# RECENT RESEARCH ACTIVITIES

## Analysis of gene expression in field grown soybean

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Plants synthesize specialized metabolites which can be counted up to 200,000 to 1,000,000 compunds. These compounds are active in defense against both biotic and abiotic stresses as well as the interactions with other organisms. Among these metabolites, flavonoids, with more than 8,000 compounds, are a chemically and functionally well-characterized group. For example, flavonoids are known to function in the regulation of auxin transport, protection against UV exposure, and modulation of ROS (reactive oxygen species). In addition to these functions in plants, flavonoids are secreted from plant roots and shown to play important roles in rhizosphere biological communications.

Flavonoid synthesis is well characterized: chalcone synthase (CHS) converts 4-coumaroyl-CoA to naringenin chalcone or, to isoliquiritigenin together with chalcone reductase (CHR). Chalcone isomerase (CHI) isomerizes naringenin chalcone to naringenin and isoliquiritigenin to liquiritigenin.

Isoflavones are a class of flavonoids, which occur predominantly in legume species. The isoflavone biosynthesis pathway is also well characterized, and known to be derived from the central flavonoid biosynthesis pathway. P450 protein, isoflavone synthase (IFS), catalyses the first reaction in isoflavone biosynthesis pathway to form 2-hydroxyisoflavanone. 2-Hydroxyisoflavanone is dehydrated to produce daidzein and genistein. This reaction is mediated either spontaneously or via 2-hydroxyisoflavanone dehydratase (HID). Soybean (*Glycine max* (L.) Merr.) synthesizes daidzein and genistein, which are further glucosylated in the cytosol to daidzin and genistin by UDP-glucose: isoflavone 7-O-glucosyltransferase (IF7GT) and malonylated to malonyldaidzin/genistin by malonyl-CoA: isoflavone 7-O-glucoside 6″ -O-malonyltransferase (IF7MaT). These products are presumably accumulated in vacuoles via unknown mechanisms. These isoflavone aglycones are suggested to be secreted from roots into the rhizosphere via two different pathways: membrane transport mediated by an ABC (ATP binding cassette)-type transporter, and secreted as isoflavone glucosides, which then to be hydrolysed to aglycones, daidzein and genistein, by an apoplast-localized ICHG (isoflavone conjugates hydrolysing beta-glucosidase). It is well known that Isoflavones in the soybean rhizosphere were shown to induce the expression of nod genes of *Bradyrhizobium japonicum*, which in turn initiate the nodule formation in the soybean roots to fix atmospheric nitrogen.

The major form of isoflavone in soybean root tissue is malonyldaidzin, but the major secreted form of isoflavone is daidzein. We previously showed that the secretion of isoflavones was developmentally regulated in hydroponic culture. Higher secretion of daidzein was observed during the vegetative stages than during the reproductive stages (Sugiyama et al. 2016). To expand our understanding of isoflavone synthesis and secretion, we analyzed isoflavone biosynthesis and contents during soybean growth in the field (Sugiyama et al. 2017). Differential expression of isoflavone biosynthesis genes was observed.

### References

[1] A Sugiyama, Y Yamazaki, K Yamashita, S Takahashi, T Nakayama, K Yazaki, “Developmental and nutritional regulation of isoflavone secretion from soybean roots” Bioscience, biotechnology, and biochemistry 80 (1), 89-94, 2016.

[2] A Sugiyama, Y Yamazaki, S Hamamoto, H Takase, K Yazaki, “Synthesis and secretion of isoflavones by field-grown soybean” Plant and Cell Physiology 58 (9), 1594-1600, 2017.