

Note

High Stearic Acid Soybean Mutant Induced by X-ray Irradiation

Shaikh Mizanur RAHMAN, Yutaka TAKAGI, Kei MIYAMOTO, and Tetsuya KAWAKITA*

Department of Applied Biological Sciences, Faculty of Agriculture, Saga University, Saga 840, Japan

*Technology and Engineering Laboratories, Ajinomoto Co. Inc., Kawasaki 210, Japan

Received September 26, 1994

Seeds of soybean [*Glycine max* (L.) Merr. var. Bay] were subjected to X-ray irradiation (21.4 kR), and the M₂ generation was evaluated for the stearic acid content in the seed oil. Treatment with X-ray irradiation significantly increased genetic variability in the stearic acid content of the oil from Bay variety in comparison with the control plants. Among the 2513 M₂ plants tested, one mutant named M25 was selected for its stearic acid content of 20.8%, about seven-fold higher than that of the original variety. An inverse relationship of stearic acid with oleic and linoleic acids was observed. Mutant M25 always had higher stearic acid content under different environmental conditions in the M₃ generation.

Stearic acid is one of the major saturated fatty acids in soybean oil, the average stearic acid content being 4.0%, with a range from 2.2 to 7.2% in the world soybean collection.¹⁾ The potential uses for soybean oil with high stearic acid are under investigation. A higher saturated fatty acid content of the oil increases its melting temperature. Peroxide tests have indicated that the stability of oil with a high stearic acid content was superior to that of the oil from current cultivars,²⁾ although little research has been done to improve the quantity of this fatty acid.³⁻⁵⁾ We identified a soybean mutant with high stearic acid content, and we evaluate the features of this mutant.

About six thousand dry seeds of soybean Bay variety were irradiated with 21.4 kR X-rays and then planted in July 1990. The M₂ seeds were harvested from 4400 randomly selected M₂ plants. On July 27th, 1991, 3000 M₂ seeds and 120 seeds from the Bay variety were sown with spaced planting. In order to select the desired mutant, 10 seeds from each of 2513 M₂ and 93 Bay control plants were analyzed for their fatty acid composition as described earlier.⁶⁾

The range of stearic acid content in the M₂ generation was from 1.4 to 20.8%, compared with the range of 2.4 to 3.8% for Bay control plants (Fig. 1), although there was no significant difference in the mean value of this fatty acid in the M₂ generation when compared with the Bay control plants. However, one M₂ plant with the highest stearic acid content of 20.8% of the total oil, which was designated as M25, was studied further.

The palmitic, stearic, oleic, linoleic, and linolenic acid contents in the M₂ generation of M25 were 9.5, 20.8, 16.8, 44.9, and 8.0%, compared with the figures for the Bay control of 11.4, 3.1, 22.3, 55.1, and 8.1%, respectively (Table I). There was no significant difference between M25 and the Bay control in linolenic acid content, but the percentages of palmitic, oleic, and linoleic acids were significantly decreased, and that of stearic acid remarkably increased in M25 when compared with the Bay control.

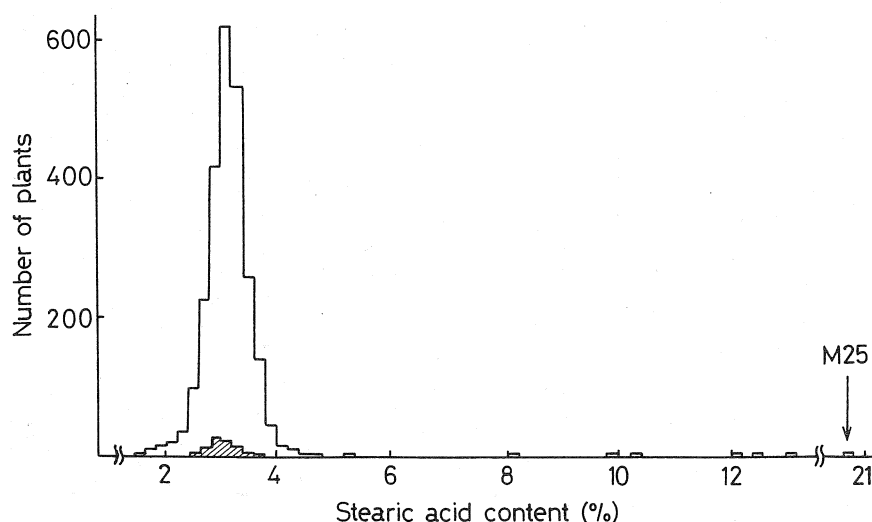


Fig. 1. Distribution of Stearic Acid Content in the M₂ and Bay (Shaded) Plants.

Table I. Fatty Acid Composition^a (% of Total Oil) in the M₂ Generation of M25 and Bay Soybean Plants

Material	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
M25	9.5 ± 0.09**	20.8 ± 0.31**	16.8 ± 0.34**	44.9 ± 0.56**	8.0 ± 0.06
Bay	11.4 ± 0.04	3.1 ± 0.03	22.3 ± 0.18	55.1 ± 0.14	8.1 ± 0.06

^a Expressed as the mean value ± standard error.

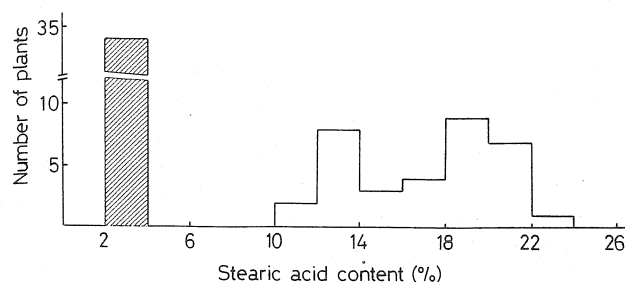
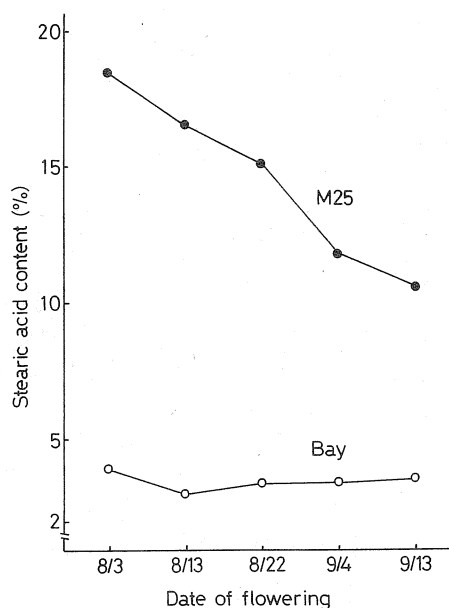
** Significant at the 1% level.

Table II. Fatty Acid Composition^a (% of Total Oil) in the M₃ Generation of M25 and Bay Soybean Plants

Material	No. of plants	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
M25	34	9.2±0.10**	17.3±0.59**	17.3±0.20**	48.2±0.48**	8.0±0.12
Bay	34	11.4±0.06	3.0±0.03	23.0±0.26	54.8±0.22	7.8±0.08

^a Expressed as the mean value±standard error.

** Significant at the 1% level.

**Fig. 2.** Distribution of Stearic Acid Content in the M₃ Plants of M25 and in the Bay Plants (Shaded).**Fig. 3.** Stearic Acid Content of the Oil from Seeds Grown in Different Environments.

M₃ seeds from the M25 mutant and seeds from the Bay control were sown on July 29th, 1992. After maturity, 10 seeds from each M₃ plant and Bay control were analyzed.⁶⁾ The stearic acid content in the M₃ generation of M25 ranged from 10.0 to 24.0%, compared with the range of 2.0 to 4.0% in the Bay control (Fig. 2). The fatty acid composition of the oil in the M₃ generation of M25 was 9.2% palmitic acid, 17.3% stearic acid, 17.3% oleic acid, 48.2% linoleic acid and 8.0% linolenic acid, compared with the Bay control plants with 11.4, 3.0, 23.0, 54.8, and 7.8% respective composition (Table II). Similar to the results for the M₂ generation, the M₃ generation of M25 had significantly lower contents of palmitic, oleic, and linoleic acids, and a significantly higher stearic acid content, which was about six-fold higher than that of the Bay control plants. An inverse relationship of stearic acid with oleic and linoleic acid contents was observed in both the M₂ and M₃ generations of the M25 mutant.

It is important to clarify the mechanism responsible for the change in fatty acid composition of M25. Desaturation of stearic acid is the primary route for the synthesis of oleic and subsequently of linoleic and linolenic acids in soybean seed oil.^{7,8)} The probable

reason for the present results is that a mutant allele could have altered the rate of stearate desaturation by modifying the activity of oleyl-ACP hydrolase, which has high specificity for oleyl-ACP hydrolysis.⁹⁾ If the specificity of that enzyme was affected by the mutant allele, stearyl-ACP hydrolysis might have been favored which would make stearic acid in M25 unavailable for desaturation. Moreover, oleic acid is the precursor of linoleic acid synthesis and has an inverse relationship with linoleic acid.^{7,8)} In the present investigation, no such relationship was observed, so the synthesis of linoleic acid in M25 might have been partially affected due to insufficient synthesis of oleic acid.

M₃ seeds from the M25 mutant and seeds from the Bay control were planted on five different dates. Figure 3 shows the stearic acid contents in the oil of M25 and the Bay control plants at the different flowering dates of August 3rd, 13th, and 22th, and September 4th and 13th associated with the planting dates of June 28th, July 10th, and 22th, and August 3rd and 15th, 1992. The stearic acid content in the oil of M25 was markedly influenced by the different flowering periods, but no such influence on the stearic acid content of the Bay control was observed. However, a distinct difference was noted in which mutant M25 had a higher stearic acid content than that of the Bay control for all the flowering periods, indicating M25 contained a different genetic system for this fatty acid.

Mutagenesis with X-ray irradiation of soybean Bay variety could effectively increase the variability of oleic,¹⁰⁾ linolenic,^{6,11)} and stearic acids. Mutant M25 resulting from this study had all the good agronomic characteristics of the Bay commercial variety. In conclusion, soybean oil with a high stearic acid content has better stability²⁾ and therefore, mutant M25 could provide better stability to soybean oil for its consumption and storage.

Acknowledgment. This work was supported in part by a grant from Ajinomoto Company Inc.

References

- 1) R. K. Downey and D. I. McGregor, *Curr. Adv. Plant Sci.*, **12**, 151-167 (1975).
- 2) P. O. Lundeen, W. R. Fehr, E. G. Hammond, and S. R. Cianzio, *Crop Sci.*, **27**, 1102-1105 (1987).
- 3) G. L. Graef, L. A. Miller, W. R. Fehr, and E. G. Hammond, *J. Am. Oil Chem. Soc.*, **62**, 773-775 (1985b).
- 4) G. L. Graef, W. R. Fehr, and E. G. Hammond, *Crop Sci.*, **25**, 1076-1079 (1985a).
- 5) D. M. Bubeck, W. R. Fehr, and E. G. Hammond, *Crop Sci.*, **29**, 652-656 (1989).
- 6) Y. Takagi, A. B. M. M. Hossain, T. Yanagita, and S. Kusaba, *Japan. J. Breed.*, **39**, 403-409 (1989).
- 7) J. R. Wilcox, J. F. Cavins, and N. C. Nielsen, *J. Am. Oil Chem. Soc.*, **61**, 79-100 (1984).
- 8) R. F. Wilson, J. W. Burton, and C. A. Brim, *Crop Sci.*, **21**, 788-791 (1981).
- 9) J. B. Ohlrogge, W. B. Shine, and P. K. Stumpf, *Arch. Biochem. Biophys.*, **189**, 382-385 (1978).
- 10) S. M. Rahman, Y. Takagi, K. Kubota, K. Miyamoto, and T. Kawakita, *Biosci. Biotech. Biochem.*, **58**, 1070-1072 (1994).
- 11) Y. Takagi, A. B. M. M. Hossain, T. Yanagita, T. Matsueda, and A. Murayama, *Agric. Biol. Chem.*, **54**, 1735-1738 (1990).