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Verification of Genetic Purity of Soybean F_1 Hybrid Using Molecular Markers

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[Introduction]
Soybeans have an extremely low success rate of artificial crossings, which requires a lot of effort from breeders. Moreover, the morphological characteristics of the F_1 hybrid seeds are difficult to assess visually if similar to the phenotypes of the paternal and maternal parents. Therefore, an accurate genetic purity test of F_1 hybrid plants is essential step for breeding purposes. We used polymerase chain reaction (PCR)-based molecular markers as a tool for hybrid seed purity determination to breed Soybean (*Glycine max* L.) varieties for natto (unripe *Cheonggukjang*).

[Materials and Methods]
We crossed eight soybean cultivars with small seed size respectively and harvested a total of 204 F_1 seeds (8 combinations). Except for distinguishable lines by phenotypical traits, a total of 96 F_1 hybrid lines in 3 combinations (*Hoseo/Kosuzu*, *Sunam/Kosuzu,* P*ungwon/Miryang355*) was analysed using molecular markers. The young leaves of each soybean F_1 line were collected and then used to extract genomic DNA using DNeasy Plant Mini Kit (Qiagen). PCR reactions with GoTaq DNA polymerase (Promega) were performed by ProFlex PCR system (Life Technologies). After PCR reaction, the products amplified by each marker were analyzed by QIAxcel advanced system (Qiagen) for confirming the soybean hybrids.

[Results and Discussion]
For confirming F_1 hybrid, nine simple sequence repeat (SSR) and three soybean Indel (Sindel) markers were screened for the parental varieties of each crossing combinations. As a result, only four SSR markers and a Sindel marker were selected as parent-specific markers. We used Satt181, Satt184, Satt187, Satt308, Sindel17-19 markers in *Hoseo/Kosuzu* line and confirmed that all plants were completely crossed. Also, Satt181, Satt187, Satt308 markers were used in *Sunam/Kosuzu* line and Satt181, Satt187, Sindel17-19 markers were used in *Pungwon/Miryang355* line. We confirmed that 5 plants (8.2%) of 61 *Sunam/Kosuzu* line and 2 plants (15.4%) of 13 *Pungwon/Miryang355* line were self-fertilized respectively and the rest were completely crossed. The result of assay showed that the five single polymorphic markers were effective tool for detection of contaminations of the soybean F_1 hybrids from their parents.

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