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Short Communication

Development of an electrochemical technology for ten *Clematis spp* varieties identification

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The identification of ornamental plants has always been a challenge. Due to the complexity of breeding development, ornamental plants often have very complex genetic relationships with native plants. In this work, we proposed the use of glassy carbon electrodes to perform voltammetric scanning of extracts of plant leaves. Ten specific varieties of *Clematis* were selected as research targets. We recorded the voltammograms of these *Clematis* varieties under different conditions and found that different extraction solvents and buffer solutions can present different profiles. Integrating these voltammograms can be used to quickly identify varieties of *Clematis*. In addition to scatter plots and 2D density maps we previously proposed, we propose a new pattern recognition method in this work.

Keywords: Electrochemical sensor; Voltammograms; Plant identification; Pattern recognition; *Clematis* spp.

1. INTRODUCTION

Ornamental plants compose a group of cultivated plants with some unique ornamental traits. Under the dual pressure of natural selection and artificial selection, they form a very broad assortment

of variants [1,2]. At present, the ornamental and horticultural industry is developing very rapidly worldwide, and the annual trade of these plants has reached 150 billion US dollars. Cultivated plant varieties are groups of plants that have a unique feature. Classification of ornamental plant varieties with specific names is a complete scientific system [3,4]. Constructing a reasonable plant variety classification system is of great significance to the production, promotion, communication and scientific research of ornamental plants. Moreover, the identification and classification of ornamental plant germplasm resources is an essential prerequisite for breeding research. Variety identification is an important basis for variety classification and lays a foundation for breeding ornamental plants [5].

The classification of ornamental plants has historically undergone the process of traditional horticultural classification, variety collection and sorting, morphological classification and comprehensive classification. At present, the main identification and classification methods include comparative morphology, cytology, palynology, quantitative taxonomy, isozyme analysis and molecular marker-based analysis [5–10].

Clematis belongs to the Ranunculaceae family, which contains 355 species worldwide. In the garden, *Clematis* plants occupy a very important position [11–13]. There are approximately 155 species of *Clematis* in China, which is the country with the richest germplasm resources [14,15]. In 1753, Linnaeus established the *Clematis* genus, described nine species, and divided these species into two categories [16,17]. The first category is the Scandentes, while the second category is the Electae. Since then, many scholars from various countries have conducted classification studies on *Clematis* plants, resulting in many classification systems. Tamura conducted in-depth research on Ranunculaceae plants [18,19]. He divided Clematis into four subgenera according to the growth state of the leaves of the seedlings, the shape of the curved flowers and the condition of the stamen coat. According to Tamura's *Clematis* classification system, the primitive group has the characteristics of a bell-shaped bell and a stamen coat. In the evolutionary group, the species have horizontal flowers bent with the glabrous stamens. According to previous studies, Johnson divided 314 species of *Clematis* into 19 groups, whereas Grey-Wilson divided 297 species of *Clematis* into 18 groups of 9 subgenera. Some *Clematis* species have reproduction problems, such as low seed set, relatively long seed germination time, and low germination rates [20–24]. These species undergo slow sexual reproduction and cannot meet the needs of garden cultivation applications [25–27]. In these case, tissue culture has been widely carried out. Due to the long-term development of ornamental varieties, the external characteristics of *Clematis* are difficult to use for variety identification. Therefore, it is necessary to develop a fast and effective variety identification technique.

In this work, we used previously established electrochemical fingerprint technology to identify 10 *Clematis* ornamental varieties. The leaves of the plants were used for these investigations. Water and ethanol were used as solvents for the simple extraction of *Clematis* leaves. Phosphate-buffered solution (PBS) and acetate buffer solution (ABS) were then used as electrolytes. A three-electrode system recorded the electrochemical voltammogram information of the *Clematis* extracts. The voltammograms were then used for constructing scatter plots, 2D density maps, and heat maps. The *Clematis* varieties could be quickly identified through these patterns.

2. EXPERIMENTAL

Leaves of Patricia ann Fretwell, Rooran, Westerplatte, Kardynal Wyszyński, Burma Star, Crystal Fountain, Romantika, Kakio(Pink Champagne), Shin-shigyoku and Isago were collected from Nanjing Botanical Garden Mem. Sun Yat-Sen (Nanjing, China) in April 2020. Table 1 shows all important information for these varities. Healthy leaves of each varieties were carefully collected and stored at -20 ℃ before analysis. KH_2PO_4, Na_2HPO_4, sodium acetate and acetic acid were purchased from Macklin Co., Ltd. All other chemicals were analytical-grade reagents and were used without further purification. The reference electrode (Ag/AgCl), counter electrode (Pt wire) and working electrode (glassy carbon electrode, 3 mm in diameter, GCE) all purchased from Gaoshi Ruilian Co.Ltd. (Wuhan, Chian) Milli-Q water (18.2 MΩ/cm) was used throughout the experiments.

Water and ethanol were directly used as a solvent for plant leaf extract preparation. Typically, 10 mL of buffer solution was added into 2 g chopped plant leaf by 1 min grinding. 0.1 M acetate buffer (ABS, pH 4.5) or phosphate buffer (PBS, pH 7) was then added for 3 min sonication. For electrochemical fingerprint recording, a GCE was polished with an alumina–water slurry and rinsed with ethanol and water. Then, a three-electrode system was inserted into the beaker for electrochemical fingerprint recording. All electrochemical fingerprints were recorded using a CHI760E working station. Differential pulse voltammetry was used for recording the electrochemical fingerprints of all plant tissue between -0.1–1.5 V with a pulse amplitude of 50 mV, pulse width of 0.05 s and pulse period of 0.5 s.

Table 1. Information of Patricia ann Fretwell, Rooran, Westerplatte, Kardynal Wyszyński, Burma Star, Crystal Fountain, Romantika, Kakio(Pink Champagne), Shin-shigyoku and Isago.

Variety name	Group	Approximate height	Country of origin	Parentage
Patricia ann Fretwell	Early large-flowered group	2.0-2.5 m	United Kingdom	Unknown
Westerplatte	Early large-flowered group	2.0 m	Poland	Unknown
Burma Star	Early large-flowered group	2.0-3.0 m	United Kingdom	Unknown
Kardynal Wyszyński	Late large-flowered group	2.5-3.0 m	Poland	Unknown
Romantika	Late large-flowered group	2.0-2.5 m	USSR/Estonia	Seeding of ‘Devjatyj Vall’
Shin-shigyoku	Early large-flowered group	1.5-2.0 m	Japan	Unknown
Isago	Early large-flowered group	2.5-3.0 m	Japan	‘Kotobuki’ x ‘Duchess of Edinburgh’
Rooran	Early large-flowered group	-	Japan	Unknown
Crystal Fountain	Early large-flowered group	1.5-2.0 m	Japan	Sport of 'H F Young'
Kakio(Pink Champagne)	Early large-flowered group	2.5-3.0 m	Japan	'Star of India' x 'Crimson King'

3. RESULTS AND DISCUSSION

Figure 1 displays the schematic process of the electrochemical recording of the *Clematis* leaves. A simple leaf extraction process was conducted using water or ethanol before the addition of any electrolyte. Small molecules were extracted from the leaf tissue for better participation in the electrochemical reaction. A GCE was then used to record the electrochemical behaviour directly in the plant extract. The voltammograms of *Clematis* recorded after different solvent extractions under either ABS or PBS were used for pattern generation. Scatter plots and 2D density patterns have been used for plant identification previously [28–36]. In this work, we further proposed an advanced identification pattern based on a heat map.

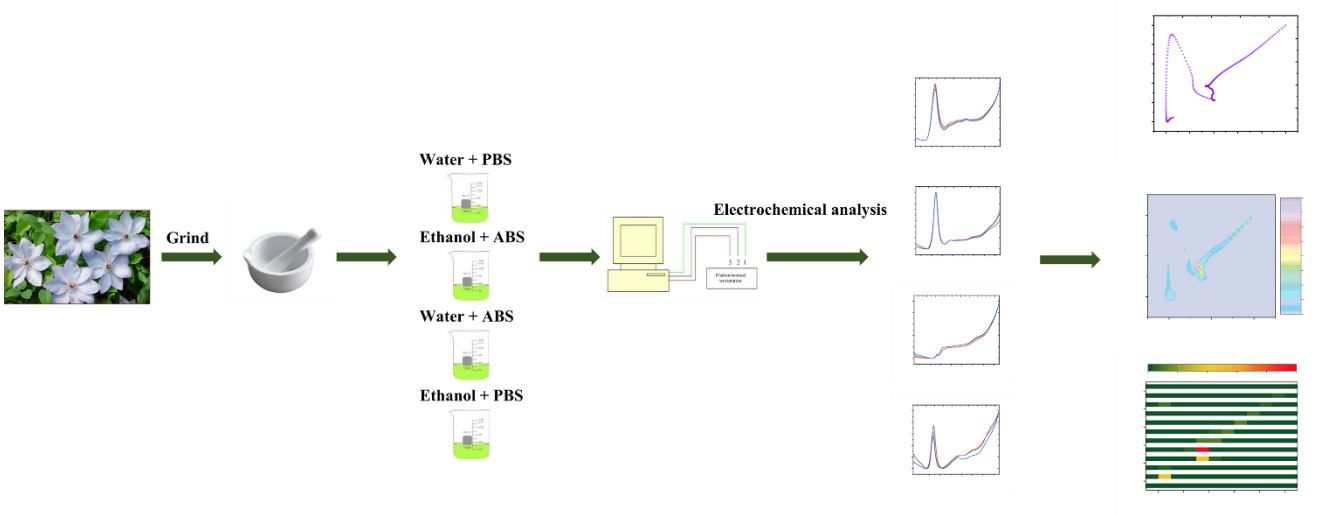

Figure 1. Schematic process of electrochemical recording voltammograms for 10 varieties of *Clematis*.

Figure 2 shows DPV profiles of 10 varieties of *Clematis* recorded using 0.1 M PBS after water extraction. Each variety exhibited several peaks between -0.1 and 1.5 V.

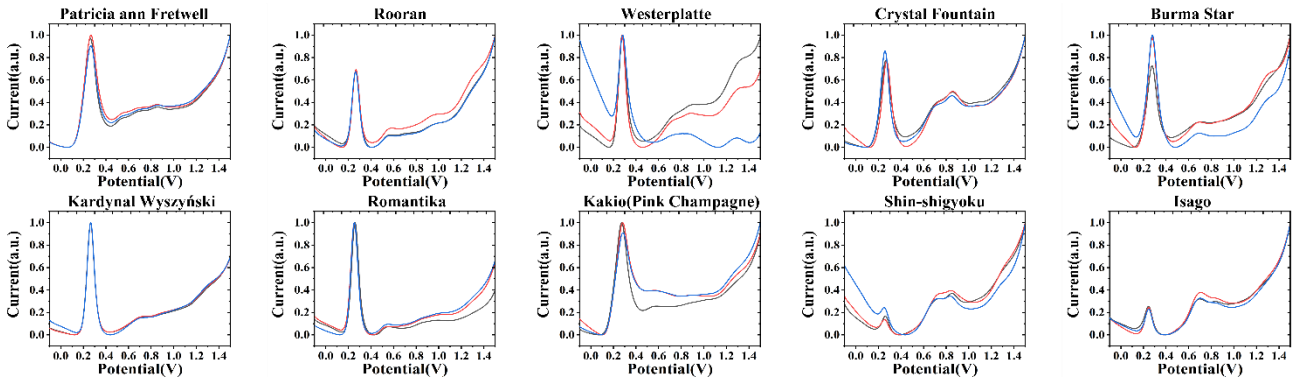

Figure 2. DPV curves of 10 varieties of *Clematis* recorded in 0.1 M PBS after water extraction.

These peaks can be attributed to the oxidation of electroactive molecules such as flavonols [37–40], phenolic acids [41–44], procyanidins [45–47], alkaloids [48–50] and pigments [51–53]. Since plant leaves have complex compositions, distinguishing specific compounds during electrochemistry cannot

be achieved. However, the total voltammetric profile contains all the information of the responsible oxidizable compounds. Therefore, this profile can be considered a fingerprint of each variety.

Figure 3 shows DPV profiles of 10 varieties of *Clematis* recorded using 0.1 M ABS after ethanol extraction. Each variety also exhibited several peaks between -0.1 and 1.5 V under these conditions. However, each variety of *Clematis* showed a different profile compared with the voltammogram profiles recorded (and shown in Figure 2). This observation indicated that the electroactive compounds participating in the electrochemical reaction were different due to the different molecules extracted using the different solvents.

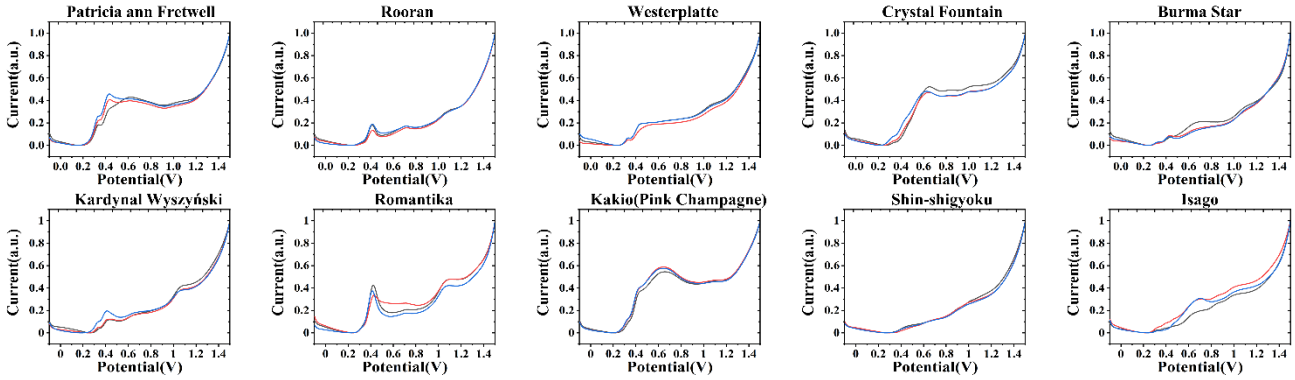

Figure 3. DPV curves of 10 varieties of *Clematis* recorded in 0.1 M ABS after ethanol extraction.

The identification of varieties of *Clematis* based on the DPV curve is an inconspicuous method because the voltammograms of some of these varieties, such as the voltammograms of Westerplatte and Kardynal Wyszyński recorded in 0.1 M PBS after water extraction, share some similar characteristics. Moreover, the voltammograms of Burma Star and Romantika recorded in 0.1 M ABS after ethanol extraction also exhibited very similar profiles. To quickly identify varieties of *Clematis*, we used voltammograms recorded from different conditions to generate different patterns.

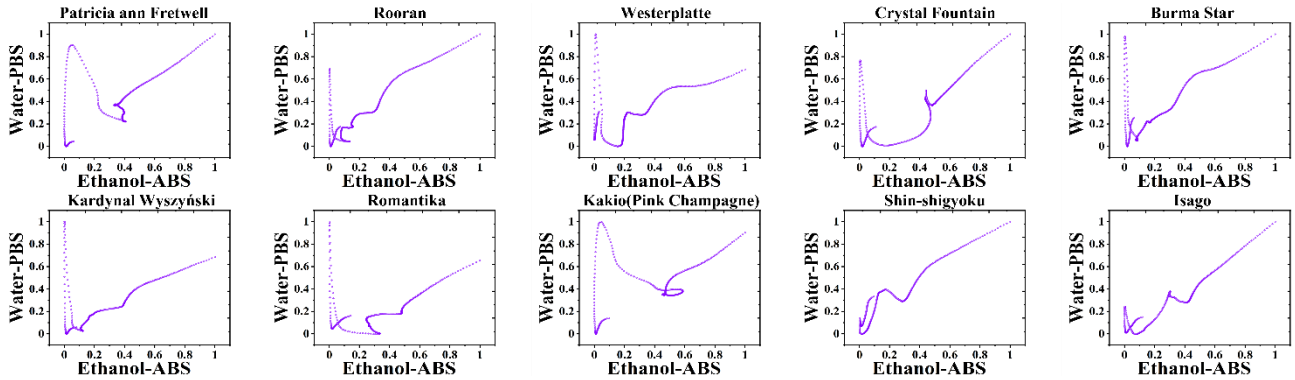

Figure 4. Scatter plots of 10 varieties of *Clematis* using data recorded from 0.1 M PBS after water extraction vs. 0.1 M ABS after ethanol extraction.

Figure 4 shows the scatter plots of the 10 varieties of *Clematis* using data recorded under the conditions of 0.1 M PBS after water extraction against data recorded under conditions of 0.1 M ABS

after ethanol extraction. It can be seen that increasing the dimensions of the data can improve the differences between varieties. For example, the difference between Westerplatte and Kardynal Wyszyński can be observed.

Although the scatter plots could be used to successfully identify some varieties that are not easy to distinguish, this technique still not lend itself to a very intuitive data presentation. Therefore, we further generate a 2D density map based on the data recorded from 0.1 M PBS after water extraction and from 0.1 M ABS after ethanol extraction. In this pattern, the area with a relatively high amount of data points is highlighted. We can distinguish different varieties by locating different hot zones. As shown in Figure 5, most varieties can be identified by locating hot zones, but there are still some varieties that have similar hot zones. In this case, we can also locate areas of other colours to distinguish these similar varieties.

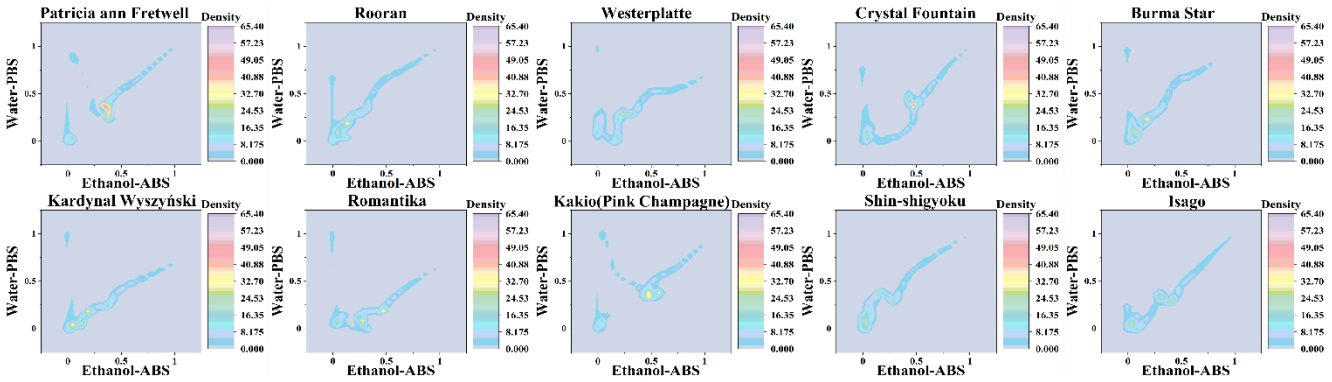

Figure 5. 2D density map of 10 varieties of *Clematis* using data recorded from 0.1 M PBS after water extraction and 0.1 M ABS after ethanol extraction.

We have proposed the above two patterns in our previous work. In this work, we once again have proposed a new pattern recognition method. A heat map was constructed using the data recorded from 0.1 M PBS after water extraction and from 0.1 M ABS after ethanol extraction. As shown in Figure 6, the heat map of each species was divided into many small cubes. Each cube represents the degree of heat of the data compared with that of the whole data. Compared with 2D density plots, this pattern recognition makes it easier to locate hot zones and to perform quantitative analysis. For example, Westerplatte and Burma Star showed very similar 2D density plots in Figure 5. However, we can easily recognize them by the 6 yellow and red cubes visible at the beginning.

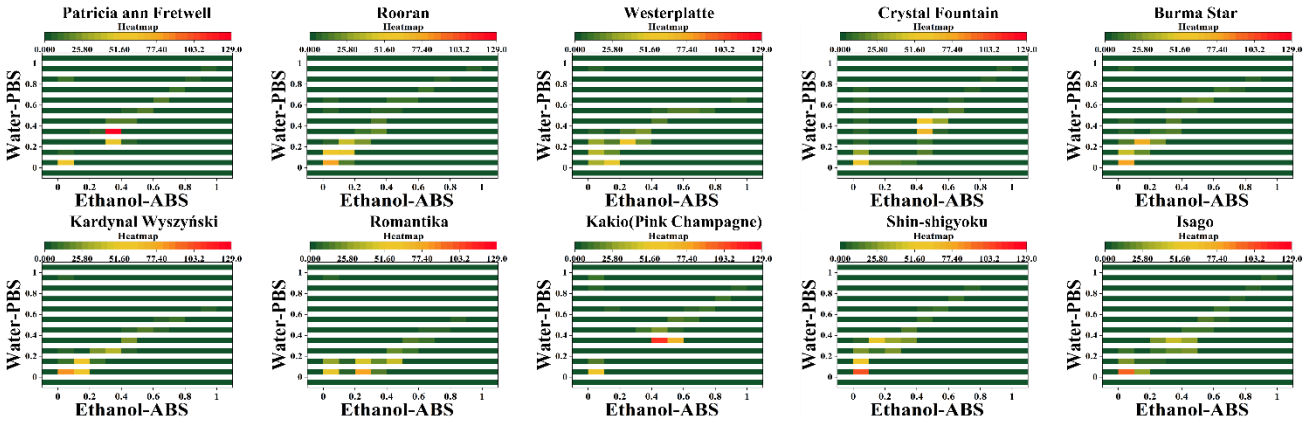

Figure 6. Hot zones of 10 varieties of *Clematis* using data recorded from 0.1 M PBS after water extraction and 0.1 M ABS after ethanol extraction.

4. CONCLUSION

In conclusion, the voltammograms of 10 varieties of *Clematis* (Patricia Ann Fretwell, Rooran, Westerplatte, Kardynal Wyszyński, Burma Star, Crystal Fountain, Romantika, Kakio (Pink Champagne), Shin-shigyoku and Isago) were recorded by a GCE using plant leaf extracts. Based on the recorded voltammograms, these varieties can be effectively identified via differences in pattern recognition. We have thus proposed a new pattern recognition method for variety identification.

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