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Polymorphic variation of seed saponin composition in Korean wild soybean (*Glycine soja* Sieb. and Zucc.)

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Introduction

Saponins are secondary metabolites containing a steroid or triterpenoid aglycone with a number of carbohydrate moieties linked through glycoside linkages at one or more sites. Soysaponins have been broadly studied for its hypocholesterolaemic, haemolytic, immunostimulatory, antitumourigenic and chemoprotective activities, as well as taste properties. Despite a great deal of attention paid on the predominant saponins, detailed studies on the biological activities of minor saponins have been hampered owing to the lack of large quantities of purified saponins. And, the lack of variations in saponin components, is one of the fact that hindering the saponin synthesis pathway studies. Since the genetic base of soybean is very narrow, screening genetic resources of wild soybean for saponin composition is an excellent tool to identify accessions that may not have undesirable taste and may have new mutations. In the present study, we have embarked on the saponin composition analysis in Korean wild soybean seeds (KWS) to determine a new saponin compound, a mutant with no group A saponins and a mutant in regard of saponin composition.

Materials and Methods

1. Seed sources

A total of 3,025 Korean *G. soja* accessions, which are being preserved in the Chung's Wild Legume Germplasm Collection (CWLGC) at the Chonnam National University (CNU), Korea, were used. These materials represented almost all the species range in South Korea (Fig. 1).

2. Extraction of Saponins

Wild soybean seeds were divided into hull, cotyledon and hypocotyls; hypocotyls were used in this study. Five hypocotyls from each accession were extracted with 80% aqueous methanol at room temperature for 24 hours.

3. Detection of saponins

Saponin extractions were analyzed by TLC and LC-PDA/MS/MS (LTQ Orbitrap XL, Thermo Fisher Scientific). TLC was performed on silica gel plates (W20 x H10 cm, Merck) with a lower phase of chloroform-methanol-water (65:35:10, v/v). Spots were visualized according to Selvam's method.

LC-PDA/MS/MS was performed in C30 reverse phase column (Develosil C30-UG-3, 2.0 mm I.D. × 150 mm) at 40ºC with acetonitrile-water gradient system (10-90% acetonitrile in 80 min) containing constant 0.1% formic acid. Saponin components were detected by MS/MS analysis which was carried out under ESI positive mode and by PDA detector at UV 205 and 292 nm..

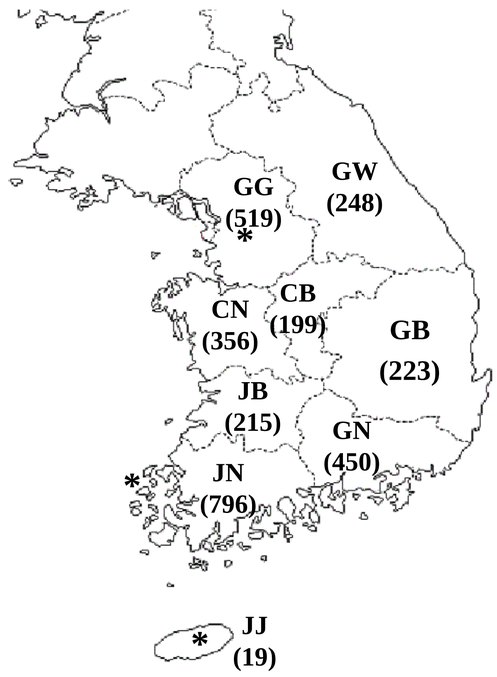

Fig. 1. Germplasm collection sites. GW, Gangwon-do; GG, Gyeonggi-do; CB, Chungcheongbuk-do; CN, Chungcheongnam-do; GB, Gyeongsangbuk-do; GN, Gyeongsangnam-do; JB, Jeollabuk-do; JN, Jeollanam-do; JJ, Jeju-do. Stars on the map indicate the location of variants.

Results and Discussion

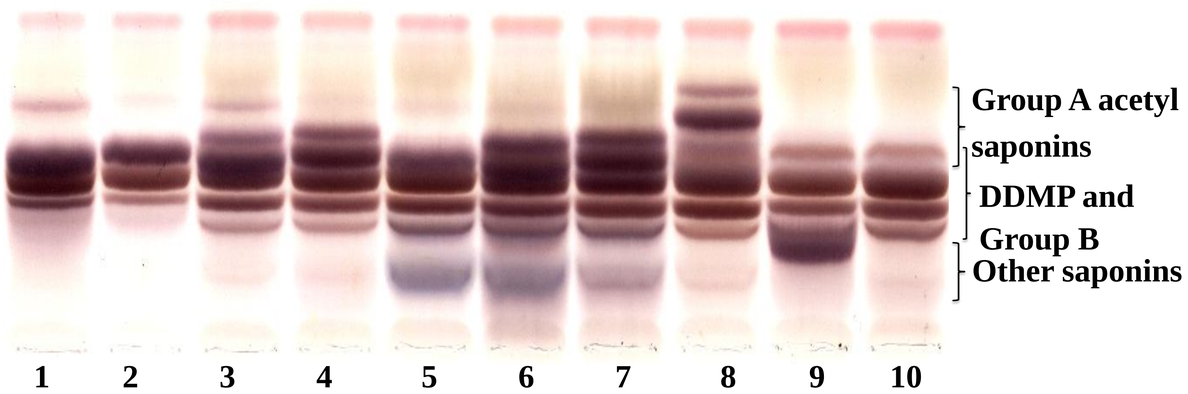

Fig. 2. Common saponin phenotypes and variants in KWS by TLC. Lanes 1 to 7 are common phenotypes, Aa, Ab, AaBc, AbBc, Aa+α, AaBc+α and AbBc+α types. Lanes 8, 9 and 10 are variants, AuAeBc (CWS0115), A0Bc+αg (CWS2133) and A0Bc-S (CWS5095) types, respectively.

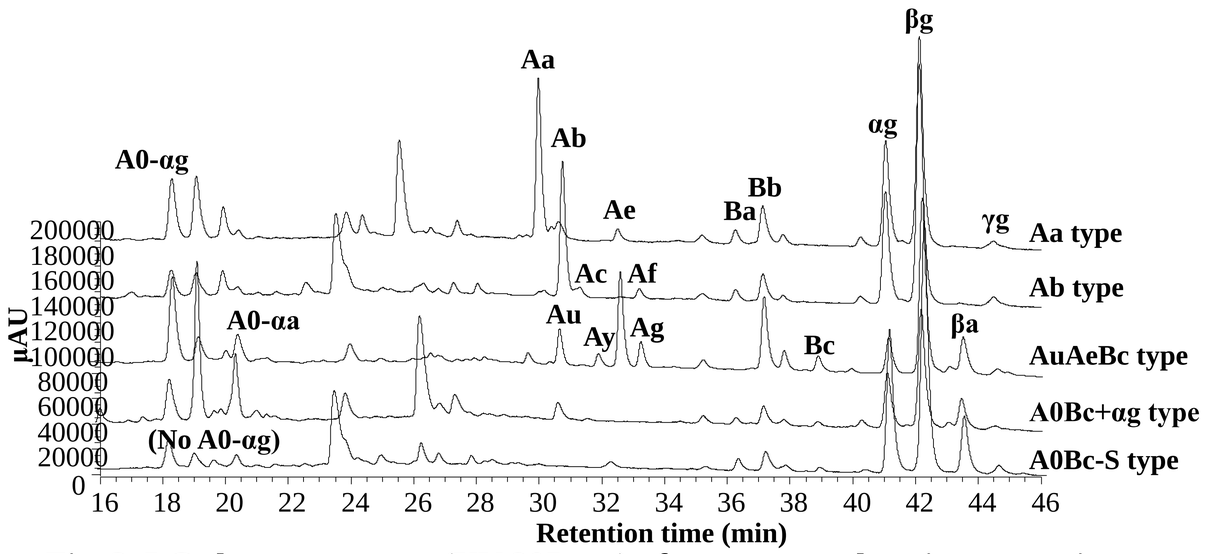

Fig. 3. LC chromatograms (UV 205 nm) of common and variant saponin types

1. Seven common saponin phenotypes (Aa, Ab, AaBc, AbBc, Aa+α, AaBc+α and AbBc+α) and three mutant phenotypes designated as AuAeBc (CWS0115), A0Bc+αg (CWS2133) and A0Bc-S (CWS5095), were identified in KWS.

2. The common phenotypes Ab, Aa, AbBc and AaBc were prevalently determined in *G. max* (71.5, 24.9, 1.8 and 0.3%) while AaBc, Aa, Ab and AbBc were in *G. soja* (58.4, 21.6, 9.7 and 4.6%). In our study we found that AaBc, Aa, AaBc+α and Aa+α were the four most commonly occurring types in Korea with the frequencies of 55, 32.8, 7.5 and 3.3 %, respectively. Phenotypes Ab, AbBc and AbBc+α were rarely found in Korea, especially the accessions from eastern regions of Korea have no Ab saponin.

3. Comparatively, the AaBc type was dominant in JB, GG, CB and CN regions (67, 64.9, 63.3 and 62.1%, respectively) and the Aa type was dominant in GB, GW and GN regions (59.2, 49.6 and 49.6%, respectively). High frequencies of AaBc+α, Aa+α, AbBc and Ab were found in JN region (17.7, 6.2, 1.4 and 1%, respectively) and AbBc+α (1.4%) in the CN region. The accessions having alpha (α) saponins are first reported as mutants in Japan, but they were frequently found in KWS (11.1%). However, they were not detected in *G. max* collections, yet.

5. LC-PDA/MS/MS results suggested that CWS0115 would carry a recessive allele *sg-3* at the *Sg-3* locus, which controls the glucosylation of the second sugar (either galactose or arabinose) of the sugar chain attached at the C-3 position of soyasapogenols A and B. Thus, this mutant consequently lacked group A saponins Aa and Ax and DDMP saponins αg and αa. It has the content of minor saponin Ae 7-fold higher than the normal wild soybeans. Hence, it will help to purify the Ae component which will be used in the evaluation of its pharmacological properties.

6. CWS2133 lacked saponin Aa nor Ab but accumulated saponin A0-αg, indicating that it would carry a recessive allele *sg-1*^{0} instead of *Sg-1*^{a} & *Sg-1*^{b} at the *Sg-1* locus. The absence of all group A saponins in CWS5095 denoted that there was no production of soyasapogenol A and thus it was presumed to carry a recessive allele *sg-5* at the *Sg-5* locus. These mutant accessions might be utilized in producing a new soybean variety with good tastes as well as in pharmacological and biosynthetic studies.

References

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This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0013600), Korea.