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Molecular Characterization and CRISPR/Cas9-mediated Mutagenesis of *GmKIX* Genes Involved in Organ Size Regulation of Soybean

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[Introduction]

Soybean (*Glycine max* (L.) Merr.) is one of the most important crop with economic value as a source of protein and vegetable oil for human food and animal feed. Seed size is one of important agronomic traits that influences seed yield of crops. Studies on organ size regulation have been mainly performed in Arabidopsis, and it has recently been reported that the KIX-PPD complex affects organ size by regulating cell proliferation and growth. However, the genetic and molecular mechanisms underlying seed size regulation are still largely unknown.

[Materials and Methods]

Information of the *GmKIX* genes were identified using the KIXBASE and SoyBase database. To expression analysis, total RNA was isolated from immature seeds (S1, stage 1; S2, stage 2; S3, stage 3) and 7-days-old grown unifoliate leaves and subjected to real-time RT-PCR. To CRISPR/Cas9-mediated mutagenesis of *GmKIX*8 genes, pECO201 vectors harboring multiplex guide RNA of *GmKIX8* was subjected to *Agrobacterium*-mediated transformation into half-imbibed seeds of soybean.

[Results and Discussions]

We identified the 3 paralogs of *GmKIX8*, 2 paralogs of *GmKIX9* and 6 non-annotated *GmKIX* genes in soybean genome. A comparative study of amino acid sequences has shown that KIX domain is highly conserved in N-terminal region of KIX8 and KIX9. The expression of *GmKIX* genes increased in Hoseo and PI86490, which has a small seed size variety, whereas decreased in Soheung-2 and KLS88035, which has a big seed size variety, in immature seeds and 7-d-old grown unifoliate leaf. The CRISPR/Cas9 vectors containing *GmKIX8* multiplex guide RNA was transformed into half seeds of soybean and induced putative transgenic shoots. To better understand the regulation and function of *GmKIX* genes, we currently are being analyzed the CRISPR/Cas9-induced transgenic plants.

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