

Gene transformation induced inherent structural changes relating to nutrient alterations in alfalfa for dairy cows

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Gene transformation is an efficient approach to alter agronomic traits of forages towards quality improvement. TT8 and HB12 are two transcriptional factors in phenylpropanoid pathway, which serves the source of many metabolites. TT8-silenced (TT8i) and HB12-silenced (HB12i) alfalfa were generated by RNAi via *Agrobacterium tumefaciens* due to their potential uses in lignin reduction. Transgenic and wild type (WT) alfalfa were grown in greenhouse and harvested before flowering (5 TT8i, 11 HB12i and 4 WT). Samples were analyzed on spectral structures by ATR-FTIR spectroscopy and chemical and nutrient profiles. Afterwards, HCA and PCA multivariate molecular analyses were conducted on structural data, and correlations and regressions between chemical/nutrient profiles and structural parameters were performed using R software. Results showed that both transgenic alfalfa had: 1) higher fibers and unavailable proteins, but lower crude protein and starch; 2) increased TC2, TC3, STC3, β -sheet heights, STC area and decreased TC1 height; HB12i increased all STC peak heights, as well as amide (total amide and amide I) and STC areas; 3) WT was separated from transgenic populations within CHO, amide and whole fingerprint regions by both HCA and PCA; TT8i and HB12i were separated from each other by PCA within amide and STC regions. 4) chemical profiles were correlated with spectral structures, which had good linear relationships with some chemical compositions. In conclusion, transformation of alfalfa with TT8 and HB12 RNAi induced alterations of inherent molecular structures of alfalfa, which were closely related to chemical/nutrient profiles, leading to changes in nutrients degradation and availability to dairy cows.

Implications: Genetic modifications of alfalfa led to changes in molecular structures of alfalfa that can be detected by vibrational molecular spectroscopy, leading to changes in its nutritive profiles.