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Electrochemical Profile Recording for *Pueraria* Variety Identification

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Abstract

Rapid identification of plant variety is valuable in both academic study and crop production. However, rapid and accurate identification has been difficult because many varieties have very similar morphological characteristics and are susceptible to the effects of the growing environment. In this work, we established an electrochemical method for recording the electro-active compounds profile in plant tissue. Because the chemical composition of different varieties is largely controlled by their genes rather than a growing environment, this method has considerable potential for variety identification. Three varieties of Pueraria with sixteen locations were collected for confirming the feasibility of the proposed methodology. Principal component analysis and peak ratio analysis have been used for grouping the sample data. The results indicate the electrochemical profiles of three varieties can be distinguished using their voltammetric data.
Introduction

Plant classification and identification are the basis of botanical research. Plant taxonomy has evolved over a long period of time into a variety of taxonomic methods. In recent years, a lot of achievements have been made in plant identification based on quantitative classification. Generally, the quantitative method is to select some relatively stable morphological characteristics of plants for identification. This identification method is quite effective at the genus level and even at the species level. However, it is difficult to recognize plants at the sub-species level due to their similar morphological characteristics. On the other hand, the economic value of different varieties of plants often varies significantly. For example, *P. montana* var. *lobata* and *P. montana* var. *thomsonii* showed very similar morphological features but the *P. montana* var. *lobata* is a medicinal plant while the *Pueraria montana* var. *thomsonii* is a food plant.

At present, in addition to the conventional identification by botanists, computer identification and classification technology is the most rapidly developed method. This kind of technology includes the classification system of machine decisions and the retrieval system of human decisions. The leaf classification system based on the machine decision is a typical pattern recognition system, by which the system can identify which kind of plant the leaf belongs to. On the other hand, the retrieval system of human decision is usually a content-based image retrieval system. Based on the image of the leaf to be identified provided by the user, the system finds a number of similar leaf images and corresponding text descriptions from the blade image database and returns them to the user for a decision. These technologies face certain challenges. For example, the leaves of some plants, especially in sub-species level, are very similar, but other organs, such as flowers, stems and roots, are different. Therefore, it is
necessary to establish an alternative way for species identification.

In plant analysis, a variety of analytical methods were used to identify plants. These methods include **infrared spectroscopy, DNA fingerprinting, high performance liquid chromatography and surface enhanced Raman scattering** \(^6\)-\(^9\). They often have excellent accuracy and reliability. However, they often suffer some drawbacks such as high instrument costs, complex preprocessing and a long-time analysis process. It is a new method to consider plant identification from the perspective of electrochemistry \(^10\)-\(^15\). Electrochemical recognition is different from traditional analytical chemistry instruments. Specifically, an electrochemical system is concerned not with the concentration of a particular compound, but with the overall intensity of the signal generated by the interaction of different components of the system. It focuses on detecting the overall feature of differences between samples.

In this work, we investigated the feasibility of the electrochemical method for identifying the plant variety. *Pueraria montana* var. *lobata*, *Pueraria montana* var. *montana* and *Pueraria montana* var. *thomsonii* were selected from sixteen locations deliberately. The disposable screen-printed electrode was used for plant tissue immobilization after the extraction process. Four different solvents were used during the extraction process for better representation of the electrochemical profile. The variety identification can be then achieved by the peak ratio recognition.

**Experimental**

**Reagents and chemicals**

All the reagents, including ethanol, glycol, dimethylformamide (DMF), KH\(_2\)PO\(_4\),...
Na$_2$HPO$_4$, were purchased from Macklin Co. Ltd. and used without purification. Screen-printed electrodes (SPEs) were purchased from Nanjing Youyun Technology Co. Ltd. The reference electrode of the SPE is made by Ag/AgCl. Both working electrode and counter electrode of the SPE are made by carbon paste. We added these information in the revised manuscript.

Plant leaf collection

Leaves of *P. montana* var. *lobata* were collected from Hefei (Anhui province), Qingdao (Shandong province), Yongji (Shanxi province) and Chongqing (Chongqing municipality). Leaves of *P. montana* var. *montana* were collected from Fuzhou (Fujian province), Guangzhou (Guangdong province), Qingyuan (Guangdong province), Sandu (Guizhou province), Leishan (Guizhou province) and Hengfeng (Jiangxi province). Leaves of *P. montana* var. *thomsonii* were collected from Fuzhou (Fujian province), Huaihua (Hunan province), Wuhan (Hubei province), Yingde (Guangdong province), Chongqing (Chongqing municipality) and Changsha (Hunan province). Figure 1 shows the locations of sample collection. All samples were collected between May and October 2018. All samples were stored frozen before electrochemical recording.

Plant tissue treatment and electrode surface modification

Four solvents include water, ethanol, glycol and DMF have been used for the leaf tissue extraction. Typically, 0.1 g of thawed plant leaf was ground with 5 mL of solvent followed with a 2 min sonication. Then, 5 µL of slurry was dip-coated on the working electrode of the SPE and dried in the room-temperature.
Electrochemical profile recording

The electrochemical profile of the plant tissue was recorded using a CHI760E electrochemical workstation with the SPE integrated a three-electrodes system. The electrolyte is a 0.1 M PBS (pH 7.0). Differential pulse voltammetry (DPV) has been used for electrochemical recording. The scan range is −0.2–1.2 V. The pulse amplitude is 50 mV. The pulse width is 0.05 s. The pulse period is 0.5 s.

Data treatment

All raw data was treated by the background current subtraction. A normalization process was carried out for recorded electrochemical signals, where the ratios between the current and the maximum peak current were obtained at different potentials. The parallel coordinate plot is constructed by the normalized current of a variety after water, ethanol, glycol and DMF. The principal component analysis (PCA) and cluster analysis were carried out using R with the ward linkage method based on combining the normalized electrochemical signals recorded from water, ethanol, glycol and DMF.

Results and Discussion

Figure 2 shows the schematic diagram of the methodology. Plant leaf tissue was firstly immobilized after the extraction. Then, the electrochemical profile of the plant has been recorded using the differential pulse voltammetry using a positive scan. The electrochemical active compounds contribute signals during the scan which corresponding to the type and concentration of the compound in the plant. These profiles are largely controlled by the gene rather than the growing environments. After the statistical analysis, these profiles can be used for the plant variety identification.
Figure 3 shows the DPV curves of the *P. montana* var. *lobata*, *P. montana* var. *montana* and *P. montana* var. *thomsonii* recorded using water, ethanol, glycol and DMF as extraction solvents. It can be seen that three varieties all showed several peaks during each voltammetric scan, suggesting electrochemical active compounds from plant tissue were oxidized. Because of the complexity of the chemical components in plant tissue, it is difficult to distinguish each compound. However, previous studies have confirmed the electrochemical activity of polyphenols\(^{16,17}\), flavonoids\(^{16,18}\) and alkaloids\(^{19,20}\) in plant tissues, which can be oxidized at low potentials. The purpose of our study is not to distinguish each compound related to the signal, but to recording the whole profile of all electrochemical active compounds of each variety, because these compounds are controlled by genes, so differences in electrochemical profile reflect differences in variety genes\(^{14,15}\). As shown in the figure, the DPV curves of a variety with different locations showed slightly different signals. This is because plants growing in different locations are influenced by their environments. In addition, each variety exhibited profile differences after extraction with different solvents due to the different extracted electrochemical active compounds with different solvents. Therefore, combining these curves can reveal a more comprehensive profile of the sample.

Figure 4A shows the parallel coordinate plot of the normalized current of *P. montana* var. *lobata*, *P. montana* var. *montana* and *P. montana* var. *thomsonii* recorded after water, ethanol, DMF and glycol extractions. The parallel coordinate plot is a common method to analyze and display multivariate data. This way of presenting data can be used to distinguish the differences of data sets obtained by different samples under different conditions. If the figure shows that different samples have significantly different trends, it means that the data obtained by the sample under different conditions are obviously different. If we cannot clearly identify different samples in the diagram, it
means that the differences between different samples are very small. It can be seen that the three varieties showed different tendencies, indicating different varieties have different electrochemical active compounds or distributions. In contrast, Figure 4B shows the parallel coordinate plot of the normalized current of *P. montana* var. *thomsonii* recorded after water, ethanol, DMF and glycol extractions with six locations. No clear tendencies can be noticed in the figure. Therefore, the electrochemical profile of the plant tissue showed the potential of using the voltammetric data for variety discrimination. As we introduced in the first section, *Pueraria* has potential commercial value because some of these species are used for food and medicine production\(^{21,22}\). *P. montana* var. *lobata* is a medicinal plant while the *P. montana* var. *thomsonii* is a food plant\(^2\).

In order to further testing the possibility of using the electrochemical method for plant variety identification, we carried out the PCA analysis of all samples. PCA is often used for dimensionality reduction of high dimensional variables. Our previous studies suggested that PCA analysis of electrochemical voltammetric data does not have a high interpretative capability\(^{23-26}\). Figure 5 shows the PCA result using the electrochemical profiles for all samples. In this case, three factors extracted within the electrochemical profiles can reach 95.2% interpretative capability, indicating the electrochemical profile between the variety showed significant differences. Moreover, all samples of each variety were in a small area, suggesting the three components could be used for variety identification.

Figure 6 shows the dendrogram of all 15 samples. It can be seen that the dendrogram divided into three clusters. Each cluster only contains one specific variety, suggesting no outlier observed in this study. Although ecology has a great influence on the type and distribution of chemicals in plant tissues, genes are still the most important
factor. As shown in the figure, the *P. montana* var. *lobata* and *P. montana* var. *montana* showed a close relationship.

Based on the above results, we proposed a simple method for these three varieties identification. As shown in Figure 7, we selected the two characteristic peaks based on the sum of four voltammetric data. It can be seen that three varieties have significant difference results. The *P. montana* var. *lobate* showed the lowest value, while the *P. montana* var. *thomsonii* showed the highest value. Therefore, we believe the voltammetric data from the plant tissue can be used as database and subsequently used for unknown variety identification.

**Conclusions** (optional)

In conclusion, the electrochemical profiles of *P. montana* var. *lobata*, *P. montana* var. *montana* and *P. montana* var. *thomsonii* were recorded using plant leaf tissue after water, ethanol, glycol and DMF extractions. Recorded profile varies between the varieties due to the presence of different content of electrochemical active compounds in plant tissue. Based on the recorded electrochemical profiles, these varieties can be effectively identified using extracted peak values. Due to the high reproducibility of the proposed methodology, this method can be used effectively for other plant variety identification.
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Figure Captions

Fig. 1 Sample locations of *P. montana* var. *lobata*, *P. montana* var. *montana* and *P. montana* var. *thomsonii*.

Fig. 2 Scheme of recording the electrochemical profile of *Pueraria* leaf tissue for variety identification.

Fig. 3 Normalized DPV curves of *P. montana* var. *lobata*, *P. montana* var. *montana* and *P. montana* var. *thomsonii* recorded using water, ethanol, DMF and glycol as extraction solvent.

Fig. 4 (A) Parallel coordinate plot of normalized currents of *P. montana* var. *lobata*, *P. montana* var. *montana* and *P. montana* var. *thomsonii*. (B) Parallel coordinate plot of normalized currents of *P. montana* var. *thomsonii* with six locations.

Fig. 5 3D PCA diagrams of twelve vinegar obtained from normalized currents recorded by voltammetric scans.

Fig. 6 Dendrogram of *P. montana* var. *lobata*, *P. montana* var. *montana* and *P. montana* var. *thomsonii* based on the voltammetric behavior recorded in four solvents.

Fig. 7 Plots of 2 peaks for *P. montana* var. *lobata*, *P. montana* var. *montana* and *P. montana* var. *thomsonii*. 
Fig. 1
Fig. 2

*P. montana* var. *lobata*

*P. montana* var. *montana*

*P. montana* var. *thomsonii*

Fig. 3
Fig. 4

Fig. 5
Fig. 6

Fig. 7
Graphical Index

The figure for the Graphical Index saved in the JPEG, PNG or TIFF format at 300 dpi should be pasted with the size adjusted to the frame below (5 cm long and 8 cm wide).