Abstract:

Cotton (*Gossypium hirustum*) is an economically important crop and widely cultivated around the globe. However, the major problem of cotton is its high vulnerability to biotic and abiotic stresses. It has been around 3 decades, since cotton plant was genetically engineered with genes encoding insecticidal proteins (mainly Cry proteins) with an aim to protect it against insects attack. Several studies have been reported on the impact of these genes on cotton production and fiber quality. However, the metabolites responsible for conferring resistance in genetically modified cotton need to be explored. The present work was aimed to unveil marker compounds responsible for the insect resistance in cotton plant. We employed ^{1}H-NMR spectroscopy conjoined with multivariate data analysis to study metabolic differences in insect resistant cotton varieties compared to non-insect resistant cotton variety. Collectively, a set of thirty-five metabolites were identified in cotton varieties used in current study. Least discriminant analysis (LDA) and Multi-layer perceptron (MLP) neural networks were applied to differentiate insect resistant and non-insect resistant cotton varieties along with identification of discriminant metabolites responsible for the separation among cotton varieties. The marker metabolites obtained from multivariate data analysis were then subjected to relative quantification. Heatmap revealed that glycine, tryptophan, tyrosine, cinnamic acid, asparagine, xylulose, valine and malic acid were highly expressed in PR-02 (Bt cotton). Furthermore, dendograms displayed that compared to NB-01(non-insect resistant), PR-02 projected close to PR-01(non-transgenic insect resistant) showing more similar metabolite profile. LDA results inferred that valine, nonanoic acid, citrulline and limonene were the major discriminating metabolites among cotton varieties. MLP results demonstrated that valine and limonene were the major metabolites in distinguishing cotton varieties. It concluded that LDA and MLP are useful techniques in differentiating cotton varieties. Moreover, relative quantification showed that elevated level of defense related metabolites like cinnamic acid, E-β-ocimene, tryptophan, uridine, dibutyl phthalate, GABA, fumarate, succinic acid, shikimic acid, arabinose, xylulose, trigonelline, glycine, di-allylic methylene, tyrosine, and maelic acid in PR-02 (Bt cotton) might be responsible for inducing resistance against insect herbivory.