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Identification of foliage plants *Heuchera* based on electrochemical profile of active molecules

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An electrochemical profile of plant tissue can reflect the genetic differences among different plants. In this work, the electrochemical profiles were used to identify 12 *Heuchera* cultivars. Leaf extracts of *Heuchera* 'Rio', *Heuchera* 'Tapestry', *Heuchera* 'Caramel', *Heuchera* 'Paris', *Heuchera* 'Huashiliu', *Heuchera* 'Cherry Cola', *Heuchera* 'Tiramisu', *Heuchera* 'Obsidian', *Heuchera* 'Midnight Ruffles', *Heuchera* 'Electra', *Heuchera* 'Georgia Peach' and *Heuchera* 'Red Wine' were prepared for recording the electrochemical profiles. These electrochemical profiles were used to construct different pattern recognition strategies and to identify cultivars. The identification of patterns was more accurate than that of individual electrochemical profiles.

Keywords: Electroanalysis; Leaf extract; Plant identification; *Heuchera*; Biometrics

1. INTRODUCTION

Broadly speaking, non-green plants with leaves that show a stable growth cycle can be called foliage plants. Foliage plants are divided into colored foliage plants and variegated foliage plants [1–4] (including spring foliage plants, autumn foliage plants, etc.). However, colored foliage plants narrowly exclude autumn foliage plants, as the latter have a uniform change in leaf color and the change only occurs in autumn [5–7]. The coloring of plant leaves originates from a variety of pigments in the leaf cells, including photosynthetic and flavonoid pigments. Photosynthetic pigments are chlorophyll a (blue-green), chlorophyll b (yellow-green) and carotenoids (orange-yellow) [8,9]. Flavonoid pigments refer to anthocyanins (red under acidic conditions and blue under alkaline conditions). Changes in the type, content and distribution of these pigments can affect the coloring of leaves. The pigment content and proportion of plants are mainly determined by genes [10,11]. At present, the research on the utilization of colored foliage plants mainly focuses on the following three aspects: (1) collecting and developing the germplasm resources of colored foliage plants, including introducing, domesticating, cultivating, managing and bringing their commercial value into play; (2) studying the effects of different environmental conditions on the change of leaf color so as to provide a theoretical basis for the application of colored foliage plants in gardens; (3) investigating the genetic mechanism of foliage plants for subsequent genetic engineering and the breeding of new cultivars [12,13].

As a kind of colored foliage plant, *Heuchera* maintains special foliage colors all year round, such as light green, dark green, gold, orange, red, purple, black and mixed colors [14–18]. With its high ornamental value, *Heuchera* can meet people's various aesthetic needs and is worth planting in gardens. At present, the research on *Heuchera* mainly focuses on propagation methods, tissue culture, photosynthetic characteristics, comparative cold resistance and adaptability [19–23]. Plant identification by electrochemical fingerprinting of plant tissues has emerged in recent years [24–30]. This technology is based on the variability of electrochemically active components in the tissues of different plants. This variability reflects the genetic differences among species to some extent. However, the recording of electrochemical fingerprints using different plant tissues shows different accuracy [24,31–37]. In this communication, the leaf extract of *Heuchera* was used for the identification of cultivars. We collected leaf extracts from 12 *Heuchera* and explored the possibility of cultivar identification. Based on the statistical differences in electrochemical fingerprints, their interspecific relationship was also discussed.

2. EXPERIMENTAL

Fresh leaves of *Heuchera* ‘Rio’, *Heuchera* ‘Tapestry’, *Heuchera* ‘Caramel’, *Heuchera* ‘Paris’, *Heuchera* ‘Huashiliu’, *Heuchera* ‘Cherry Cola’, *Heuchera* ‘Tiramisu’, *Heuchera* ‘Obsidian’, *Heuchera* ‘Midnight Ruffles’, *Heuchera* ‘Electra’, *Heuchera* ‘Georgia Peach’ and *Heuchera* ‘Red Wine’ were supplied by Nanjing Botanic Garden and Hangzhou Botanic Garden. All of the leaves were collected in July 2021. All the samples were kept frozen before analysis. All electrochemical fingerprint recordings were conducted at a CHI760 electrochemical workstation (CHI760 software was used for data acquisition). A commercial glassy carbon electrode (GCE, 3 mm in diameter), an Ag/AgCl electrode and a Pt electrode were used as the working electrode, reference electrode and counter electrode, respectively. Differential pulse voltammetry (DPV) was used for the recording of electrochemical profiles. Phosphate buffer solution (PBS) was prepared with 0.1 M KH_2PO_4 and Na_2HPO_4 and the pH reached 7.0 s required. Acetic acid buffer solution (ABS) was prepared with 0.1 M acetic acid and sodium acetate and the pH hit 4.5 as required.

All raw data files were first treated with background current subtraction. The recorded electrochemical signals were normalized to obtain the ratios between the current and the maximum peak current at different potentials. The hierarchical clustering method was employed to analyze the interspecific relationship. By using this algorithm, the data set was divided into different clusters by iteratively merging or splitting clusters based on a dendrogram. The clustering algorithm provided information about the data to be classified in a compact and graphical way. They can be agglomerative or divisive, depending on the procedure used to create the dendrogram. Agglomerative clustering follows a bottom-up strategy, in which, initially, each data point is assumed as a cluster and then, the two most similar clusters are iteratively merged according to an objective function until the final dendrogram is obtained. Divisive clustering follows an opposite approach. All data points are initially considered as one cluster and then, the selected cluster is iteratively partitioned into two new subclusters. In the completely linked clustering, the distance between two clusters is the distance between their closest or two most distant data points.

3. RESULTS AND DISCUSSION

Heuchera leaves are very similar in shape, but are very different in color. Figure 1 shows digital photos of each cultivar. It can be seen that the color of different cultivars may be different, but some species are still very similar, such as *Heuchera* 'Obsidian'/ *Heuchera* 'Huashiliu', *Heuchera* 'Paris'/ *Heuchera* 'Tiramisu', *Heuchera* 'Rio'/ *Heuchera* 'Georgia Peach'.

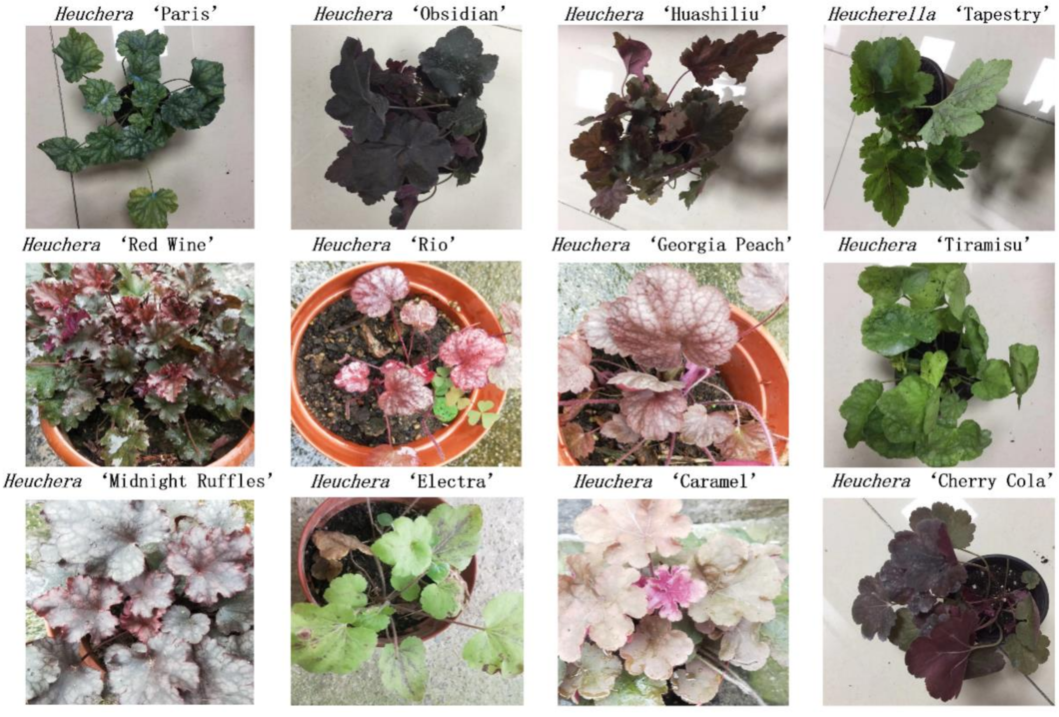

Figure 1. Digital photo of *Heuchera* 'Rio', *Heuchera* 'Tapestry', *Heuchera* 'Caramel', *Heuchera* 'Paris', *Heuchera* 'Huashiliu', *Heuchera* 'Cherry Cola', *Heuchera* 'Tiramisu', *Heuchera* 'Obsidian', *Heuchera* 'Midnight Ruffles', *Heuchera* 'Electra', *Heuchera* 'Georgia Peach' and *Heuchera* 'Red Wine'.

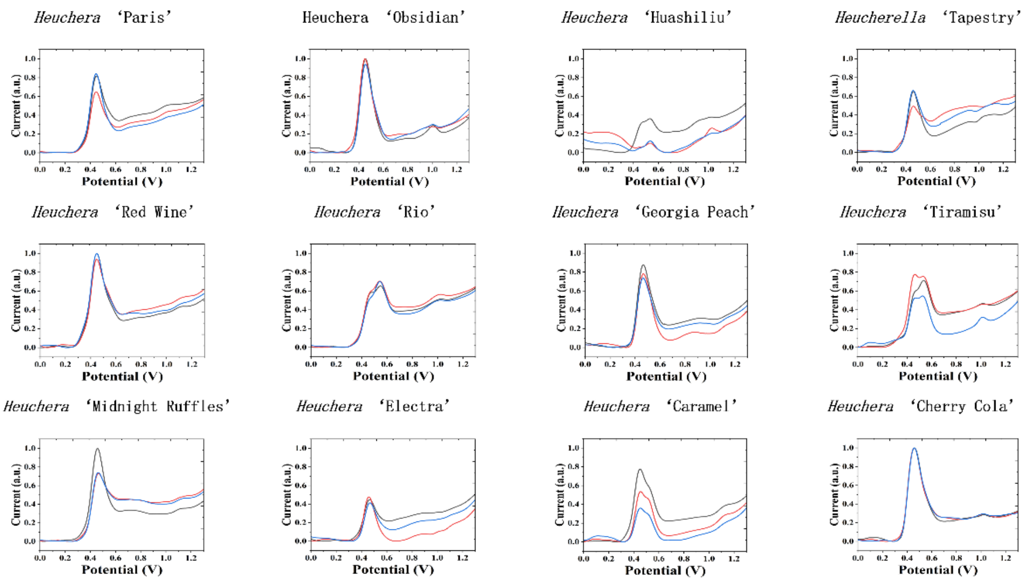

Figure 2. Electrochemical profiles of *Heuchera* 'Rio', *Heuchera* 'Tapestry', *Heuchera* 'Caramel', *Heuchera* 'Paris', *Heuchera* 'Huashiliu', *Heuchera* 'Cherry Cola', *Heuchera* 'Tiramisu', *Heuchera* 'Obsidian', *Heuchera* 'Midnight Ruffles', *Heuchera* 'Electra', *Heuchera* 'Georgia Peach' and *Heuchera* 'Red Wine' after water extraction and recorded under ABS condition.

Figure 2 shows the electrochemical profiles of all *Heuchera* cultivars recorded after water extraction and using ABS as electrolyte. All cultivars showed a series of oxidation peaks. These peaks can be ascribed to the oxidation of flavonols, phenolic acids, procyanidins, alkaloids and pigments in plant tissue [38–41]. There were significant differences in electrochemical profiles among different cultivars. However, some of these cultivars showed very similar profiles. For example, the electrochemical profiles of *Heuchera* 'Obsidian', *Heuchera* 'Red Wine' and *Heuchera* 'Midnight Ruffles' were very similar.

It was difficult to directly identify these cultivars based on a single electrochemical profile. Therefore, the profile of ethanol extract under PBS was also recorded. Figure 3 shows the electrochemical profiles of all *Heuchera* cultivars recorded after ethanol extraction and using PBS as electrolyte. It can be seen that the curve of the electrochemical profile was similar to that of the profiles in Figure 2, and all cultivars displayed a series of oxidation peaks. Comparing Figure 2 and Figure 3, it can be found that different cultivars exhibited different profiles under the two conditions, which proved that the electrochemically active molecules involved in the electrochemical reaction were different. The electrochemical profiles of *Heuchera* 'Obsidian', *Heuchera* 'Red Wine' and *Heuchera* 'Midnight Ruffles' recorded in Figure 3 showed low similarity. Therefore, the electrochemical profile can be potentially used for identifying the cultivars of *Heuchera*.

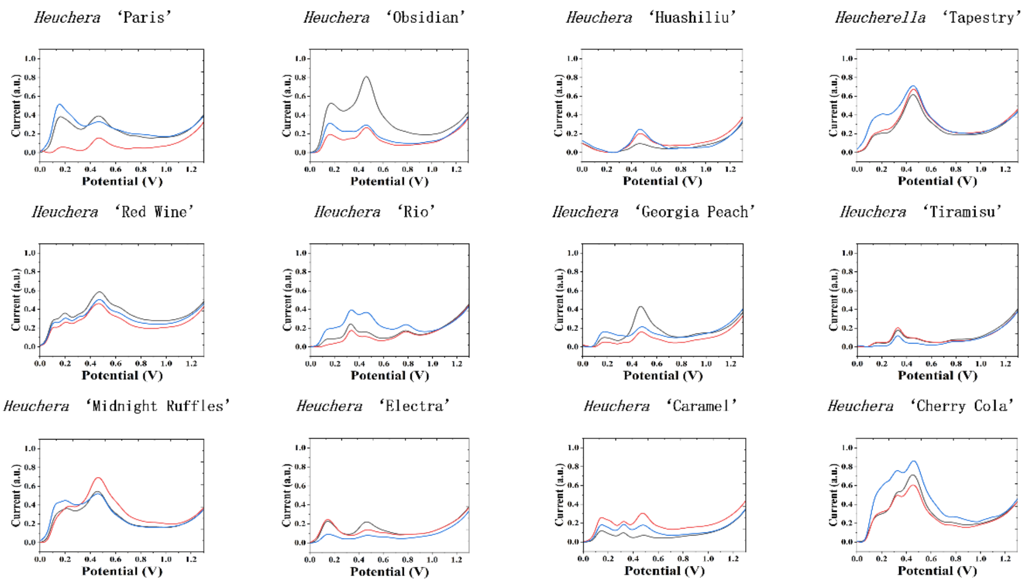

Figure 3. Electrochemical profiles of *Heuchera* 'Rio', *Heuchera* 'Tapestry', *Heuchera* 'Caramel', *Heuchera* 'Paris', *Heuchera* 'Huashiliu', *Heuchera* 'Cherry Cola', *Heuchera* 'Tiramisu', *Heuchera* 'Obsidian', *Heuchera* 'Midnight Ruffles', *Heuchera* 'Electra', *Heuchera* 'Georgia Peach' and *Heuchera* 'Red Wine' after ethanol extraction and recorded under PBS condition.

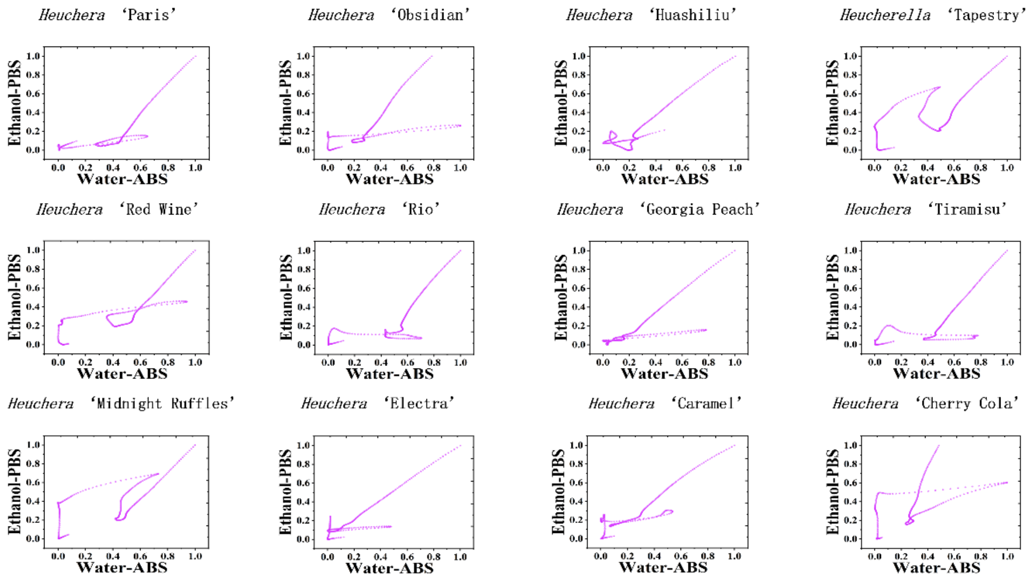

Figure 4. Scatter plot pattern of *Heuchera* 'Rio', *Heuchera* 'Tapestry', *Heuchera* 'Caramel', *Heuchera* 'Paris', *Heuchera* 'Huashiliu', *Heuchera* 'Cherry Cola', *Heuchera* 'Tiramisu', *Heuchera* 'Obsidian', *Heuchera* 'Midnight Ruffles', *Heuchera* 'Electra', *Heuchera* 'Georgia Peach' and *Heuchera* 'Red Wine'.

Different pattern identification maps can be obtained by combining the electrochemical profiles under different conditions. The accuracy of pattern identification is higher than that of individual electrochemical profiles. As shown in Figure 4, a scatter pattern plot can be generated by combining the electrochemical profiles in Figure 2 and 3. In this pattern identification, the differences among different cultivars were amplified for easier identification. The patterns of *Heuchera* 'Obsidian', *Heuchera* 'Red

Wine' and *Heuchera* 'Midnight Ruffles' showed significant differences from the electrochemical profiles in the scatter plot.

Although the scatter plot represented the differences among different species more intuitively than the electrochemical profiles, it was not easy to calculate the scatter points in the scatter plot directly. In addition, a two-dimensional density pattern was also used for the identification of cultivars. In the two-dimensional density pattern, the closer the clustering, the darker the color of the data points (Figure 5). Therefore, the cultivars can be identified by locating the position of these key regions [42]. The advantage of this pattern identification is that the amount of data used for image recognition can be reduced.

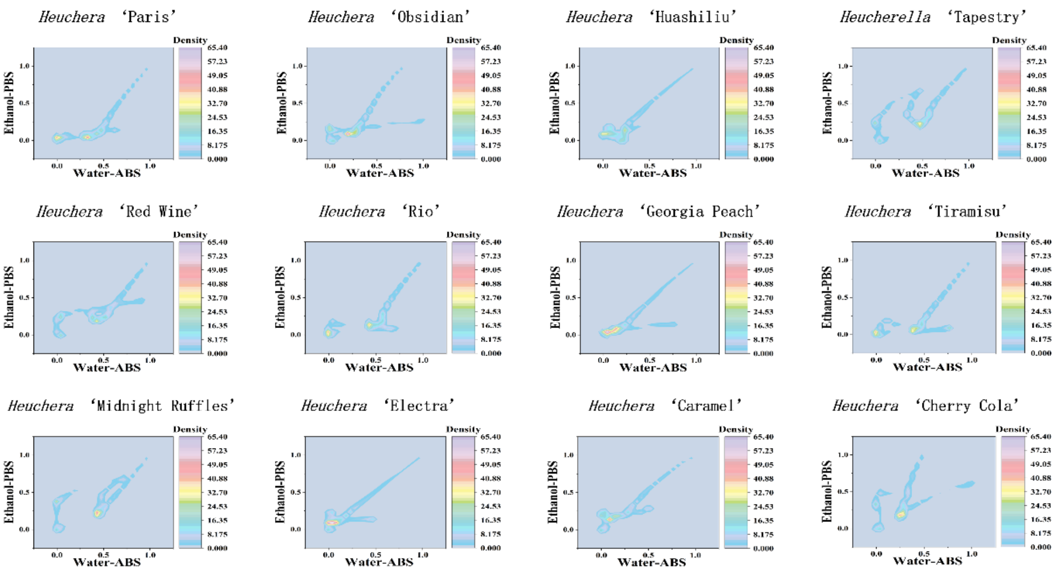

Figure 5. 2D density pattern of *Heuchera* 'Rio', *Heuchera* 'Tapestry', *Heuchera* 'Caramel', *Heuchera* 'Paris', *Heuchera* 'Huashiliu', *Heuchera* 'Cherry Cola', *Heuchera* 'Tiramisu', *Heuchera* 'Obsidian', *Heuchera* 'Midnight Ruffles', *Heuchera* 'Electra', *Heuchera* 'Georgia Peach' and *Heuchera* 'Red Wine'.

In addition to determining the focus regions by the two-dimensional density map, all image regions can be divided. Based on the number of data points in each region, the corresponding heat map can be formed. As shown in Figure 6, the heatmap can be used to calculate the similarity among different cultivars. It can be seen from the above results that an electrochemical profile has a very strong ability to identify plant cultivars.

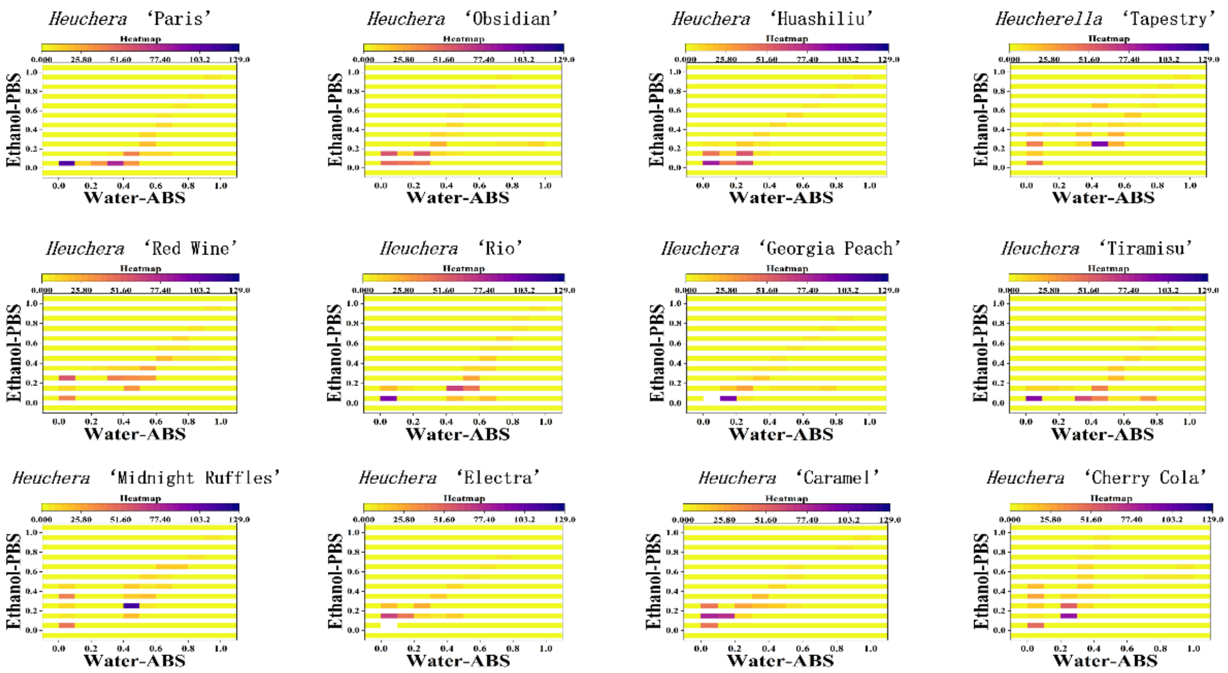

Figure 6. Heatmaps of *Heuchera* 'Rio', *Heuchera* 'Tapestry', *Heuchera* 'Caramel', *Heuchera* 'Paris', *Heuchera* 'Huashiliu', *Heuchera* 'Cherry Cola', *Heuchera* 'Tiramisu', *Heuchera* 'Obsidian', *Heuchera* 'Midnight Ruffles', *Heuchera* 'Electra', *Heuchera* 'Georgia Peach' and *Heuchera* 'Red Wine'.

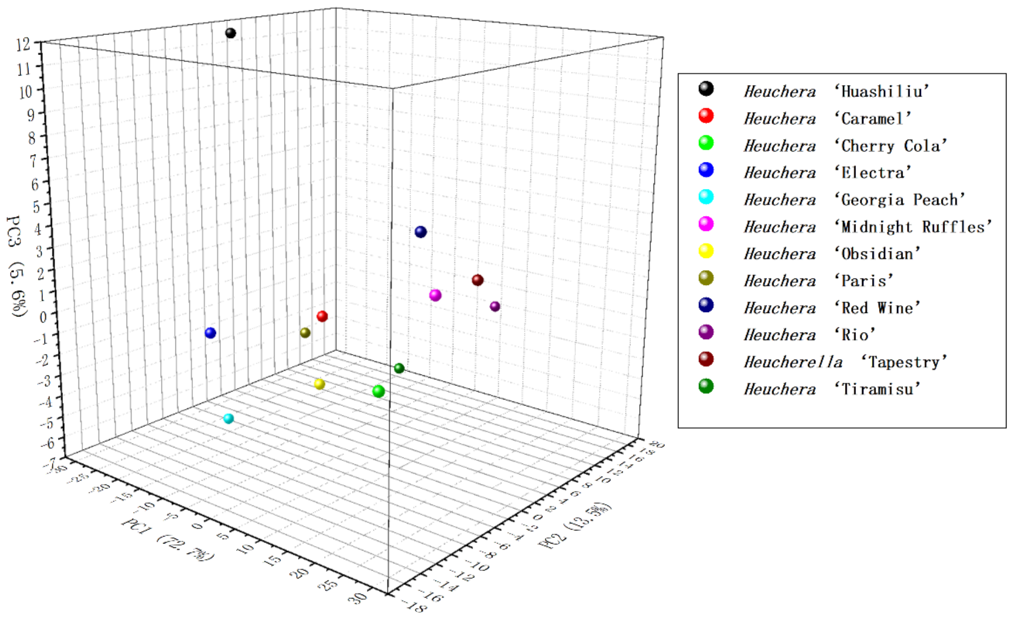

Figure 7. PCA analysis of *Heuchera* 'Rio', *Heuchera* 'Tapestry', *Heuchera* 'Caramel', *Heuchera* 'Paris', *Heuchera* 'Huashiliu', *Heuchera* 'Cherry Cola', *Heuchera* 'Tiramisu', *Heuchera* 'Obsidian', *Heuchera* 'Midnight Ruffles', *Heuchera* 'Electra', *Heuchera* 'Georgia Peach' and *Heuchera* 'Red Wine'.

The differences in electrochemical profiles can be used not only to identify different species, but also to explore the relationship among different cultivars. The electrochemical profiles of different cultivars were first studied by principal component analysis (PCA). As shown in Figure 7, after extracting three factors, PCA reached more than 92% interpretation. Thus, the electrochemical profiles contained representative information points that can be used to represent different data sets. This further indicated that electrochemical profiles using leaf extracts can be applied for the identification of cultivars.

4. CONCLUSION

In conclusion, the electrochemical profiles of *Heuchera* 'Rio', *Heuchera* 'Tapestry', *Heuchera* 'Caramel', *Heuchera* 'Paris', *Heuchera* 'Huashiliu', *Heuchera* 'Cherry Cola', *Heuchera* 'Tiramisu', *Heuchera* 'Obsidian', *Heuchera* 'Midnight Ruffles', *Heuchera* 'Electra', *Heuchera* 'Georgia Peach' and *Heuchera* 'Red Wine' were recorded using leaf extracts under PBS and ABS. The electro-active compounds in different cultivars were different because of different oxidation at different potentials. It was very inaccurate to directly use a signal electrochemical profile recorded in one condition for the identification of cultivars. Our results indicated the pattern constructed using these electrochemical profiles can be used more effectively for different identification strategies.

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