A11

Proteome Approach as a Tool for the Efficient Isolation of Basic Seed Proteins from Soybean (*Glycine max* L. Merr.)

Sun-Hee Woo*, Se-Young Kim, Keun-Yook Chung[1], Sun-Lim Kim[2],
Seung-Keun Jong and Hong-Sig Kim
Department of Agronomy, Chungbuk National University, Cheongju, 366-763 Korea;
[1]Department of Agricultural Chemistry, Chungbuk National University, Cheongju, 366-763 KOREA;
[2]National Institute of Crop Science, RDA, Suwon 441-100, KOREA

Background and Research Objectives

Recently, plant biology in the field of plant proteomics has been increasing interest in the proteins expressed by the genome, the proteome. We have so far faced the difficulties in the separation of basic proteins from soybean. To clarify how the separation of glycine subunit of basic proteins affect soybean properties, we are currently developing and improving the methods needed for two-dimensional electrophoresis(2-DE) techniques. We report here the separation and analysis of 2-DE electroblotted from the gels onto PVDF membranes for the mature cotyledon of soybean, but also on providing an overview of protein components in the various stages for proteome analysis of the soybean. Especially, rehydration buffer, water, and TCA were used as possible solvents for the extraction of basic proteins and also their extraction efficiencies were compared in this study.

Materials and Methods

Soybean (*Glycine max* L.) cv. Hwangkum was used for this experiment. Soluble proteins of cotyledons were examined by two-dimensional gel electrophoresis according to the protocol of O'Farrell (1975). The new instrument used in this study allows the easy separation by IEF tube gels in adaption 27cm length. Also, rehydration buffer, water, and TCA were used for the extraction of basic protein and the evaluation of their extraction efficiencies.

Results

The basic proteins from the soybean cotyledon, *Glycine max*L. Merr. were better separated and isolated through the use of two-dimensional electrophoresis (2-DE) and by searching *N*-terminal sequencing data of proteins obtained compared to the previous methods used. The proteins extracted from the cotyledon were better separated and observed on the use of hexane for the removal of impurities. Using this method, the highly reproducible isoelectric focusing (IEF) can be obtained from polyacrylamide gels, and IEF from the polyacryl amide gel at all the possible pH range (4.0-9.8) was more easily separated than IPG (immobilized pH gradient) gels. The polyacrylamide gels in the first dimension in 2-DE was used to separate and identify a number of soybean cotyledon proteins in the proteome analysis. In this new method using 2-DE, 27cm in length of plate coated with polyacrylamide gel was used and the experiment was further investigated under the various conditions. In conclusion, the nine basic proteins were separated from soybean in this study. It appears that the glycine subunit separated played the important role in soybean breeding and biochemical characterization.

*Corresponding author : Tel: 043-261-2515 E-mail: shwoo@chungbuk.ac.kr

Fig.1) Rehydration buffer, TCA, Water 추출방법 따른 이차원전기영동 의한 단백질 분리

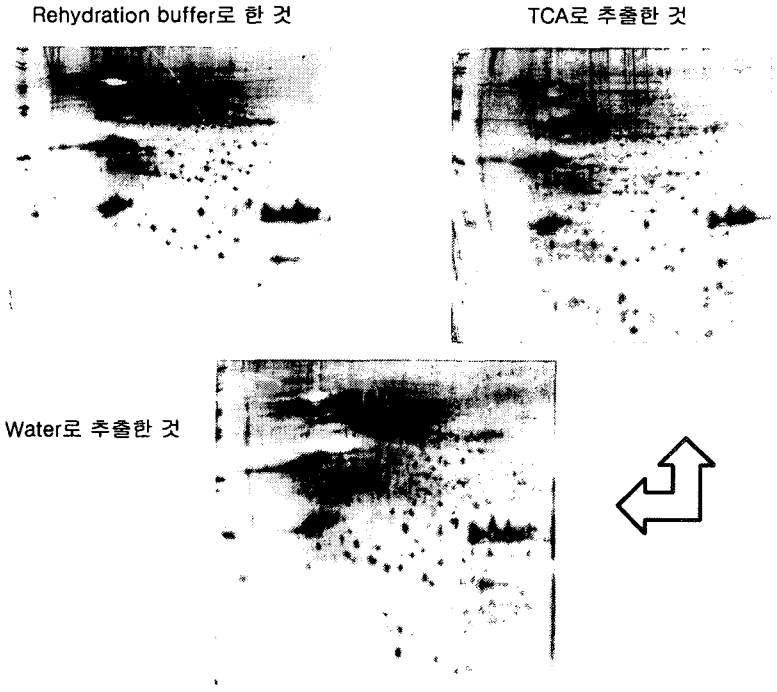

Fig. 2) TCA 와 Water 추출방법에 따른 SDS-PAGE에 의한 단백질 분리