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# Metabolomic Analysis of Soybean Leaves and Seeds at High Temperatures and Elevated $CO_2$ Concentrations

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**[Introduction]**
Soybean is one of the most important crops for supplying proteins and fats to humans and is increasingly in high demand in South Korea. However, there is little information about the growth responses of soybean to climate change in South Korea. This study was carried out to investigate the impact of high temperature and elevated air CO2 concentration on growth and metabolites of soybean crops through semi-chamber experiments using the Soil-Plant-Atmosphere-Research system(SPAR)

**[Materials and Methods]**
The soybean cultivar, Daewon, was used in this study for leaf and seed metabolite analysis. Soybean leaves were harvested on July 30, during the vegetative growth stage, and soybean seeds were collected during the final harvest on October 20. Each extract was analyzed using ultra-high performance (UPLC-Q-TOF MS) and gas chromatography mass spectrometry-triple quadrupole 8030 (GCMS-TQ 8030). Metabolite data sets were analyzed using SIMCA-P+ version 12.0.1, and MetaboAnalyst software was used for principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA), permutation test, enrichment, and pathway analysis.

**[Results and Discussion]**
The evaluation parameters of the PLS-DA model for each treatment group were R2X = 0.480, R2Y = 0.662, and Q2 = 0.625 in leaves and R2X = 0.356, R2Y = 0.594, and Q2 = 0.347 in seeds. In leaves, all groups were statistically discriminated by two components of PLS-DA score plots, but not clearly discriminated in seeds.
Forty-eight metabolites, including sugars (sucrose, fructose, galactose, mannose, myo-inositol, and pinitol), amino acids (tryptophan, phenylalanine), organic acids (manic acid, aspartic acid, malic acid, citric acid, fumaric acid, glutamic acid), lipid metabolites (LPC(16:0), glyceric acid, diacylglycerols), isoflavones (malonyl genistein, malonyl daidzin), and soyasaponins (DDMP type: gamma-a; B type: Bb) were identified with VIP > 1.0 in leaf by LC-MS and GC-MS analysis. Moreover, seventy metabolites, including isoflavone (genistein, daidzein, malonyl genistein, genistin, malonyl daidzin, daidzin) and soyasaponins (DDMP type: gamma-g, gamma-a; B type: Bb, Bc) were identified with VIP > 1.0 in seeds by LC-MS and GC-MS analysis. The metabolites of seeds were clearly distinguished by high-temperature treatment in PLS-DA, but not by elevated CO2 treatment. Seed metabolites tended to be affected by temperature levels, but they were less affected by CO2 levels. In the high temperature treatment, regardless of CO2 levels, 16 metabolites increased in seeds while 27 metabolites decreased. In particular, secondary metabolites, such as daidzin, daidzein, and genistin were significantly reduced in seeds after high-temperature treatment.

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