From Discovery to Biotechnological Application: LCMS-based approaches reveal a constitutive biosynthesis pathway of flavonols in insect pest-resistant soybean genotypes

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INTRODUCTION: Soybean is one of the most important crops in Brazil\'s economy. However, soybean crops often suffer damage from insect pests, such as Anticarsia gemmatalis. Some resistant genotypes were used to decipher resistance mechanisms by evaluating defense compounds, including protease inhibitors (PIs) and flavonoids. This study aimed to evaluate gene expression and metabolic pathways involved in soybean resistance to insect attack. METHODS: Firstly, an LC/MS QqQ-based strategy was applied for broad-range flavonoid profiling of soybean genotypes contrasting in resistance to the pest insect. Product ion and precursor ion scan modes were combined with pseudoMS3 fragmentation to map flavonoid classes (aglycones) and their glycoconjugates. Secondly, LCMS untargeted analysis was used to discover new metabolites involved in plant resistance. High abundance proteins were depleted by PEG, and 2DE-LCMS was used to map low-abundance dysregulated proteins. Finally, RNM and LCMSn were used to confirm the structure of deregulated metabolites. Biological activities were confirmed by chemical synthesis and caterpillar survival assays. RESULTS: The metabolic profiles generated by LC/MS allowed reconstruction of the flavonoid biosynthetic pathways, revealing a constitutive nature of herbivory resistance in the resistant genotype IAC-17 and metabolic regulation for the rechanneling of Quercetin and Kaempferol and their conjugates in soybean. Highest relative abundances were detected for glycosides such as Rutin, Quercetin 3-O-rhamnosylglycoside-7-O-glucoside, and Quercetin-3-O-rhamnopyranosyl-glucopyranoside-rhamnopyranoside in the leaves of the resistant genotype. LCMS untargeted analysis revealed that the methylated form of Rutin, Isorhamnetin Rutinoside, was produced constitutively in the resistant genotypes IAC-17. Likewise, genes encoding flavonol synthase and methyltransferases were highly up-regulated in IAC-17. Overall, some herbivory defense responses appeared to be constitutive characteristics, while others were induced or JA-independent, as verified for flavonol levels. Conversely, salicylic acid levels were higher in IAC-17 and IAC-100 genotypes. Proteins not yet characterized for plant-insect interactions, such as transmembrane receptors and transcription factors, were positively regulated in the resistant genotype IAC-17. LCMS QqQ-based analysis confirmed that constitutive flavonol biosynthesis was inherited genetically from the PI229358 ancestor and maintained in the resistant genotypes IAC-17 and IAC-100. The structure of Isorhamnetin 3-O-Rutinoside was confirmed by RNM and MSn analysis. The importance of methylation in the resistance mechanism was confirmed by reduced caterpillar survival when fed diets supplemented with molecules of Rutin containing multiple methylations generated by chemical synthesis. CONCLUSION: IAC-17 and IAC-100 appear to be genetic sources for studying flavonol biosynthesis and its relationship with insect resistance. Genetic manipulation of flavonol biosynthesis regulation, driving the production of methylated flavonols, may be a strategy for developing new soybean genotypes showing high levels of resistance to insect attack.

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