Identification and Characterization of Anthocyanins in Adzuki Bean (*Vigna angularis*) by HPLC-DAD-ESI/MS Analysis

Tae Joung Ha1*, Seok Bo Song1, Jeeyeon Ko1, Chang-Hwan Park1, Myeong-Eun Choe1 and Doyeon Kwak1

1Upland Crop Breeding Research Division, Department of Southern Area Crop Science, NICS, RDA, 20th, Jeompiljaero, Miryang, 50424, KOREA

**[Introduction]**
Adzuki bean (*Vigna angularis*) is indigenous to the tropical region of Asia; currently, it is one of the important crops in Korea, Japan, and China. To our knowledge, the pigments of its black seeds have still not been fully characterized. This prompted us to identify the anthocyanins occurring in the black seed coats (cv. Geomguseul) of adzuki beans by employing reversed-phase C-18 column chromatography and high-performance liquid chromatography with diode array detection and electrospray ionization/mass spectrometry analysis.

**[Materials and Methods]**
Adzuki bean (*Vigna angularis*, cv Geomguseul) with black seed coat cultivar were cultivated within an experimental field at the Department of Southern Area Crop Science, NICS, RDA at Miryang, in 2016. The anthocyanins present in the adzuki beans were characterized by HPLC-DAD-ESI/MS analysis. A 10 μL sample of the crude acidic methanolic extract was injected onto an analytical Zorbax SB-C18 column. The mobile phase was composed of water (A) and methanol (B). The gradient conditions were as follows: 0-2 min, 15-20% B; 2-30 min, 20-35% B and then held for 10 min before returning to the initial conditions. The other HPLC conditions were as follow: a flow rate of 0.8 mL/min; column temperature, 25 °C; and detection, 530 nm. The mass spectrometer used a SCIEX API 2000 LC/MS/MS (Applied Biosystems) equipped with an electro spray ionization (ESI) source and an ion trap mass analyzer. The MS revealed the positive molecular ions, and MS2 was used to break down the most abundant species by collision-induced dissociation.

**[Results and Discussions]**
Anthocyanins play an important role in physiological functions related to human health. The objective of this study was to investigate the profiles of anthocyanins in the black seeds of adzuki beans using HPLC-DAD-ESI/MS analysis. The individual anthocyanins were identified by comparing their mass spectrometric data and retention times. From the black seed coat of the adzuki beans, ten anthocyanins were identified, including delphinidin-3,5-diglucoside(1), cyanidin-3,5-diglucoside(2), petunidin-3,5-diglucoside(3), delphinidin-3-galactoside(4), delphinidin-3-rutinoside(5), cyanidin-3-glucoside(6), petunidin-3-glucoside(7), petunidin-3-(6”-coumaroyl)glucoside(8), delphinidin-3-(malonoyl)glucoside(9), and delphinidin-3-glucoside(10). In this study, we report for the first time anthocyanin profiles for the seed coat of adzuki beans.

**[Acknowledgements]**
This work was supported by a grant from AGENDA Program (No. PJ011346022017), Rural Development Administration, Republic of Korea.

*Corresponding author: Tel. +82-55-350-1222, E-mail. taejoung@korea.kr