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Short Communiction

Electrochemical Fingerprint for Species Identification in *Acer* Linn

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Aceraceae is the largest family in broad-leaved deciduous and evergreen forests, widely distributed in Asia, Eastern North America, and Europe. The interspecific hybridization and introgression occur extensively among closely related species of *Acer* Linn, causing transitional intraspecific morphological variations, such as similar shapes of leaves, inflorescences and samaras. The adoption of electrochemical fingerprinttechnology for the identification of plant species is an emerging application in the field of biosensors. In this work, leaves of *Acer sinense, A. oliverianum, A. wilsonii, A. tonkinense, A. kuomeii, A. elegantulum, A. henryi, A. sinopurpurascens, A. ceriferum, A. linganense, A. changhuaense, A. palmatum, A. davidii* subsp. *grosser, A. davidii, A. metcalfii, A. tataricum*subsp. *ginnala, A. negundo, A. amplum* subsp. *catalpifolium* and *Dipteroniasinensis* were selected for the recording of electrochemical fingerprint. Different electrochemical fingerprint were obtained according to the differences of electrochemically active substances in the leaf tissue. These electrochemical fingerprint can be used to construct different pattern recognition strategies and further used for the identification of species.

Keywords: Electrochemical sensor, Electrochemical fingerprint; Electroactive compounds; *Acer* Linn.; Phylogenetics

1. INTRODUCTION

The representative of forest plant family Aceraceae has prominent floral elements, with mixed broad-leaved deciduous and evergreen forests and species found particularly in the temperate regions

of eastern Asia, eastern North America, and Europe. It is widely spread in climatically and ecologically diverse habitats with approximately 156 species [1–3]. The genus can be easily identified by opposite leaves and winged schizo-carpic fruits (samaras), but other morphological characteristics are also highly diverse. Although species of Acer aremainly distributed in temperate to subtropical areas, several species expand their distribution to the tropics, such as *A. decandrum*Merr in Hainan, China, and *A. laurinum*Hassk in Thailand and Vietnam [4]. Different classification systems for Aceraceae have been put forward. In addition to the difficulties in species delimitation, the phylogenetic schemes and intra-genus configuration of the genus by different workers are very controversial [5].For example, Ackerly and Donoghue [6] explored the evolutionary relationships of three morphological characteristics of *Acer* plants in relation to ITS sequence information. Some studies have also analyzed the phylogenetic relationships among some groups of *Acer* using ITS sequences [7–11]. Due to the high homology of maples' ITS sequences and the potential for homologous evolutionary incompleteness caused by long generations and hybridization, it is difficult to conduct taxonomic and phylogenetic studies of maples with a single ITS sequence [12,13]. In addition,maples are extensively cultivated and developed due to their rapid growth rates, great importance for landscaping and adaptability to various harsh environmental conditions, [14,15].Since seeds and pollen are dispersed by wind and birds, interspecific hybridization and introgression occur widely among closely related species, leading to transitional intraspecific morphological variations, such as similar shapes of leaves, inflorescences and samaras. Therefore, it is difficult to recognize and delimitate maple species accurately through the existing morphological characteristics [16,17].

The identification of species using electrochemical fingerprint technology of plant tissues is an identification method that has emerged in recent years [18–24]. The principle of this technology is due to the variability of electrochemically active components in the tissues of different plants. This variability reflects, to some extent, the genetic differences among species. However, the recording of electrochemical fingerprintof different plant tissues has different accuracy [25–28]. This is due to the fact that the components in some plant organs vary considerably with the change of the growth environment. For example, the reproducibility of electrochemical fingerprint in plant bulbs is poor due to the interference of starch [29]. Although the electrochemical fingerprint of plants and flowers have good reproducibility, these fingerprintcannot reflect genetic differences among species well [30]. This is because the electrochemical signal of pigments in plants and flowers is too strong to cover up other electrochemically active substances. In this work, this technology was employed to study the 18 species of *Acer* with an exo-taxa that had been selected for the recording of electrochemical fingerprint. Based on the statistical differences of electrochemical fingerprint, their interspecific relationships were also explored.

2. EXPERIMENTAL AND INSTRUMENTS

Leaves of *Acer sinense, A. oliverianum, A. wilsonii, A. tonkinense, A. kuomeii, A. elegantulum, A. henryi, A. sinopurpurascens, A. ceriferum, A. linganense, A. changhuaense, A. palmatum, A. davidii* subsp. *grosser, A. davidii*, *A. metcalfii, A. tataricum*subsp. *ginnala, A. negundo, A. amplum* subsp.

catalpifolium and *D. sinensis* were supplied by Nanjing Botanic Garden. All of the leaves were collected in June 2021, when mature and healthy leaves were harvested. All of the samples were frozen before analysis. Other reagents were analytical grade and used without further purification. The extraction process was conducted with ethanol or water as solvent. Phosphate buffered solution (PBS, pH=7) and acetate buffered solution (ABS, pH=4.5) were used to collect electrochemical fingerprint. The recording of all electrochemical fingerprintwas carried out in a CHI760 electrochemical workstation. A commercial glassy carbon electrode (GCE), an Ag/AgCl electrode and a Pt electrode were used as the working electrode, reference electrode and counter electrode, respectively. Before biometric recognition, all data were first treated with a normalization process to obtain the ratios between the current and the maximum peak current at different potentials. These normalized data were also used for the generation of phylogenetic trees.An electrochemical peak from voltammetric scanning should belong to one specific reaction when a simple analyte system was involved. However, the voltammetric peaks could overlap in the presence of similarly structured molecules. In our work, we deliberated on avoid discussing the specific electrochemical reaction because we tried to find out the relationship of overall profile with species identification and classification. This hypothesis ws based on the electrochemical profile of plant tissues that could reflect the integrated information of the distribution of oxidized compounds. As our work was not a quantitative analysis of the leaf extracts of a particular plant, the analysis of oxidation peaks at specific potentials was avoided.

3. RESULTS AND DISCUSSION

The electrochemical fingerprint of the 18 species of *Acer* with an exo-taxaafter water extraction wererecorded under PBS first (Figure 1). A series of oxidation peaks were seen between -0.1 and 1.5 V for either species, representing the involvement of substances in the electrochemical oxidation reaction of the leaf extracts. These peaks could be ascribed to the oxidation of flavonols, phenolic acids, procyanidins, alkaloids and pigments in plant tissues [31–34].The electrochemical fingerprint varied considerably among the different species, because there were remarkable differences ofthe genetic levels between different species and then the differences of the components of plant tissues [35,36]. However, some other species showed very similar fingerprint, such as *A. palmatum, A. ceriferum,* and *A. sinense*. The direct identification of these species based on single DPV profile was difficult. Therefore, we collected electrochemical fingerprintof these species under the other three conditions. Figure 2 shows the electrochemical fingerprint of the 18 species of *Acer* with an exo-taxaafter water extraction recorded under ABS. Figure 3 shows all species recorded after ethanol extraction under PBS. Figure 4 shows all species recorded after ethanol extraction under ABS. These DPV curves werevery similar to those in Figure 1, and all species had a series of oxidation peaks. The DPV curves of *A. palmatum, A. ceriferum,* and *A. sinense* recorded in Figure 1 showed high similarity because the substances involved in the electrochemical reaction under PBS were very similar in three species after extraction with water. This does not mean that the electrochemically active substances in these three species were the same because some electrochemically active substances not soluble in water were not extracted [37]. In addition, some electrochemically active substances were also not involved in the

reaction in the neutral electrolyte. Therefore, we observed different DPV profiles of these three species in Figure 2, 3 and 4.

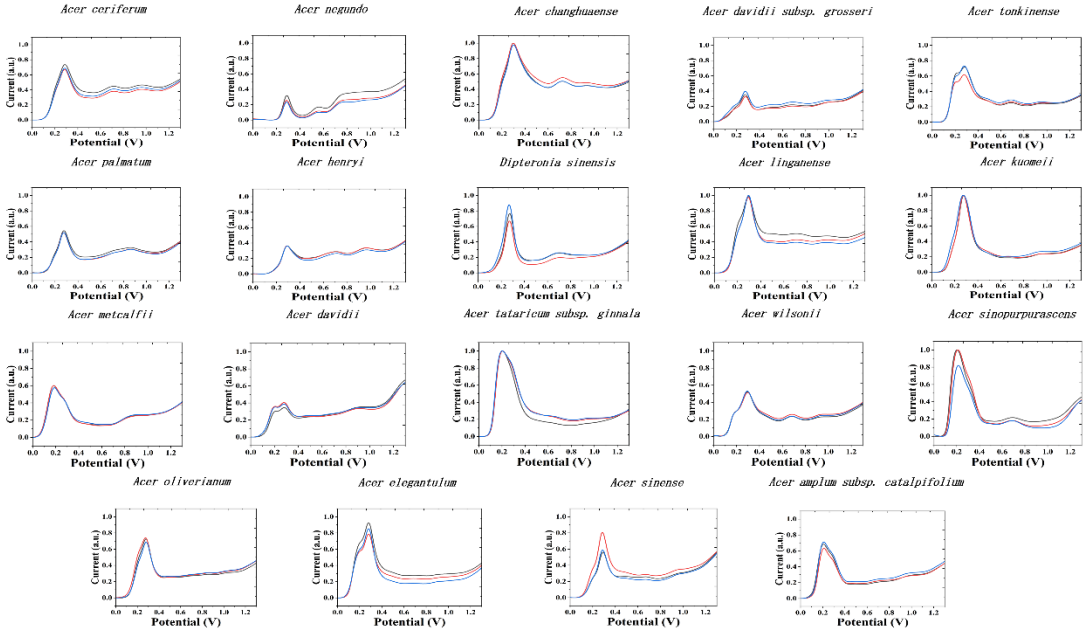

Figure 1. Electrochemical fingerprint of *A. sinense*, *A. oliverianum*, *A. wilsonii*, *A. tonkinense*, *A. kuomeii*, *A. elegantulum*, *A. henryi*, *A. sinopurpurascens*, *A. ceriferum*, *A. linganense*, *A. changhuaense*, *A. palmatum*, *A. davidii* subsp. *grosser*, *A. davidii*, *A. metcalfii*, *A. tataricum* subsp. *ginnala*, *A. negundo*, *A. amplum* subsp. *catalpifolium* and *D. sinensis* after water extraction and recorded under PBS condition.

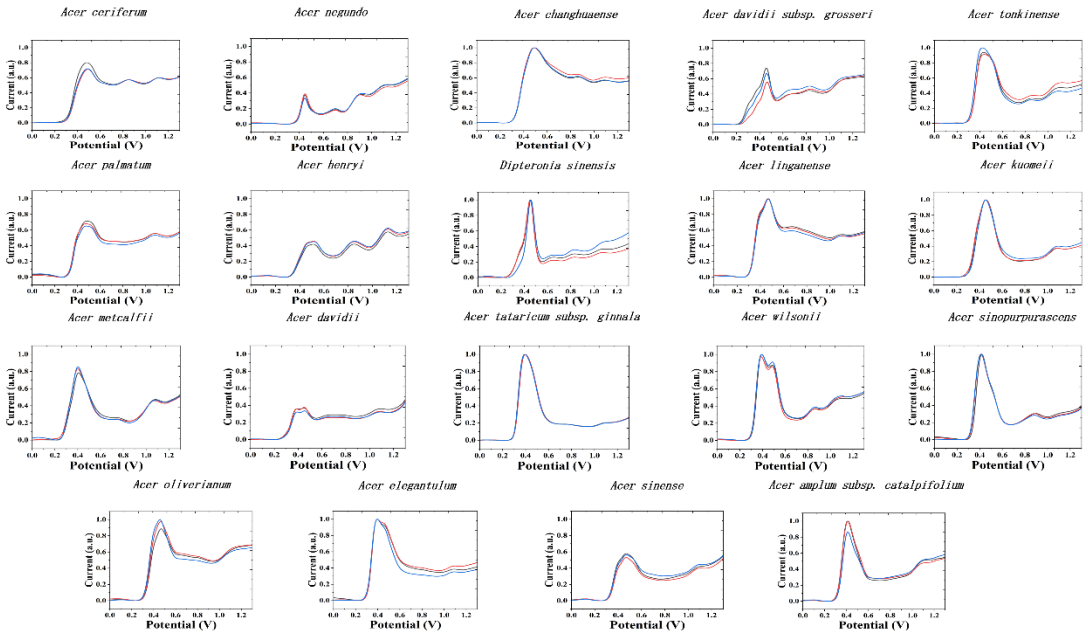

Figure 2. Electrochemical fingerprint of *A. sinense*, *A. oliverianum*, *A. wilsonii*, *A. tonkinense*, *A. kuomeii*, *A. elegantulum*, *A. henryi*, *A. sinopurpurascens*, *A. ceriferum*, *A. linganense*, *A. changhuaense*, *A. palmatum*, *A. davidii* subsp. *grosser*, *A. davidii*, *A. metcalfii*, *A. tataricum* subsp. *ginnala*, *A. negundo*, *A. amplum* subsp. *catalpifolium* and *D. sinensis* after water extraction and recorded under ABS condition.

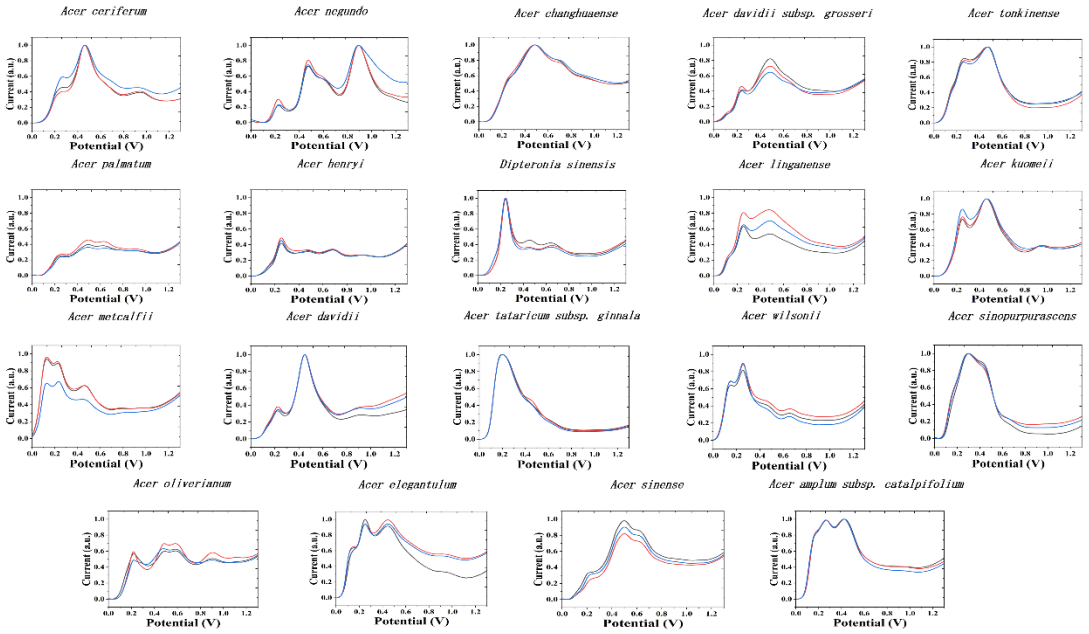

Figure 3. Electrochemical fingerprint of *A. sinense*, *A. oliverianum*, *A. wilsonii*, *A. tonkinense*, *A. kuomeii*, *A. elegantulum*, *A. henryi*, *A. sinopurpurascens*, *A. ceriferum*, *A. linganense*, *A. changhuaense*, *A. palmatum*, *A. davidii* subsp. *grosser*, *A. davidii*, *A. metcalfii*, *A. tataricum* subsp. *ginnala*, *A. negundo*, *A. amplum* subsp. *catalpifolium* and *D. sinensis* after ethanol extraction and recorded under PBS condition.

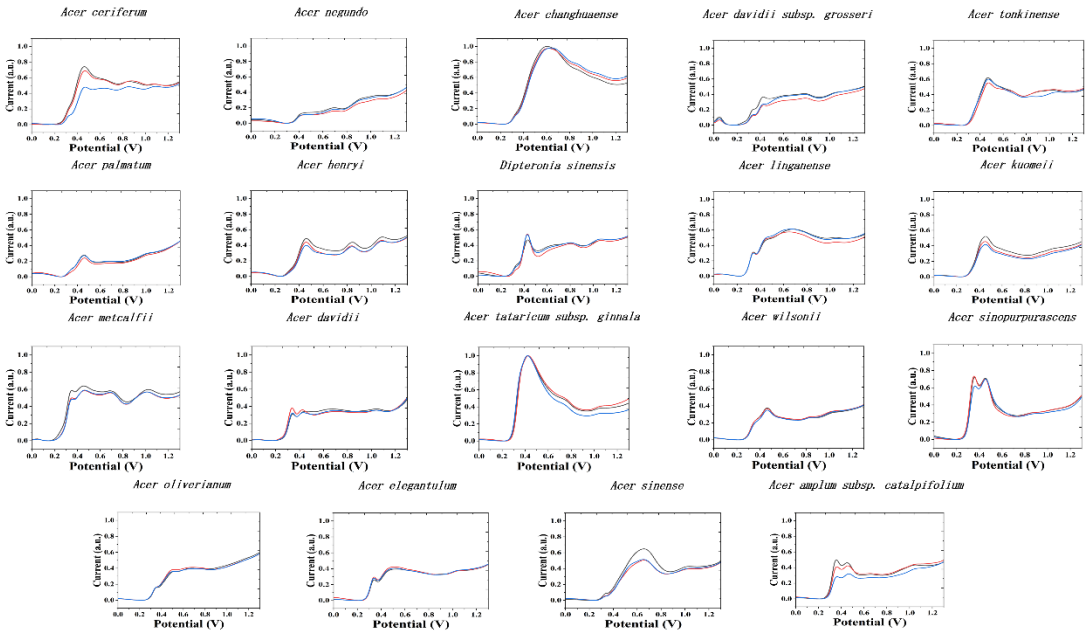

Figure 4. Electrochemical fingerprint of *A. sinense*, *A. oliverianum*, *A. wilsonii*, *A. tonkinense*, *A. kuomeii*, *A. elegantulum*, *A. henryi*, *A. sinopurpurascens*, *A. ceriferum*, *A. linganense*,*A. changhuaense*, *A. palmatum*, *A. davidii* subsp. *grosser*, *A. davidii*, *A. metcalfii*, *A. tataricum* subsp. *ginnala*, *A. negundo*, *A. amplum* subsp. *catalpifolium* and *D. sinensis* after ethanol extraction and recorded under ABS condition.

It is difficult to directly identify these species by observing their electrochemical fingerprint because they had a certain similarities [38]. However, when we obtained multi electrochemical fingerprint data sets, they can be combined to improve the accuracy of identification. As shown in Figure 4, a scatter pattern plot can be generated by combining the fingerprint recorded after water extraction under PBS and ABS. In this pattern recognition, the differences among specieswere amplified for better identification. The scatter plots of *A. palmatum*, *A. ceriferum*, and *A. sinense*were greatly differentfrom an DPV profile.

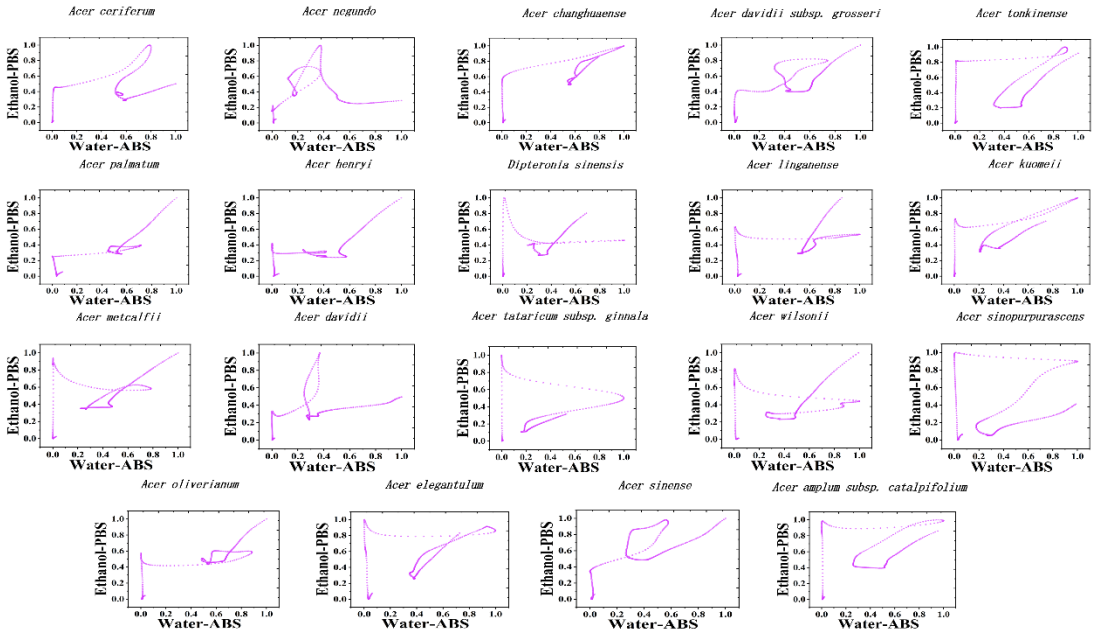

Figure 5. Scatter plot pattern of *A. sinense, A. oliverianum, A. wilsonii, A. tonkinense, A. kuomeii, A. elegantulum, A. henryi, A. sinopurpurascens, A. ceriferum, A. linganense, A. changhuaense, A. palmatum, A. davidii* subsp. *grosser, A. davidii, A. metcalfii, A. tataricum* subsp. *ginnala, A. negundo, A. amplum* subsp. *catalpifolium* and *D. sinensis*.

In addition, a two-dimensional density pattern was used for species identification. In the two-dimensional density pattern, the closer data points were clustered,the darker the color they were (Figure 6). Therefore, the identification of species were achieved by locating the position of these key regions [39].The pattern could be divided into different areas so that it can be more easily located [40]. Therefore, a heatmap pattern for the identification of species was generated. As shown in Figure 7, the heatmap can be used to calculate the similarity between different species. Based on above results, it can be seen that the electrochemical fingerprint technology has a very strong ability to identify plant species.

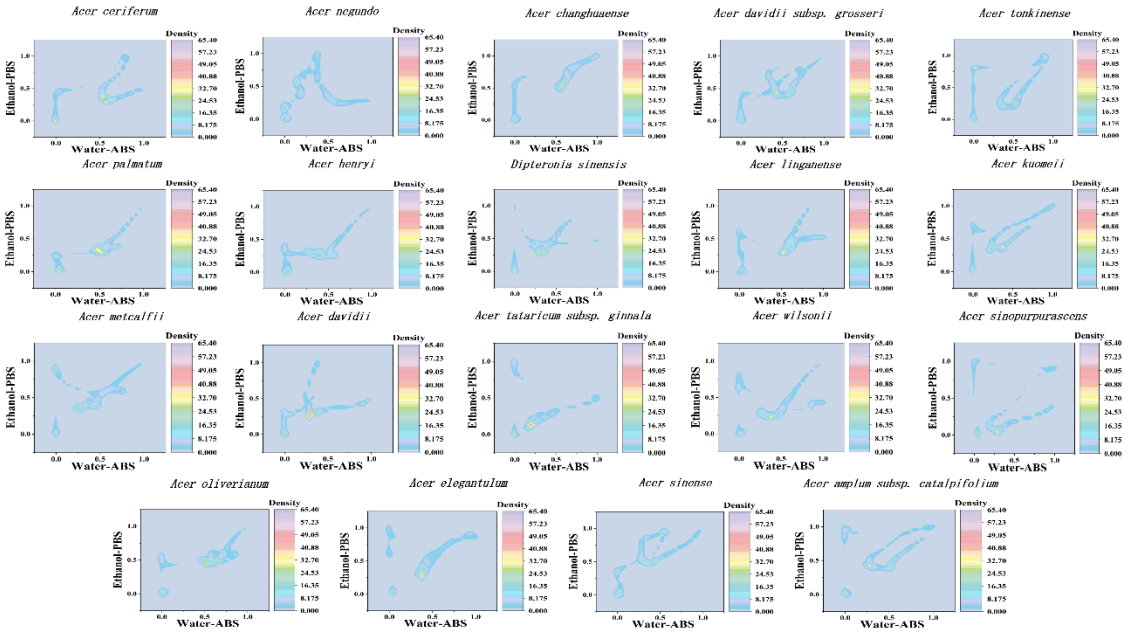

Figure 6. 2D density pattern of *A. sinense, A. oliverianum, A. wilsonii,A. tonkinense, A. kuomeii, A. elegantulum, A. henryi, A. sinopurpurascens, A. ceriferum, A. linganense, A. changhuaense, A. palmatum, A. davidii* subsp. *grosser, A. davidii, A. metcalfii, A. tataricum* subsp. *ginnala, A. negundo, A. amplum* subsp. *catalpifolium* and *D. sinensis*.

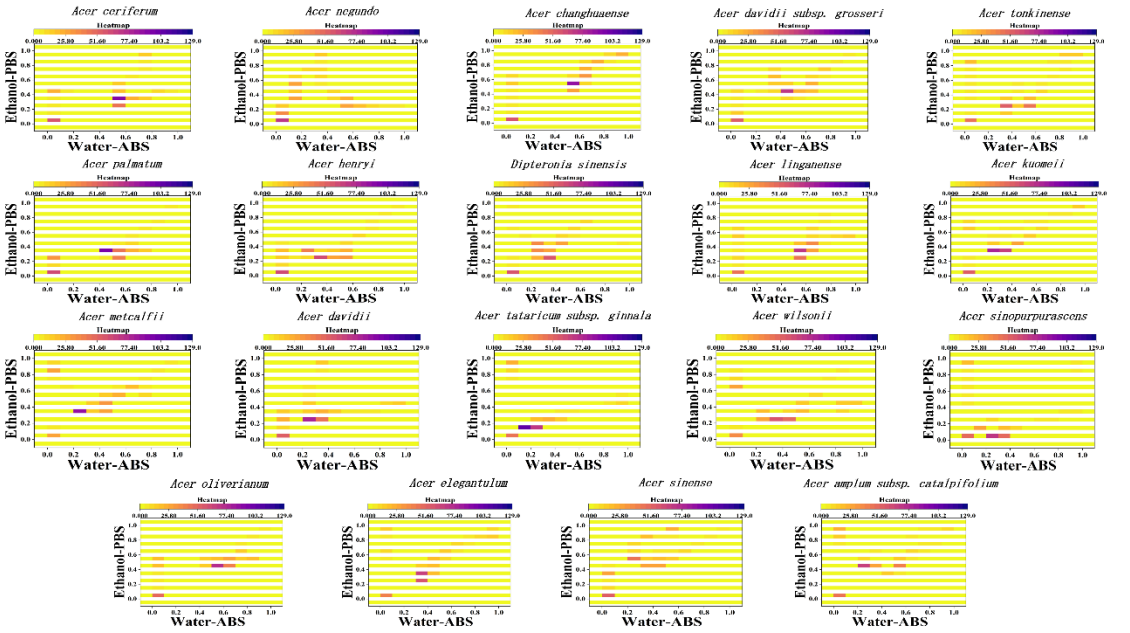

Figure 7. Heatmaps of *A. sinense, A. oliverianum, A. wilsonii,A. tonkinense, A. kuomeii,A. elegantulum, A. henryi, A. sinopurpurascens, A. ceriferum, A. linganense, A. changhuaense, A. palmatum, A. davidii* subsp. *grosser, A. davidii, A. metcalfii, A. tataricum* subsp. *ginnala, A. negundo, A. amplum* subsp. *catalpifolium* and *D. sinensis*.

Acer and *Dipteronia*are classified as Aceraceae as theyare found having many common morphological characteristics. Recent studies have verified the close phylogenetic relationship between *Acer* and *Dipteronia* [41]. In this work, *Dipteronia sinensis* was treated as an exo-taxa. A previous work had studied the interspecific relationships of *Acer*via HPLC data [42]. However, there was a large discrepancy between the HPLC results and results derived from molecular phylogenetics, which was mainly because the tracking and identification of a limited number of alkaloids in the plants that weakened the representativeness of HPLC. However, the information in the electrochemical fingerprint map was a collection of all electrochemically active substances and was therefore more representative. Figure 8 shows a phylogenetic tree constructed based on the electrochemical fingerprint technology. The entire phylogenetic tree was divided into three main clades. The first clade contained *A. ceriferum, A. linganense* and *A. changhuaense*. The second clade contained *A. sinopurpurascens, A. amplum* subsp. *catalpifolium, A. metcalfii, A. wilsonii, A. tonkinense, A. elegantulum* and *A. kuomeii*. The third clade contained *A. negundo, A. palmatum, A. henryi, A. davidii* subsp. *grosser, A. davidii,A. sinense, A. oliverianum* and *D. sinensis*.

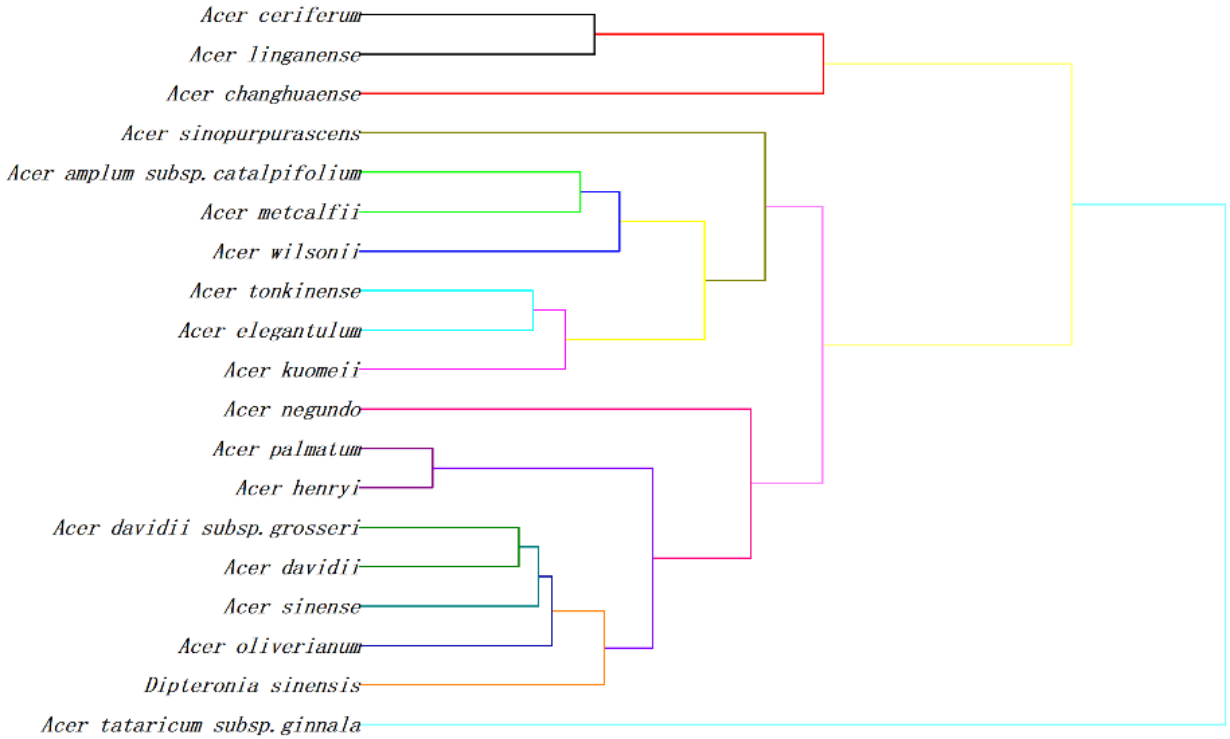

Figure 8. Dendrogram of *A. sinense, A. oliverianum, A. wilsonii, A. tonkinense, A. kuomeii, A. elegantulum, A. henryi, A. sinopurpurascens, A. ceriferum, A. linganense, A. changhuaense, A. palmatum, A. davidii* subsp. *grosser, A. davidii, A. metcalfii, A. tataricum* subsp. *ginnala, A. negundo, A. amplum* subsp. *catalpifolium* and *D. sinensis*.

4. CONCLUSION

In conclusion, the electrochemical fingerprintof *A. sinense, A. oliverianum, A. wilsonii, A. tonkinense, A. kuomeii, A. elegantulum, A. henryi, A. sinopurpurascens, A. ceriferum, A. linganense, A.*

changhuaense, A. palmatum, A. davidii subsp. *grosser, A. davidii, A. metcalfii, A. tataricum*subsp. *ginnala, A. negundo, A. amplum* subsp. *catalpifolium* and *D. sinensis*were recorded using leaf extracts under PBS and ABS. The electro-active compounds in different species were different because of oxidation at different potentials. The patterns constructed using these electrochemical fingerprintwere more effective for identification. These electrochemical fingerprintcould be used for phylogenetic investigation. The entire phylogenetic tree was divided into three main clades. The first clade contains *A. ceriferum, A. linganense* and *A. changhuaense*. The second clade contained *A. sinopurpurascens, A. amplum* subsp. *catalpifolium, A. metcalfii, A. wilsonii, A. tonkinense, A. elegantulum*and*A. kuomeii*. The third clade contained *A. negundo, A. palmatum, A. henryi, A. davidii* subsp. *grosser, A. davidii, A. sinense, A. oliverianum* and *D. sinensis*.

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