Metabolite profiling of Huaiyang *Medicago polymorpha* with different mowing crops

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ABSTRACT

The main purpose of our study was to identify and compare secondary metabolites due to different mowing in order to make better use of Huaiyang *Medicago polymorpha*. The metabolite profiling of Huaiyang *Medicago polymorpha* with two mowing crops was performed using a rapid resolution liquid chromatography system with quadrupole time-of-flight mass spectrometer (RRLC-QTOFMS) followed by multivariate statistical analyses. Principal Component Analysis (PCA) results showed a clear distinction between two mowing crops. The major metabolites that contributed to mowing discrimination were identified. The results also showed that the content of major active compounds in *Medicago polymorpha* from the second crop are higher significantly than the first crop. This study suggests that the strategy is a reliable and simple method for the rapid discrimination of subtle variations due to different mowing crops.

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1. Introduction

*Medicago polymorpha* Linn. (Chinese name, Yangcao) is one of the agronomically most important medic species and mainly spread Jiangsu province and Zhejiang province in China. In the theory of Traditional Chinese Medicine, it has been known to “heat-clearing and detoxifying and remove-dampness” and used for treating indigestion, urinary tract calculi, jaundice and intestinal inflammation (Editorial Board of Flora of China of Chinese Academy of Sciences 1998). It has also been used to one of the best vegetables in the area of Jiangsu and Zhejiang Province. Currently, studies on the compounds and biological activity in the genus Medicago are reported.(Hanif et al. 2015; Gholami A et al. 2014; Bora and Sharma 2011; Krakowska et al. 2017) However, there were little studies on the compounds of *Medicago polymorpha* Linn. and mainly representative compounds are saponins and flavonoids (Kinjo et al. 1994; He et al. 2005; Yin and Qin 2008; Tava et al. 2011). Moreover, the researches on differences of compounds in *Medicago polymorpha* between the first and the second crop have not been reported. It is well known that the type and quantity of compounds vary as a function of the mowing crops of *Medicago polymorpha*. Obviously, this has a significant impact on the corresponding ingredients and contents. Thus, it is very important to investigate different mowing crops in the metabolite profiling of *Medicago polymorpha*.

Metabolomics has emerged as a valuable technology for the comprehensive profiling and comparison of metabolites in biological systems, especially in plant systems (De Vos et al. 2007). LC-MS-based approaches are expected to be of particular importance in plants, owing to the highly rich biochemistry of plants, which include many semi-polar compounds, including key secondary metabolite groups, which can be easily separated and detected by LC-MS techniques. In this study, an approach using RRLC-QTOFMS, along with multivariate statistical analysis, was developed to rapidly find variables (markers) that contribute most to mowing crops. This analysis method has demonstrated its potential for the discrimination of subtle variations, within the same plant species or strains, due to different mowing crops.

2. Results and discussion

2.1. Multivariate statistical analysis

The representative BPI chromatograms of *Medicago polymorpha* collected in ESI negative and positive mode are illustrated in Figure S1. To compare difference in the metabolite profiling of *Medicago polymorpha*, unsupervised principal component analysis was performed. All observations acquired in both ion modes were analyzed using PCA. PCA, a common method to handle metabolomics data, can discriminate samples from different groups and indicate ions responsible for the discrimination. In this work, PCA exhibit satisfactory classification (Figure 1), which indicated that the content and distribution of components were highly varied in *Medicago polymorpha* collected from different mowing crops.

2.2. Identity assignment and confirmation of chemical markers

Variables (metabolites) significantly contributed to the clustering and discrimination was identified according to Independent-Samples T-test (SPSS 17.0) analysis. The
critical p-value was set to 0.05 for significantly differential variables in this study. The potential markers together with the major peaks detected in *Medicago polymorpha* extracts from different mowing crops, were identified or tentatively assigned by comparison with the reference compounds or by matching the empirical molecular formula and MS/MS data with that of previously published compounds. Details of the identified components are summarized in Tables S1–S4. First, the possible elemental compositions of the selected compounds were generated by using the software Masshunter. The structures of the potential biomarkers were elucidated with the high-resolution MS and MS/MS spectra with that of previously published compounds. As a result, 18 components of *Medicago polymorpha* are identified, and 12 of them are varied with mowing crops. Among these compounds, Cynaroside, Apigenin-7-O-β-D-glucopyranoside, Daidzein, Luteolin, Apigenin, Chryseriol, Isoliquiritigenin, Formononetin, Soyasaponin Bb, Genkwanin, α-Hederin and Oleic acid were identified by comparing the mass spectra and retention time with that of reference compounds. For example, Figure S2 and Figure S3 showed how luteolin and soyasaponin Bb were confirmed by MS spectra and MS/MS with external standard in both positive and negative mode. We found this mass spectral interpretation of luteolin and soyasaponin Bb in real *Medicago polymorpha* samples in clear agreement with reference standards, which demonstrated reliable identification.

To get an overview of distributions of potential biomarkers for two groups, we performed PCA biplot (Figure 2) of the potential biomarkers. The PCA biplot is aimed to optimally display the potential biomarkers distributed in different samples. The arrows are good indicators of the correlations among the variables. The position of the samples of two groups relative to the arrows provides good indications as to which variable(s) have had the largest effect. Except Soyasaponin Bb, Cynaroside, Apigenin-7-O-β-D-glucopyranoside, other biomarkers were shown more abundant in the second mowing group than the first mowing group (Figure 2). Meanwhile, the levels of Isoliquiritigenin was shown no significance in these two groups.
All these findings were consistent with Supplementary Figure S4, data distributions by ESI$^+$ and ESI- of these biomarkers were displayed based on T-test analysis.

3. Conclusions

In this study, a novel strategy to rapidly differentiate and identify the complex Traditional Chinese Medicine (TCM) extracts, which have subtle variations within the same plant species or strains due to different mowing crops, by using RRLC-QTOFMS coupled with multivariate statistical analysis were proposed, and validated using *Medicago polymorpha* as a model herb. The result from this study indicates that the proposed method is reliable for the rapid analysis of a group of metabolites present in herbal medicine and applicable in the differentiation of complex samples that share similar chemical ingredients.

Disclosure statement

The authors declare that they have no competing interests.

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