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Proteometabolic analysis revealed alteration in flavonoid and isoflavonoid metabolism upon ethylene and abscisic acid treatments in *Glycine max* leaves

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Phytohormones play a central role in plant's physiology. Despite the significant understanding in the hormone signaling, a deep understanding of downstream targets is missing, especially at the protein and metabolite levels. In this direction, here we used an integrated proteomics, phosphoproteomics and metabolomics approach to investigate the ethylene, ABA and combined ABA+ethylene signaling in soybean leaves. A protamine sulfate precipitation (PSP) method was employed to enrich the low-abundance proteins followed by their identification and quantification using label-free quantitative proteomics. This approach allowed the identification of 5171 unique proteins and 1182 differentially modulated in one or more treatments. Moreover, phosphoproteome analysis led to the identification of 716 class 1 phosphorylation sites (localization probability ≥0.75, score difference ≥5) belonging to 532 unique phosphoproteins. Increased phosphorylation of MPK3/6 was observed after ethylene treatment while ABA resulted in dephosphorylation of these MPKs. Functional annotation of the identified proteins showed an increased abundance of proteins related to the flavonoid and isoflavonoids biosynthesis in response to ethylene treatment and a shift in the fatty acid metabolism upon ABA treatment. HPLC analysis showed an accumulation of isoflavones (Genistin, Daidzein and Genistein) upon ethylene treatment, validating the proteomics results. Further, metabolome analysis using LC-MS/MS confirmed the accumulation of flavonoids and isoflavonoids in response to ethylene treatment and accumulation of lipids in response to ABA treatment. Taken together, our results showed potential cross-talks between ethylene and MPK-signaling and ABA and lipid signaling pathways.

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