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Note

Analysis of γ-glutamyl dipeptides in F_2 hybrid of blackgram and mungbean by high-performance liquid chromatography

SHIH-HSIUNG WU
Institute of Biological Chemistry, Academica Sinica, Taipei (Taiwan)
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Mungbean [*Vigna radiata* (L.) Wilezek] is a common grain legume in Asia, especially in the Indian sub-continent. The nutritive value of mungbean protein is limited by sulphur-containing amino acids (methionine and cysteine) with a chemical score of about 32 (ref. 1). Rat feeding experiments indicated that the nutritive value of mungbean can be improved by increasing its methionine content[2]. Blackgram [*Vigna mungo* (L.) Hepper] is a close relative of mungbean. These two species can be crossed inter-specifically[3,4] and the methionine content of blackgram was found to be much higher than that of mungbean[5]. Protein fractionation experiments revealed that about 46% of blackgram methionine is in the form of smaller molecules which are permeable to a cellophane membrane[6]. Otoul *et al.*[7] found that γ-glutamylmethionine is the predominant form in blackgram and γ-glutamyl-S-methylcysteine is the major form in mungbean. Luyeye[5] confirmed the findings of Otoul *et al.* and found that γ-glutamyl-S-methylcysteine is dominant in the F_1 hybrid of mungbean and blackgram. Improving mungbean protein quality through an inter-specific breeding programme seems to be a feasible approach if a relatively simple mass screening technique can be developed to detect these two dipeptides in the F_2 hybrid of mungbean and blackgram. We report here a method for analysing these two dipeptides by high-performance liquid chromatography (HPLC). This method could be useful in developing mass screening techniques for genetic studies.

EXPERIMENTAL

Glutamic acid, methionine and S-methylcysteine were all of L-configuration and purchased from E. Merck (Darmstadt, G.F.R.). γ-Glutamyl-S-methylcysteine and γ-glutamylmethionine were synthesized as described previously[8]. F_2 hybrid beans of mungbean and blackgram were harvested in the fields of the Asian Vegetable Research and Development Centre (AVRDC).

Preparation of bean extract

A single bean was ground carefully with a mortar and pestle in 3 ml water. The aqueous mixture was sedimented by centrifugation at 9220 *g* for 20 min and the supernatant was collected and lyophilized. The lyophilized powder was dissolved in 0.3 ml of distilled water and the insoluble material was filtered off with a Millipore

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filter-paper (HA type) and a Sep-Pak C_{18} cartrdige (Waters Assoc., Milford, MA, U.S.A.). A 20-μl volume of the filtrate was analysed by HPLC as described below.

High-performance liquid chromatography system

The HPLC system consisted of two Waters Model 6000 pumps, a Waters UK-6 valve-loop injector, a Waters Model 450 variable-wavelength UV detector, a Waters refractive index detector and a Model 660 solvent programmer. A μBondapak C_{18} reversed-phase column (Waters Assoc.) was used for all chromatographic separations. All runs were performed at ambient temperature with UV detection (230 nm). The eluent was 0.05 *M* phosphate buffer (pH 3.2) and the flow-rate was 2.0 ml/min in the isocratic mode.

RESULTS AND DISCUSSION

Paper chromatography may be an alternative, rapid method for the analysis of γ-glutamyl-S-methylcysteine and γ-glutamylmethionine in F_2 hybrid, after the interspecific breeding programme, but it has limitations with respect to quantitation. In this and previous studies[8] we analysed these dipeptides by HPLC and found that the peak height bears a linear relationship with amount. From the analysis of many bean extracts of F_2 hybrid by HPLC, two kinds of F_2 beans could be distinguished: one contains only γ-glutamyl-S-methylcysteine and the other contains both γ-glutamyl-S-methylcysteine and γ-glutamylmethionine, as shown in Fig. 1.

The nutritive value of mungbean seems to have been improved through the

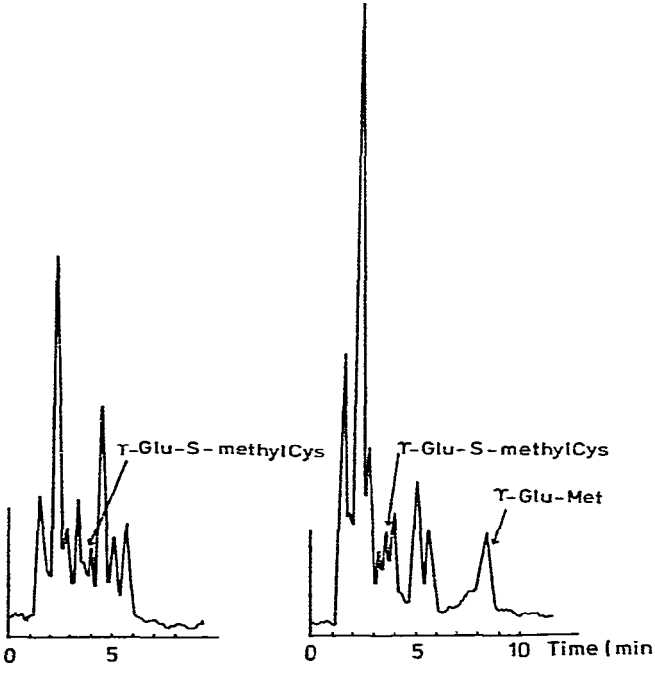

Fig. 1. HPLC profile of bean extracts of F_2 hybrid.

inter-specific breeding programme, and the HPLC system provides a simple and convenient method for the mass screening of these beans in the breeding programme.

REFERENCES

1 *Amino-Acid Content of Foods and Biological Data on Proteins*, Food Policy and Food Science Service, Nutrition Division, Food and Agriculture Organization, 1970.
2 C. S. Tsou and M. S. Hsu, in *Proceedings of 1st International Mungbean Symposium*, AVRDC, Shanshua, Taiwan, 1978, pp. 40–45.
3 C. S. Ahn and R. W. Hartman, in *Proceedings of 1st International Mungbean Symposium*, AVRDC, Shanhua, Taiwan, 1978, pp. 240–246.
4 N. C. Chen, J. F. Parrot, T. Jacobs, L. R. Baker and P. S. Carlason, in *Proceedings of 1st International Mungbean Symposium*, AVRDC, Shanshua, Taiwan, 1978, pp. 247–252.
5 L. Luyeye, *Dissertation*, Docteur en Sciences Agronomiques, Faculté des Sciences Agronomiques de Letat Gembloux, Belgium, 1979.
6 C. S. Tsou, M. S. Hsu, S. T. Tan and H. G. Park, *Acta Hortic.*, 93 (1979) 279.
7 E. Otoul, R. Marechal, G. Dardenne and F. Desmedt, *Phytochemistry*, 14 (1975) 173.
8 S. H. Wu, K. T. Wang and C. S. Tsou, *Proc. Natl. Sci. Counc. Rep. China (A)*, 6 (1982) 34.