Short Communication

Phosphoenolpyruvate Carboxylase Level in Soybean Seed Highly Correlates to Its Contents of Protein and Lipid

Toshio SUGIMOTO, Kiyoshi TANAKA,*
Michiko MONMA, Yukio KAWAMURA
and Kyoko SAIO

*Protein Science Laboratory,
National Food Research Institute,
Ministry of Agriculture, Forestry,
and Fisheries, Tsukuba,
Ibaraki 305, Japan*

**National Institute for Environmental Studies,
Tsukuba, Ibaraki 305, Japan*

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Soybean seed is one of the most important sources of vegetable protein as well as of oils for humans. The inverse correlation between the contents of protein and lipid in soybean seeds of many cultivars is known,^{1,2)} and suggests that some metabolic step(s) are in common in regulating protein and lipid contents in seed of each cultivar.

Phosphoenolpyruvate carboxylase (EC 4.1.1.31) exists universally in plant tissues³⁾ and catalyzes carboxylation of phosphoenolpyruvate to oxalacetic acid, which is one of the anaplerotic reactions proposed by Kornberg⁴⁾ to connect glycolysis with the TCA cycle and to increase concentrations of TCA cycle members for supplying carbon skeletons of amino acids. Due to such a possible role of the enzyme, it is probable that PEPCase is one of the candidates for regulating protein contents in soybean seeds.

In this communication, we discuss the role of PEPCase in soybean seeds depending upon the results on PEPCase activity and contents of protein and lipid in matured seeds of vari-

ous Japanese domestic soybean cultivars.

Ten soaked soybean seeds were homogenized with 50 ml of 50 mM Tris, 20 mM MgCl₂, and 10 mM 2-ME, pH 8.1, at 0°C by the method of Singal and Singh⁵⁾ and were dialyzed against the same buffer overnight. The dialyzate was centrifuged at 18,000 rpm for 20 min. The supernatant was assayed for PEPCase activity by the method of Hatch.⁶⁾ Protein was measured by the Kjeldhal method or the method of Bradford⁷⁾ with BSA as a standard for the extracts for the enzyme assay. Lipids were measured by weighing the oil extracted with a mixture of CHCl₃:CH₃OH=3:1 three times.

To check whether an inverse relationship^{1,2)} exists between protein and lipid contents in soybean seeds, the seeds of 13 cultivars were collected which are different in protein contents and in the place of cultivation.

The cultivar of the highest protein content was Enrei from Nagano prefecture (protein content was 43.47% of dry weight), and the lowest was Tamahomare from Yamaguchi prefecture (38.41%). The highest lipid content was observed in Akishirome from Yamaguchi prefecture (21.96%) and the one of lowest was Miyagishirome from Miyagi prefecture (19.85%) which was the cultivar with the 2nd highest protein content. The correlation between protein content (X) and lipid content (Y) was expressed as $Y = -0.39X + 36.78$. The correlation coefficient was -0.8417 with a significance at 1%. Thus, an inverse relationship between protein and lipid content was valid for these thirteen different cultivars.

The relationship between the PEPCase activity and the protein and lipid contents in soybean seeds are shown in Fig. 1. PEPCase activity correlated positively to protein contents in seeds of the 13 soybean cultivars (Fig. 1 A and B). The correlation coefficients were 0.8395 on a dry weight basis and 0.8660 on a soluble protein basis. On the other hand, the enzyme activity clearly showed an inverse correlation to lipid contents in the seeds (Fig. 1 C

Abbreviations: DW, dry weight; MDH, malate dehydrogenase; 2-ME, 2-mercaptoethanol; NAD, nicotinamide adeninedinucleotide, PEP, phosphoenolpyruvate; PEPCase, phosphoenolpyruvate carboxylase.

Fig. 1. Relationships between PEPCase Activity and Contents of Protein and Lipid in Soybean Seed.

Cultivar names and produced prefectures of soybean seeds harvested in 1986 are as follows; 1, Fukuyutaka (Fukuoka); 2, Tamahomare (Yamaguchi); 3, Akishirome (Yamaguchi); 4, Akishirome (Hiroshima); 5, Akiyoshi (Kagawa); 6, Enrei (Nagano); 7, Enrei (Niigata); 8, Tachisuzunari (Tochigi); 9, Suzuyutaka (Yamagata); 10, Miyagishirome (Miyagi); 11, Shirosennari (Akita); 12, Nanbushirome (Iwate); 13, Okushirome (Aomori).

A: Relationship between PEPCase activity per 1 g of dry seed (X) and protein content (Y) in soybean seed.

B: Relationship between PEPCase activity per 1 mg of soluble protein (X) and protein content (Y) in soybean seed.

C: Relationship between PEPCase activity per 1 g of dry seed (X) and lipid content (Y) in soybean seed.

D: Relationship between PEPCase activity per 1 mg of soluble protein (X) and lipid content (Y) in soybean seed.

The equations of regression lines and the coefficients of A ~ D were as follows: A, $Y = 0.062X + 31.727$, 0.8395;

B, $Y = 1.99X + 32.29$, 0.8660; C, $Y = -0.029X + 24.884$, -0.8411; D, $Y = -0.91X + 24.55$, -0.8494.

and D). The coefficients were -0.8411 and -0.8494 on the dry weight basis and the soluble protein basis, respectively, with a significance at the 1% level.

These results suggest that PEPCase is crucial in setting the protein and lipid contents in soybean seeds. These results also provide an important index for breeding soybean cultivars with high protein content, because PEPCase activity is measured very rapidly, easily, and precisely with a small specimen.

The physiological reason for the close relationship between PEPCase activity and protein contents (and also lipid contents) in soybean seeds is uncertain yet, although PEPCase observed in pods of legumes^{8~11)} is assumed to reflux CO₂ formed by active respiration in seeds.⁸⁾ But we know that PEPCase activity in matured seed was almost the same as that at

the late developing stage,¹²⁾ it means that the enzyme activity in matured seeds well reflects the state of seed maturation. Thus, the following explanation: the higher enzyme activity produces the higher amounts of oxalacetic acid formed from phosphoenolpyruvate, which increases the supply of amino acids for the synthesis of storage protein. Further studies on the mechanism of controlling contents of storage substances in soybean plants and on the role of PEPCase are now in progress.

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References

- 1) H. Taira and H. Taira, *Proc. Crop Sci. Soc. Japan*, **40**, 530 (1971).
 - 2) In "Soybeans: Improvement, Production, and Uses," ed. by B. E. Caldwell, American Society of Agronomy, Inc., Publisher, Madison, Wisconsin, U.S.A., 1973.
 - 3) M. H. O'Leary, *Ann. Rev. Plant Physiol.*, **33**, 297 (1982).
 - 4) K. L. Kornberg, "Essays in Biochemistry," Vol. 2, Academic Press, London, 1966.
 - 5) H. Singal and R. Singh, *Plant Physiol.*, **80**, 369 (1986).
 - 6) M. D. Hatch, *Anal. Biochem.*, **47**, 174 (1972).
 - 7) M. M. Bradford, *Anal. Biochem.*, **72**, 248 (1976).
 - 8) Hedelley, CL, DM Harvey and RJ Kelly, *Nature*, **258**, 352 (1975).
 - 9) Y. P. Luthra, I. S. Sheoran and R. Singh, *Photosynthetica*, **17**, 210 (1983).
 - 10) R. K. Chopra and S. K. Sinha, *Photosynthetica*, **16**, 509 (1982).
 - 11) K. Shimotubo, Y. Hojyo and S. Kato, *Japanese J. Crop Sci.*, **48** (extra issue 2), 81 (1979).
 - 12) T. Sugimoto, K. Tanaka, M. Monma, K. Hashizume and K. Saio, unpublished.
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