（学位第３号様式）

学 位 論 文 要 旨

氏　名	Md. Abdur Rauf Sarkar
題　目	Studies on genetic improvement of isoflavone biosynthetic pathway in soybean (ダイズのイソフラボン生合成経路の遺伝的改良に関する研究)

Soybean isoflavones are secondary metabolites accumulated in seeds and have an important role in microbe-plant interactions for nodulation and defense against pathogens and have health benefits as part of the human diet. Therefore, genetically improved high isoflavone content soybean line development is a great challenge in the soybean breeding program. Many enzymes related to the isoflavone biosynthetic pathway have been well-characterized and their transcription is coordinately regulated by transcriptional factors including MYB. Two approaches are available for genetic improvement of the isoflavone biosynthetic pathway in soybean seeds. One approach is a direct modification of gene-encoded isoflavone biosynthesis enzyme and the second one is the modification of genes encoded transcriptional factors regulate the expression levels of isoflavone biosynthetic genes.

In this study, several genes were identified that are useful for the genetic improvement of isoflavone content in soybean seeds using both approaches. Firstly, identify the responsible gene in the soybean mutant line, F333ES017D9, displaying a low accumulation of daidzein derivatives instead of genistein derivatives in seeds. A mutant locus was identified on chromosome 18 using a mapping experiment and also found a single base deletion resulting in a frameshift mutation in the coding sequence, at third exon of a chalcone reductase gene (GmCHR5) by next-generation sequencing technique. Chalcone reductase is a key enzyme involved in switching the divergence of daidzein and genistein derivatives. Ectopic expression of GmCHR5 in soybean hairy roots showed the partly compensated mutant phenotype and increased daidzein derivatives content in the transgenic hairy roots. These results suggest that the mutant allele of F333ES017D9 would be valuable in the breeding programs to change the ratio of daidzein and genistein derivatives in soybean seeds.

Secondly, MYB transcriptional factors were identified that are regulating the expression level of genes encoded enzymes related to the isoflavone biosynthesis by a novel screening system combining agroinfiltration technique into N. benthamiana plants and hairy root transformation system in soybean. Reporter gene (ß-glucuronidase: GUS) construct was developed which fused with the promoter sequences of three isoflavone biosynthesis genes (Chalcone synthase; CHS, isoflavone synthase 1 and 2; IFS1 and IFS2), respectively. Hereafter, co-transformed the reporter gene plasmid with a plasmid that induces the constitutive expression of candidate soybean MYBs through agroinfiltration into N. benthamiana leaf and identified novel candidate MYB genes that are functional for isoflavone biosynthesis. Three candidate MYB genes, GmMYB102, GmMYB280 and GmMYB502 were enhanced the expression of GUS genes with agro-infiltration system. The expression profiles of these three MYB genes in soybean developing seeds are highly correlated with isoflavone accumulation pattern. Also confirmed the accumulation of isoflavones via the over-expression of these three MYB genes in transgenic soybean hairy roots. The developed TFs screening system in this study would be useful to identify the functional transcriptional factors regulating a specific biosynthetic pathway for phytochemicals that beneficial for human health.

The findings of this study would be valuable for the genetic improvement of isoflavone content in soybean seeds.