hydroperoxidation of unsaturated fatty acids with cis,cis-1,4-pentadiene (-CH = CH-CH_2-CH = CH-) moieties, and is present in many tissues of numerous higher plants and animals.

Soybean seeds contain three lipoxygenase isozymes, called L-1, L-2 and L-3[2] and they are particularly abundant in soybean seeds (ca. 1% of the total seed protein). Lipoxygenase activity is ubiquitous in seeds. When soybean seeds are crushed to meal with water, the enzymes and soybean oil are mixed, activating the enzymes. Soybean lipoxygenases oxidize various substrates including linoleic acid, methyl linoleate and unfractionated seed oils. The oxidation products consist of middle chain aldehydes and alcohols which have been associated with the development of an undesirable grassy-beany flavor as well as a bitter taste in soybean products[1,15,16].

In food processing, heat treatment has been used to inactivate these enzymes. Heat treatment does not necessarily eliminate the grassy-beany flavor. The genetic elimination of lipoxygenases from the seeds is one of the most effective solutions to this problem. Mutant soybean lines lacking one of the seed lipoxygenase isozymes have been reported[3,8,12,13] and from the crosses among these mutant lines, a line lacking L-1 and L-3 and a line lacking L-2 and L-3 have been bred[10,12]. However, no lines lacking L-1, L-2 and L-3 have been detected.

In this study, we report the induction of a line lacking L-1, L-2 and L-3 by gamma-ray irradiation and the results of a preliminary field test of this mutant line. Furthermore, we developed crosses between a normal soybean line and the line lacking L-1, L-2 and L-3, and between a line lacking L-3 and a line lacking L-1, L-2 and L-3 to study the genetic relationships among the genes for L-1, L-2 and L-3 isozymes.

Materials and methods

1) Induction of line lacking L-1, L-2 and L-3

The seeds irradiated with gamma-ray were the F_2 seeds obtained from crosses between the seeds lacking L-1 and L-3 and the seeds lacking L-2 and L-3. The irradiation was conducted at the Institute of Radiation Breeding, Ibaraki, Japan in 1989 and the irradiation level was 15KR. The M_3 seeds were analyzed and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique[11] was used for the analysis of lipoxygenases.

2) Crossing tests

The following crosses were made, (1) Suzuyutaka (normal) × the line lacking L-1, L-2 and L-3, (2) Bragg (normal) × the line lacking L-1, L-2 and L-3, (3) the line lacking L-3 × the line lacking L-1, L-2 and L-3. The line lacking L-3 was a BC_4F_6 plant derived from the following crosses: Suzuyutaka (recurrent parent) × Wasenatsu (lacking L-3).

Results

1) Induction of line lacking L-1, L-2 and L-3

Of the 1,813 seeds analyzed by SDS-PAGE, we identified only one seed lacking L-1, L-2 and L-3 isozymes (Plate 1). Of the other 1,812 M_3 seeds, 789 lacked L-2 and L-3, 246 lacked L-3, and 777 lacked L-1 and L-3.

The single M_3 seed embryo with cotyledons lacking L-1, L-2 and L-3 was immediately planted in a greenhouse. It germinated normally, grew and matured to produce 30 M_4 seeds. Analysis of ten of the seeds by SDS-PAGE enabled to confirm that they lacked all the seed lipoxygenases. Based on the above results, we confirmed that the induced trait consisting of the lack of L-1, L-2 and L-3 is a truly inherited trait.

In addition, we planted 18 M_4 seeds in a field at the Kyushu National Agricultural Experiment Station, Kumamoto, Japan in the summer of 1990. They grew and matured to produce M_5 seeds (Table 1). The soybean lines lacking L-1, L-2 and L-3 went through more than two generations without displaying any physiological abnormalities, and showed a similar growth to that of standard soybean cultivars in the field. The fact that the seeds lacking L-1, L-2 and L-3 did not display any detectable physiological and/or agronomical abnormalities suggests that seed lipoxygenases do not contribute significantly to the plant life cycle. However the elucidation of the physiological role of seed lipoxygenases will require further studies.

2) Crossing tests

A total of 148 F_2 seeds from the crosses between Suzuyutaka and the line lacking L-1, L-2 and L-3 and 517 F_2 seeds from the cross between Bragg and the line lacking L-1, L-2 and L-3 were analyzed. The cross between Suzuyutaka and the line lacking L-1, L-2 and L-3 gave a segregation ratio of 74 : 36 : 29 : 9 (normal : lacking L-3 : lacking L-1 and L-2 : lacking L-1, L-2 and L-3) and the cross between Bragg and the line lacking L-1, L-2 and

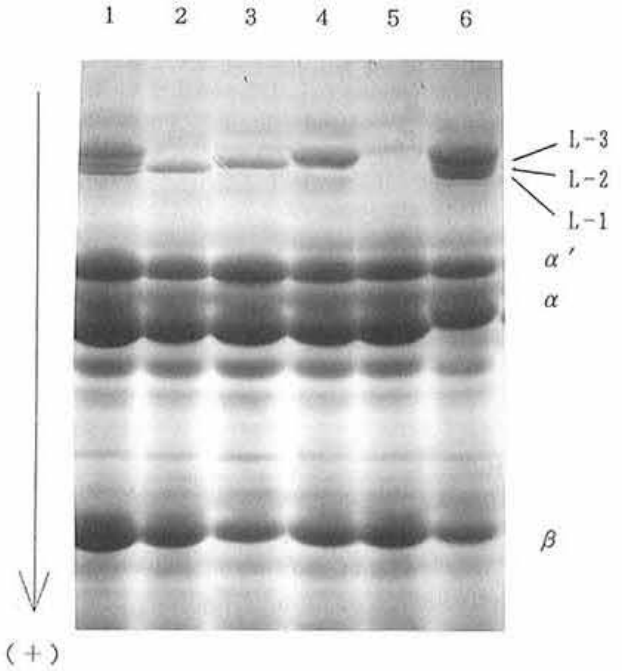

Plate 1. Resolution of the lipoxygenase isozymes in soybean seed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)
1) L-1: lipoxygenase-1, L-2: lipoxygenase-2, L-3: lipoxygenase-3, α, α′ and β: subunits of 7S globulin (β-conglycinin).
2) Lanes 1,6: Suzuyutaka (normal), 2: Kanto 101 (line lacking L-2 and L-3), 3: Kanto 102 (line lacking L-1 and L-3), 4: Kyukei 114 (line lacking L-1 and L-3), 5: Kyushu 111 (line lacking L-1, L-2 and L-3).

Table 1. Growth characteristics of the line lacking L-1, L-2 and L-3

Soybean line	Flowering date	Main stem length (cm)	Maturing date	Yield (kg/a)
Lacking L-1, L-2, L-3	July 20	35.5	Oct. 2	28.7
Lacking L-1, L-3	July 21	37.1	Oct. 4	31.8
Lacking L-2, L-3	July 20	31.3	Oct. 3	25.3
Suzuyutaka (normal)	July 20	31.1	Oct. 4	21.7

Table 2. Observed and expected segregation of F_2 seeds from the cross between normal soybeans and a line lacking L-1, L-2 and L-3 for the presence or absence of L-1, L-2, L-3, suggesting the presence of a close linkage between the Lx_1-lx_1 and Lx_2-lx_2 loci

Phenotype			Seed number observed (expected)	Chi-square value (9 : 3 : 3 : 1)		Probability
L-1	L-2	L-3				
Suzuyutaka × the line lacking all lipoxygenases						
+	+	+	74 (83.25)	1.028	3.544	P > 0.2
+	+	−	36 (27.75)	2.453		
−	−	+	29 (27.75)	0.056		
−	−	−	9 (9.25)	0.007		
Bragg × the line lacking all lipoxygenases						
+	+	+	287 (290.81)	0.050	0.782	P > 0.8
+	+	−	95 (96.94)	0.039		
−	−	+	98 (96.94)	0.012		
−	−	−	37 (32.31)	0.681		

Table 3. Observed and expected segregation of F_2 seeds from the cross between a line lacking L-3 and a line lacking L-1, L-2 and L-3 for the presence or absence of L-1, L-2 and L-3, suggesting the presence of a close linkage between the Lx_1-lx_1 and Lx_2-lx_2 loci

Phenotype			Seed number observed (expected)	Chi-square value (3 : 1)		Probability
L-1	L-2	L-3				
+	+	−	830 (849)	0.425	1.701	P > 0.5
−	−	−	302 (283)	1.276		

L-3 gave a segregation ratio of 287 : 95 : 98 : 37 (Table 2).

A total of 1,132 F_2 seeds of the cross between the line lacking L-3 and the line lacking L-1, L-2 and L-3 gave a segregation ratio of 830 : 302 (normal : lacking L-3). Neither the seeds lacking L-1 and L-3 nor the seeds lacking L-2 and L-3 segregated from these crosses (Table 3).

These results indicate that there is a close linkage between the lx_1 and lx_2 loci.

Discussion

In the early 1980s, three mutant soybean lines lacking L-1[8], L-2[3,12], and L-3[13], respectively, were identified. It was anticipated that a soybean line lacking all lipoxygenases isozymes would emerge from crosses among these mutant soybean lines.

The analysis of F_2 seeds from the crosses between these mutants and normal soybeans indicated that the absence of L-1, L-2 and L-3 from seeds is controlled by the single null-alleles, lx_1, lx_2 and lx_3, and that the lx_3 locus is independent of the lx_1 and lx_2 loci respectively[9,10,12].

Kitamura et al.[12] reported the results of further crosses to study the relationship between the lx_1 and lx_2 loci. The results indicated that the lx_2 locus was not independent of the lx_1 locus and it was assumed that there was a close linkage between the lx_1 and lx_2 loci. However due to the limited information about the physiological role of seed lipoxygenases, the possibility of a physiological role for seed lipoxygenases could not be ruled out.

In these studies we successfully induced a line lacking L-1, L-2 and L-3[4,5]. Furthermore, the crossing test between the line lacking L-3 and the line lacking L-1, L-2 and L-3, revealed that no lines lacking L-1 and L-3 and no lines lacking L-2 and L-3 could be detected and the segregation ratio was nearly 3 : 1 (lacking L-3 : lacking L-1, L-2 and L-3) which was expected from the theoretical ratio based on the close linkage between the lx_1 and lx_2 loci[6]. These facts suggest that the unsuccessful breeding of a line lacking L-1 and L-2 may be due to the close linkage between the lx_1 and lx_2 loci and not related to the physiological requirement for the enzymes[4]. In addition, the probability of crossing over between the lx_1 and lx_2 loci is very low or inexistent. The close linkage between the lx_1 and lx_2 loci is a very useful trait in breeding new soybean varieties lacking seed

lipoxygenases, because it results in a high segregation ratio of seeds lacking L-1, L-2 and L-3 from the cross between normal soybean and the line lacking L-1, L-2 and L-3, and it allows a wider selection for other breeding traits.

Up to now, the role of lipoxygenases in soybean has not been elucidated. There are three major fields of plant physiology where lipoxygenases have been implicated[7,14]: (1) growth and development, (2) senescence, and (3) wound response and pest resistance. There is no definite evidence for the role of lipoxygenases in soybean.

The fact that the line lacking all the seed lipoxygenase isozymes grew without abnormalities and the results of preliminary field tests suggest that soybean seed lipoxygenases do not play an essential role in the growth and development of the plant. However, to elucidate the true role of seed lipoxygenases, more experiments will be required, and the line lacking all seed lipoxygenases may enable to determine the role played by seed lipoxygenases in the physiology of soybeans.

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