Short Communication

A Line Lacking all the Seed Lipoxygenase Isozymes in Soybean [*Glycine max* (L.) MERRILL] Induced by gamma-ray Irradiation.

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Soybean seeds contain three lipoxygenase isozymes which are responsible for the generation of grassy beany flavors. Double mutant soybeans lacking both L-1 and L-3, and L-2 and L-3, respectively were already obtained, but neither double mutant soybean seeds lacking both L-1 and L-2 nor triple mutant soybean seeds lacking all the isozymes have been obtained.

In this study we successfully induced a line lacking all the lipoxygenases by gamma-ray irradiation. The soybeans lacking all the lipoxygenases went through two generations without displaying any physiological abnormalities, and we concluded that the induced trait characterized by the lack of all the isozymes is a truly inherited trait.

KEY WORDS: *Glycine max*, gamma-ray, lipoxygenase, mutant.

Introduction

Soybean seeds contain three lipoxygenase isozymes, called L-1, L-2, and L-3 (AXELROD *et al.* 1981). The isozymes are responsible for the generation of grassy beany flavors which hamper a wider utilization of soybean products (WOLF, 1975; RACHIS *et al.* 1979; SESSA 1979).

Recently, mutant soybeans lacking L-1 (HILDEBRAND and HYMOWITZ, 1981; HILDEBRAND and HYMOWITZ, 1982), L-2 (KITAMURA *et al.* 1985; DAVIES and NIELSEN 1986), and L-3 (KITAMURA *et al.* 1983), respectively, have been detected. Genetic studies have demonstrated that the absence of L-1, L-2, and L-3 from the seeds is due to the operation of single null-alleles, lx_1 , lx_2 , and lx_3 , which are recessive to the alleles, Lx_1 , Lx_2 , and Lx_3 , controlling the presence of L-1, L-2, and L-3, respectively, and that the Lx_3 - lx_3 locus is independent of the Lx_1 - lx_1 and Lx_2 - lx_2 loci, respectively.

So far, two types of double mutants which lack both L-1 and L-3, and L-2 and L-3 have been obtained (KITAMURA *et al.* 1985). But, neither double mutant seeds lacking both L-1 and L-2 nor triple mutant seeds lacking all the isozymes have been identified yet, presumably due to the close linkage between the Lx_1 - lx_1 and Lx_2 - lx_2 loci. The present study describes the successful production of a soybean line lacking all the lipoxygenase isozymes and some characterization of the triple mutant soybean.

Materials and Methods

About 1200 F_2 seeds obtained from crosses between the soybean lines lacking L-1 and L-3, and L-2 and L-3, respectively, were irradiated with gamma-rays at the National Institute of Radiation Breeding in Japan. The irradiation level was 15 kR. The treated seeds were planted to obtain M_2 seeds in the summer of 1989. A total of 2,289 single M_3 seeds were harvested from individual M_2 plants which were grown in the greenhouse in the autumn of 1989. The presence or absence of the isozymes in 1,813 individual M_3 seeds was analyzed using distal portions of the seeds subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Results and Discussion

Of the 1,813 seeds analyzed by SDS-PAGE, we identified only one seed lacking all the lipoxygenase isozymes. Of the other 1,812 M_3 seeds, 789 were lacking L-2 and L-3, 246 were lacking L-3, and 777 were lacking L-1 and L-3. This segregation ratio ($\approx 7:2:7$) suggests that the Lx_1-lx_1 locus is very closely linked to the Lx_2-lx_2 one.

The single M_3 seed embryo with cotyledons lacking all the isozymes was immediately planted in a greenhouse. It germinated normally, grew and matured to produce 30 M_4 seeds. We confirmed by SDS-PAGE that ten individuals of the M_4 seeds lacked all the isozymes (Fig. 1). From the above results, we concluded that the induced trait characterized by the lack of all the isozymes identified in the single M_3 seed is a truly inherited trait.

Soybeans lacking all the seed lipoxygenases went through two generations without displaying any physiological abnormalities. This fact suggests that the seed lipoxygenases do not contribute significantly to the plant life cycle.

No information is available on the mutation mechanisms involved in the induction of a seed lacking all the lipoxygenases. It is possible to consider that point or gene mutation, or chromosomal deletion may have occurred. Although the possibility of crossing-over is unlikely due

Fig. 1. Analysis of lipoxygenase isozymes in soybean seed by SDS-polyacrylamide gel electrophoresis.

L-1: lipoxygenase-1, L-2: lipoxygenase-2, L-3: lipoxygenase-3, α , α' , and β : subunits of 7S globulin (β -conglycinin). Lane 1: a line (M_3) lacking all the lipoxygenase isozymes, 2: a line (Kanto 101) lacking L-2 and L-3, 3: a line (Kanto 102) lacking L-1 and L-3, 4: Suzuyutaka (normal).

to the close linkage between the Lx_1-lx_1 and Lx_2-lx_2 loci, such a mechanism cannot be totally ruled out. To elucidate the mechanism of mutation and inheritance of the trait characterized by the lack of the three lipoxygenases in soybean seeds, further studies should be carried out.

Literature Cited

- AXELROD, B., T. M. CHEESEBOURGH, and C. LAAKSO 1981. Lipoxygenase from soybeans. *Methods Enzymol.* **71**: 441-451.
- DAVIES, C. S. and N. C. NIELSEN 1986. Genetic analysis of a null allele for lipoxygenase-2 in soybean. *Crop Sci.* **26**: 460-463.
- HILDEBRAND, D. F. and T. HYMOWITZ 1981. Two soybean genotypes lacking lipoxygenase-1. *J. Am. Oil Chem. Soc.* **58**: 583-586.
- and ——— 1982. Inheritance of lipoxygenase-1 activity in soybean seeds. *Crop Sci.* **22**: 851-853.
- KITAMURA, K., C. S. DAVIES, N. KAIZUMA and N. C. NIELSEN 1983. Genetic analysis of a null-allele for lipoxygenase-3 in soybean seeds. *Crop Sci.* **23**: 924-927.
- , T. KUMAGAI and A. KIKUCHI 1985. Inheritance of lipoxygenase-2 and genetic relationships among genes for lipoxygenase-1, -2 and -3 isozymes in soybean seeds. *Japan. J. Breed.* **35**: 413-420.
- RACHIS, J. J., D. J. SESSA, and D. H. HONIG 1979. Flavor problems of vegetable food proteins. *J. Am. Oil Chem. Soc.* **56**: 262-271.
- SESSA, D. J. 1979. Biochemical aspects of lipid-derived flavors in legumes. *J. Agric. Food Chem.* **27**: 234-239.
- WOLF, W. J. 1975. Lipoxygenase and flavor of soybean products. *J. Agric. Food Chem.* **23**: 136-141.

ガンマー線照射によるリポキシゲナーゼ全欠失ダイズの作出

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ダイズ種子には青くさみの原因となる3種類のリポキシゲナーゼアイソザイムが含まれている。これまでL-1・L-3欠及び、L-2・L-3欠のそれぞれ2種類のアイソザイムが欠失したダイズは得られていたが、3つの酵素全てを欠失している系統は得られていなかった。

本研究ではガンマー線を照射することにより、全てを欠失している系統を作出することに成功した。この系統は生育期間を通じて正常に生育し、その特性は後代に遺伝することが確かめられた。また、これまで全リポキシゲナーゼ欠失ダイズが作出されない理由が、L-1支配座とL-2支配座の強連鎖にあるのか、全リポキシゲナーゼを欠失することによる何らかの生理障害によるものか特定できなかったが、全欠失系統が作出されたことにより、この理由が単なる強連鎖にあることが明かとなった。