Characterization of Transgenic Soybean Seedlines by Protein Expression with the Agilent 2100 Bioanalyzer

Application

Food

Authors

Debra Dempsey
E.I. DuPont de Nemours
DuPont Experimental Station
Wilmington, DE 19880
USA

Mark Jensen
Agilent Technologies
2850 Centerville Road
Wilmington, DE 19808-1610
USA

Abstract

This application note describes how the Agilent Technologies 2100 Bioanalyzer can be used to analyze protein extracts from transgenic seedlines. Accuracy and precision in the determination of protein size and concentration was sufficiently good to allow for the characterization of experimental seedlines based solely on expressed protein profiles.

Introduction

ß-conglycinin(7S) and glycininin(11S) are the primary seed storage proteins in soybean, comprising about 70% of the total storage proteins. Because these proteins make up such a large portion of soya protein, they are of critical economic importance. Characterizing the expression of these proteins in various soybean seed lines is also essential in expanding the range of soy protein applications in food. The relative levels of these two proteins have been shown to significantly impact the nutrition, taste, and texture of food products derived from soy protein extracts [1]. For this reason, soybean lines that preferentially express the 11S or 7S proteins continue to be an active target in the efforts to improve seed quality.

Both conglycinin and glycinin are complex aggregates made of smaller protein subunits. β-conglycinin is a 7S protein with a trimeric structure and is composed of 53, 70, and 76 kDa units. Glycinin is an 11S hexameric protein consisting of six monomer units, where each monomer is made up of 40 and 20 kDa subunits [2].

Given the sizes of protein subunits, it is relatively straightforward to characterize the levels of these two proteins by electrophoresis. The Agilent 2100 Bioanalyzer, an automated microfluidic electrophoresis platform, is well suited for the analysis of proteins in this size range. The Protein 200 LabChip has a size range of 14-200 kDa. Samples of soy protein isolate can be loaded, separated, and analyzed for relative protein composition in less than 45 minutes. In this application we describe the use of the Agilent 2100 Bioanalyzer in the analysis of soybeans, to determine the level of expression of 7S and 11S proteins.

Experimental

Extraction Protocol

Grind the seed into a fine powder. Place a 30 mg sample in an Eppendorf® tube and add 1000 µL of extraction buffer (50 mM Tris [pH 7.5], 10 mM 2-mercapto-ethanol, 0.1% SDS). Agitate the mixture on a rotary shaker for 30 minutes and then centrifuge at 15,000 g for 10 minutes. Remove the supernatant and introduce the extract directly into the Protein 200 LabChip to begin the assay protocol.

If the extracts contain an excessive amount of oil, the supernatant may be removed and further diluted prior to beginning the Protein 200 protocol.

Methodology

To determine if a transgenic line of soybeans preferentially expressed the β-conglycinin or glycinin protein groups, seed extracts were compared to extracts made from wild-type seed lines that strongly expressed either the 7S or the 11S group. Twenty extracts from the unknown seedline were prepared as described above. The protein profiles were determined by separating the proteins in the Agilent 2100 Bioanalyzer. Because of the size range of the proteins, the Protein 200 Plus chip (14-200 kDa) was used for the separation. The concentration of the individual proteins was determined by a comparison with an internal concentration marker (myosin). The ratio of 7S/11S proteins was then calculated from those values and the ratio was compared to the ratio of wild-type seedlines that preferentially expressed either 7S or 11S protein groups. Figure 1 shows the electropherogram for a control seedline, a 7S wild-type, an 11S wild type, and a representative sample from the unknown transgenic seedline. A simulated gel image of the same traces is shown in Figure 2.

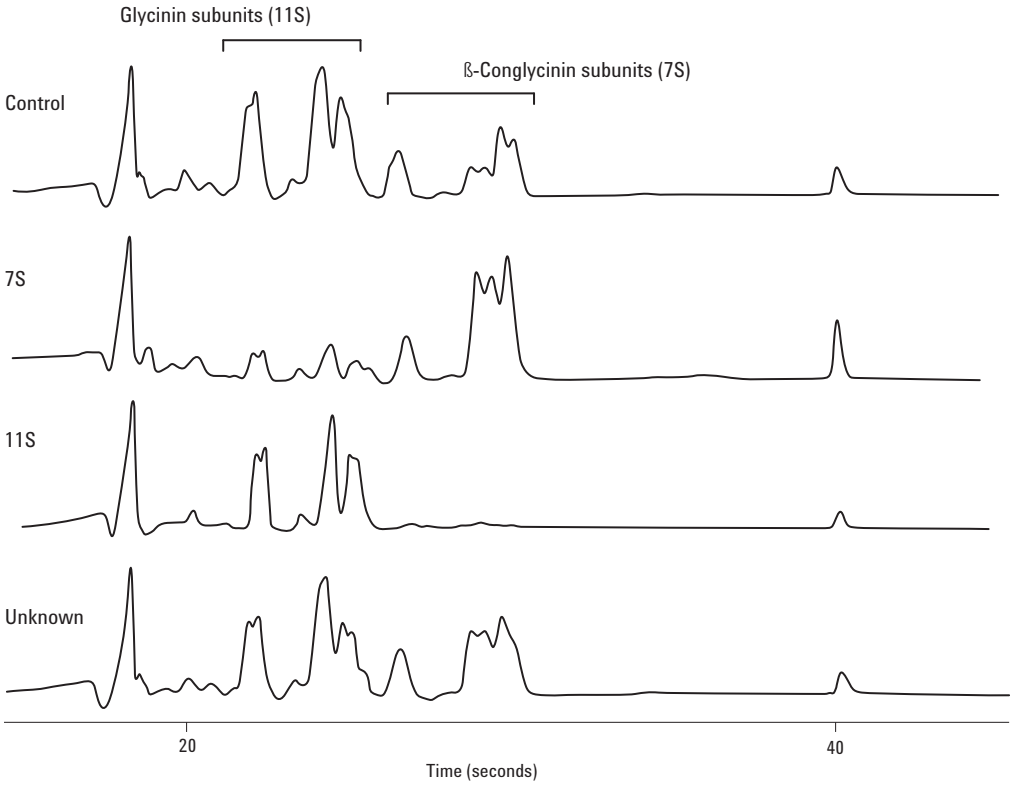

Figure 1. Electropherograms of soya protein extracts.

The ratio of 7S to 11S for the 20 sample extracts was calculated by integrating the individual components comprising the 7S and 11S groups and then determining the summed areas of the two groups. The levels of extracted protein and the 7S/11S ratios for the control 7S, 11S, and unknown groups are summarized in Table 1.

The ratios determined for the high 11S and high 7S seedlines indicate the range of expected 7S/11S ratios should fall between 0.04-3.4. The ratios determined for both the controls and unknown extracts both fall within this range. All 20 unknown samples showed a higher 7S/11S ratio than the control. Average ratio values for 20 unknown extracts and 5 control extracts was 0.72 and 0.39, respectively. Measurement precision was excellent for both sample sets.

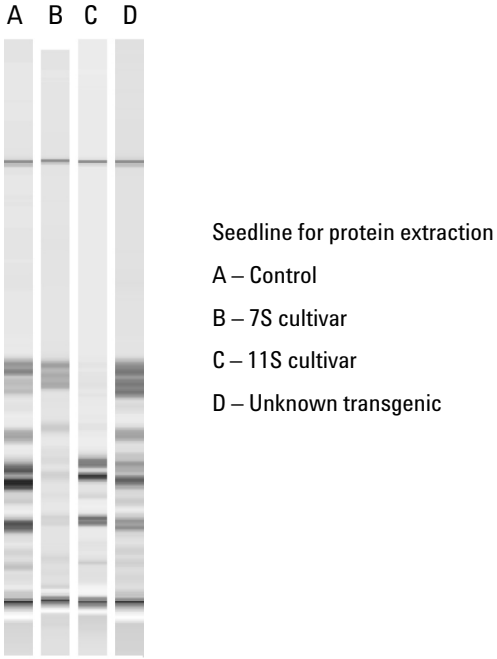

Figure 2. Gel simulation of electropherograms for soya protein extracts.

Table 1. Summary of Extracted Protein Levels and 7S/11S Protein Ratios

SeedLine	Extracted protein level µg/mL	7S/11S Ratio
Control	14,000	0.39 ±0.004 (n=5)
7S	5,200	3.4
11S	14,000	0.04
Unknowns	13,000	0.72 ±0.1 (n=20)

Conclusions

This application note describes the use of the Agilent 2100 Bioanalyzer and the Protein 200 LabChip Kit for evaluating the relative expression of β-conglycinin and glycininin in unknown seedlines. In the 20 protein extracts taken from the unknown seedline, the average ratio was 0.72 ±0.1. This ratio lays in range that is characteristic of high 7S expression seedlines. Given the precision of the ratio determination, it is clearly apparent that the assignment of this unknown seedline to the high 7S group is statistically significant. This conclusion is further supported by a comparison to a normal control seedline where the ratio of 7S/11S is 0.39 ±0.004.

The Agilent 2100 Bioanalyzer together with the Protein 200 LabChip Kit are quick and efficient tools for the determination of relative protein expression. The resulting protein expression profiles are in turn a highly effective means for the characterization of new transgenic seedlines.

References:

1. Kitamura, K., (1995) "Genetic Improvement of Nutritional and Food Processing Quality in Soybean," *Jpn. Agric. Res. Quart.*, **29(1)**, 1-8.
2. Yaklich, R. W., (2001) "-Conglycinin and Glycinin in High-Protein Soybean Seeds," *J. Agric. Food Chem.* **49**, 729-735.

For More Information

For more information on our products and services, visit our web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
May 9, 2003
5988-9441EN

Agilent Technologies