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Feasibility of Electrochemical Fingerprinting for Plant Phylogeography Study: A Case of *Chimonanthus praecox*

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Investigation of plant relationships is very important in phylogeny and plant culture. In this work, we proposed an electrochemical fingerprint-based method for the investigation of *Chimonanthus praecox* from different locations. This is the first time that electrochemical fingerprinting technology has been applied to the study of the relationships between subspecies. Three buffer solutions were used as a solvents along with an electrolyte during the *Chimonanthus praecox* leaf extraction and fingerprint recording. The voltammetric curves of *Chimonanthus praecox* from different locations exhibited very similar profiles, confirming the feasibility of species identification based on the electrochemical fingerprint. A two-dimensional scatter pattern deduced from two sets of fingerprints was used to distinguish the locations. In addition, this phylogeographic study based on electrochemical fingerprinting suggested that *Chimonanthus praecox* has multisite and multitime origins.

Keywords: Electrochemical fingerprints; Plant identification; Taxonomic sensing; Solid state electrochemistry; *Chimonanthus praecox*

1. INTRODUCTION

Cultivated fruit trees, vegetables and flowers all originated from corresponding wild plants. Generally, management of useful wild plants, such as collection of these plants or removal of useless wild plants, gradually evolves into domestication and cultivation of useful wild species [1–7]. The cultivation history of ornamental plants is not as long as that of crops. Studying the cultivation origin of ornamental plants can reveal the changes in the genetic background of domesticated plants and better elucidate the process of plant domestication. Current research on the cultivation origin of ornamental plants has focused on *Chrysanthemum morifolium*, *Paeonia suffruticosa* and *Armeniaca mume* [8–13].

Chimonanthus is native to China. In recent years, research on *Chimonanthus* has mainly focused on species resource investigation, plant taxonomy, systems evolution and genetic relationships. However, up to now, due to the lack of in-depth investigation of the wild resources of this species, the classification of the subspecies of *Chimonanthus* has not yet been determined [14–19]. There is no unified standard for recording species and terms for character description.

Avise et al. [20] proposed a new discipline related to the principles and processes of determining the geographical distribution of species, namely, phylogeography, which mainly discusses the correlation between the evolution of a species and its geological history. Cytoplast genomes are commonly used for phylogeography to study the genetic structure and gene flow of populations [21–27]. Plant cpDNA is a single, nonrecombined genetic unit similar to animal mtDNA. There is no recombination in the chloroplast genome, and the same haplotype can be maintained in the next generation. Therefore, the chloroplast genome is an effective tool to study the origin of plant cultivation, population variation and phylogeography [28–31]. However, gene sequencing requires high-cost and complex sample processing. Our previous work explored the application of electrochemical fingerprinting of plant tissues in plant phylogeny [32–35]. The differences in plant chemical composition partly reflect differences in genes. The species and contents of electrochemically active substances in plant tissues were used to study the relationships between species. In this work, we attempt the application of electrochemical fingerprinting for the study of plant phylogeography. *Chimonanthus praecox* collected from seven locations was subjected to investigation. Electrochemical fingerprints of *Chimonanthus praecox* leaf tissue at three pH conditions were recorded to examine the differences in electrochemically active compound composition and content. These signals were then superimposed and standardized to study the genetic relationships between *Chimonanthus praecox* from different regions.

2. MATERIALS AND METHODS

Figure 1. Map of *Chimonanthus praecox* collection locations.

All chemicals used in this work were of analytical grade. *Chimonanthus praecox* leaves were collected from Emeishan, Guizhou, Hangzhou, Hubei, Hunan, Chengdu and Chongqing. Figure 1 shows the sample location sites. All samples were freeze-dried immediately after collection.

For a typical electrochemical fingerprint recording process, 0.2 g plant leaves were ground in a mortar with 10 mL of a buffer solution of acetate buffer (ABS, pH 4.5), phosphate buffer (PBS, pH 7) or Tris buffer (Tris, pH 9.0). Then, 5 min of sonication was carried out for extraction. The slurry was transferred in an electrolytic cell with the insertion of a three-electrode system, where a glassy carbon electrode, a Pt wire and an Ag/AgCl (3M) electrode acted as a working electrode, a counter electrode and a reference electrode, respectively. The electrochemical fingerprint was recorded using differential pulse voltammetry (DPV) with a pulse amplitude of 50 mV, a pulse width of 0.05 s and a pulse period of 0.5 s. Figure 2 shows a schematic diagram of the entire process.

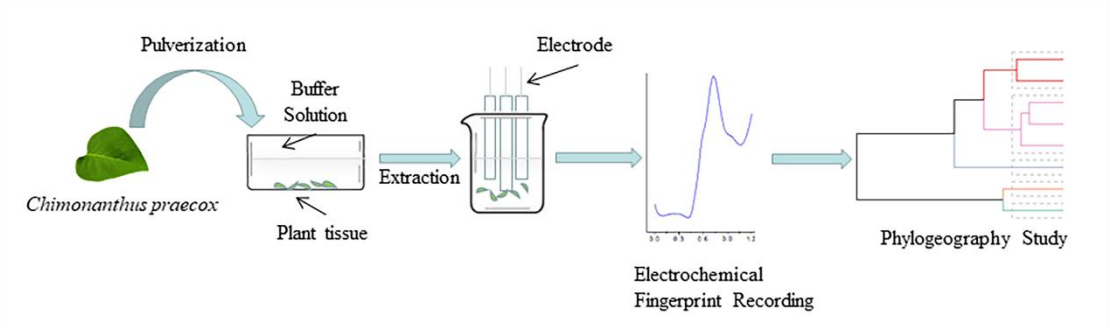

Figure 2. Schematic diagram of the electrochemical fingerprinting of *Chimonanthus praecox* and the phylogeographic study.

3. RESULTS AND DISCUSSION

Electrochemical fingerprinting can reveal profiles of electrochemically active substances in plant tissues. Because the genetic differences between the same species are very small, the same species should have very similar electrochemical fingerprints even in different regions. Figure 3 shows the electrochemical fingerprints of *Chimonanthus praecox* from seven regions in three different buffer solutions. All *Chimonanthus praecox* exhibited similar profiles with either ABS, PBS or Tris buffers. These results indicate that the electrochemical fingerprint can be used for plant species identification regardless of geographic origin. Specifically, all samples exhibited a distinct oxidation peak at 1.1 V with ABS. With PBS, all samples exhibited two major oxidation peaks at 0.27 V and 0.7 V. With Tris, all samples also showed two major oxidation peaks at 0.42 V and 0.88 V. These oxidation peaks corresponded to oxidizable compounds, such as phenolic acids [36], alkaloids [37], pigments [38], flavonols [39], and procyanidins [38]. Although the electrochemical fingerprint does not indicate exactly which electrochemically active substances were oxidized, their distribution can be reflected in the overall curves. Slight differences can be noticed between *Chimonanthus praecox* samples collected from different locations.

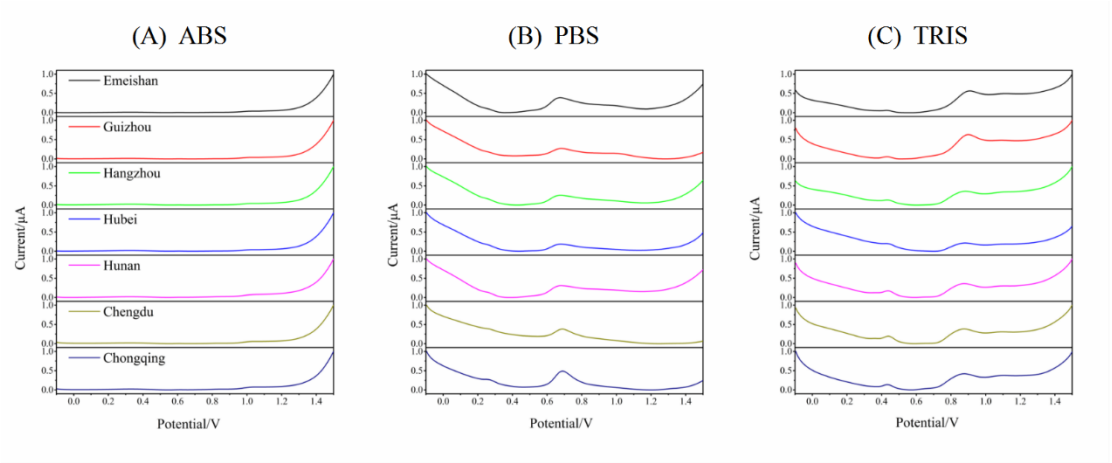

Figure 3. Electrochemical fingerprints of *Chimonanthus praecox* collected from Emeishan, Guizhou, Hangzhou, Hubei, Hunan, Chengdu and Chongqing in (A) ABS, pH 4.5, (B) PBS, pH 7 and (C) Tris, pH 9.0.

To better show the differences among *Chimonanthus praecox* from different regions, we composed a scatter diagram of two sets of electrochemical fingerprints. Figure 4 shows scatter patterns prepared using electrochemical fingerprints recorded with ABS, PBS and Tris buffers. As shown in the figure, each sample of *Chimonanthus praecox* showed a similar pattern trend, while differences were also noticed. These differences are due to subtle differences in electrochemically active compounds. The 2D scatter patterns deduced from two sets of electrochemical fingerprints could amplify the differences between samples. Specifically, as shown in Figure 4A, all samples showed an almost straight line after a complex curve, which can be clearly used for identifying the location of the sample.

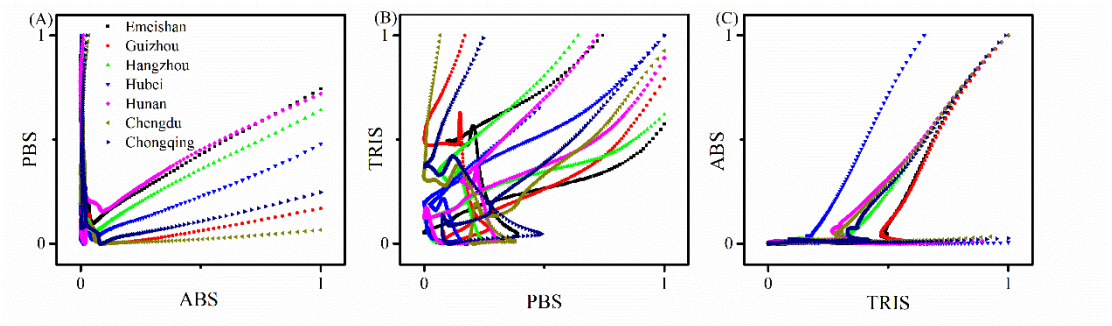

Figure 4. Two-dimensional scatter patterns of *Chimonanthus praecox* collected from Emeishan, Guizhou, Hangzhou, Hubei, Hunan, Chengdu and Chongqing using electrochemical fingerprints deduced with (A) ABS/PBS, (B) PBS/Tris and (C) Tris/ABS.

Plant domestication is a gradual process, and artificial cultivation and domestication can change the genetic diversity of crops. The genetic diversity of the earliest selected wild populations is generally relatively high. In the subsequent planting process, only the seeds most suitable for human needs are further sown. Such selection results in a decrease in genetic diversity in today's cultivated species and subsequently reduces the genetic diversity across the entire genome [40]. Many studies have shown that

the genetic diversity of cultivated populations is lower than that of wild populations [41–43]. Genetic diversity based on cpDNA is usually measured by haplotype diversity and nucleotide diversity [44]. The variation of a single base can generate new haplotypes but has little effect on nucleotide diversity. Changes in nucleotide diversity take longer to accumulate, so nucleotide diversity is more representative of the genetic diversity of a population [45].

Zhou et al. [46] reported that genetic differentiation was found in *Chimonanthus praecox* cultivated in Nanjing and Wuhan. Humans often collect plants from nearby wild and cultivated communities when introducing seeds. This is especially true for *Chimonanthus praecox*, a tree with high ornamental value, that has been widely discussed, artificially spread and popularized. Therefore, we used the electrochemical fingerprints recorded with three buffer solutions for the cluster analysis. As shown in Figure 5, all samples were divided into two clusters. According to the geographic representation in Figure 1, the distribution of *Chimonanthus praecox* seems to be related to latitude. Specifically, the *Chimonanthus praecox* collected from Hubei and Chengdu were clustered together. The remaining *Chimonanthus praecox* from lower latitudes were placed in another cluster. There are two main hypotheses for the origin of cultivation: (1) single origin (the domestication of cultivated species from a defined area through continuous transplanting to other areas) and (2) multiplace origin (the domestication of multiple cultivation groups occurred after long-term introduction along the whole distribution area of wild ancestor species). According to the cluster analysis, our results suggest that *Chimonanthus praecox* has a multisite and multitime origin. There are also some shortcomings in using electrochemical fingerprints to study plant phylogeography. First, electrochemical fingerprints represent only information about electrochemically active substances, so they differ less than direct molecular markers. Furthermore, the recording accuracy of the electrochemical fingerprint is limited, so some errors may be introduced. These deficiencies lead to electrochemical fingerprinting technology that cannot completely solve the problem of the origin of *Chimonanthus praecox* cultivation. Further investigation based on molecular technology is necessary to confirm these results.

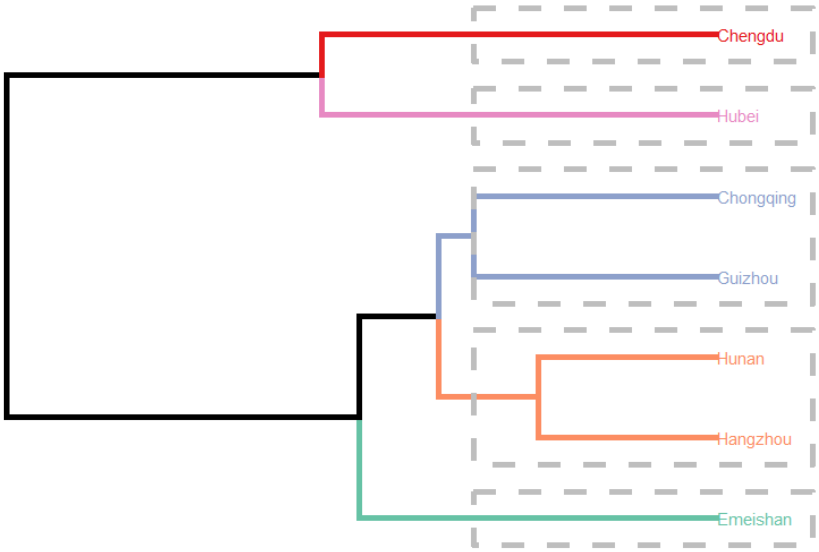

Figure 5. Dendrogram of *Chimonanthus praecox* with different locations based on the voltammetric fingerprints recorded in ABS, PBS and Tris.

4. CONCLUSION

In conclusion, electrochemical fingerprinting shows potential in plant phylogeographic studies. We sampled *Chimonanthus praecox* from seven locations and recorded their electrochemical fingerprints under three buffer solutions. Based on the recorded electrochemical fingerprints, we successfully identified *Chimonanthus praecox* in different locations. At the same time, the electrochemical fingerprinting was analyzed by cluster analysis. The results show that *Chimonanthus praecox* may have multiple independent origins, and its origin is related to latitude.

References

1. S. Angelova, K. Varbanova, I. Peeva, Y. Guteva, D. Dimitrova, *J. Herbs Spices Med. Plants*, 2 (1994) 3–8.
2. A. Ensslin, V.D.V. Ann, T. Vanderborght, S. Godefroid, *J. Appl. Ecol.*, 55 (2018) 1145–1154.
3. H.R. Juliani, A.R. Koroch, J.A. Zygadlo, V.S. Trippi, *Ind. Crops Prod.*, 34 (2011) 1353–1357.
4. I.B. Karhagomba, M.T. Adhama, T.B. Mushagalusa, V.B. Nabino, K. Koh, H.S. Kim, *Nutr. Res. Pract.*, 7 (2013) 510–518.
5. M. Liu, K. Cui, M. Zheng, *J. Northeast For. Univ.*, 32 (2004) 22–28.
6. V. Scariot, W. Gaino, M. Devecchi, *Acta Hortic.*, 952 (2012) 265–272.
7. M. Urbano, V. Tomaselli, V. Bisignano, G. Veronico, K. Hammer, G. Laghetti, *Genet. Resour. Crop Evol.*, 64 (2017) 1465–1472.
8. J. Avdic, B. Becic, N. Sarajlic, *Hydrol. Earth Syst. Sci. Discuss.*, 5 (2012) 529–541.
9. K.D. Bowman, F.G. Gmitter, *Econ. Bot.*, 44 (1990) 165–173.
10. F. Cheng, X. Yu, *Acta Hortic.* (2008) 375–382.
11. D.J.V.D. Gaag, F.R.V. Noort, L.H.M. Stapelcuijpers, C.D. Kreij, A.J. Termorshuizen, E.V. Rijn, S. Zmoranahum, Y. Chen, *Sci. Hortic.*, 114 (2007) 289–297.
12. Z. Li, Z. Hong, Z. Jie, G.U. Zhong, C. Gong, *J. Hubei Inst. Natl.*, 29 (2004) 106–11.
13. J.A.J. Veenenbos, *Eppo Bull.*, 11 (2010) 235–237.
14. Q.J. Li, M.L. Wang, X.S. Yang, L. Ma, X.J. Hao, *J. Asian Nat. Prod. Res.*, 15 (2013) 270–275.
15. T. Ouyang, X. Mai, *J. Chin. Med. Mater.*, 33 (2010) 385–387.
16. R.G. Shu, L.I. Sha-Sha, H.U. Hao-Wu, P.Z. Zhang, *Chin. Pharm. J.*, 45 (2010) 1134–1135.
17. W. Tong, H.H. Luo, X. Mai, J.J. Wei, L. Lin, *Zhong Yao Cai*, 34 (2011) 202–205.
18. X. Zhang, X. Meng, J. Zhang, L. Wu, J. Liu, J. Si, *Ind. Crops Prod.*, 102 (2017) 164–172.
19. M.Q. Zhou, L.Q. Chen, R. Rui, *Sci. Hortic.*, 136 (2012) 38–42.
20. J.C. Avise, J. Arnold, R.M. Ball, E. Bermingham, T. Lamb, J.E. Neigel, C.A.R. And, N.C. Saunders, *Annu. Rev. Ecol. Syst.*, 18 (1987) 489–522.
21. J. Duminil, M. Heuertz, J.L. Doucet, N. Bourland, C. Cruaud, F. Gavory, C. Doumenge, M. Navascués, O.J. Hardy, *Mol. Ecol.*, 19 (2010) 5469–5483.
22. P. Li, M. Li, Y. Shi, Y. Zhao, Y. Wan, C. Fu, K.M. Cameron, *Am. J. Bot.*, 100 (2013) 801–814.
23. J. Lihová, H. Kudoh, K. Marhold, *Am. J. Bot.*, 97 (2010) 1058–1070.
24. J. Shaw, J.H. Craddock, M.A. Binkley, *Castanea*, 77 (2012) 186–211.
25. Æ.T. Thórsson, S. Pálsson, M. Lascoux, K. Anamthawatjónsson, *J. Biogeogr.*, 37 (2010) 2098–2110.
26. der M.M. Van, H. Mcpherson, J. Siow, M. Rossetto, *Mol. Ecol. Resour.*, 14 (2013) 199–208.
27. F.D.A. Vieira, J.A.D.S. Santana, R.M.D. Santos, C.G. Fajardo, G.A.D.O. Coelho, D.D. Carvalho, *Rev. Caatinga*, 23 (2010) 69–74.
28. H. Dvořáková, T. Fér, K. Marhold, *Flora*, 205 (2010) 418–423.

29. L.M. Gao, M. Möller, X.M. Zhang, M.L. Hollingsworth, J. Liu, R.R. Mill, M. Gibby, D.Z. Li, *Mol. Ecol.*, 16 (2010) 4684–4698.
30. R.G. Hodel, E. Gonzales, *J. Hered.*, 104 (2013) 656–665.
31. H.Y. Jian, K.X. Tang, H. Sun, *Plant Syst. Evol.*, 301 (2015) 1819–1830.
32. L. Fu, M. Wu, Y. Zheng, P. Zhang, C. Ye, H. Zhang, K. Wang, W. Su, F. Chen, J. Yu, *Sens. Actuators B Chem.*, 298 (2019) 126836.
33. L. Fu, Y. Zheng, P. Zhang, J. Zhu, H. Zhang, L. Zhang, W. Su, *Electrochem. Commun.*, 92 (2018) 39–42.
34. L. Fu, Y. Zheng, P. Zhang, H. Zhang, W. Zhuang, H. Zhang, A. Wang, W. Su, J. Yu, C.-T. Lin, *Biosens. Bioelectron.*, 120 (2018) 102–107.
35. L. Fu, Y. Zheng, P. Zhang, H. Zhang, M. Wu, H. Zhang, A. Wang, W. Su, F. Chen, J. Yu, *Bioelectrochemistry*, 129 (2019) 199-205.
36. M.G. Campos, S. Bogdanov, L.B. de Almeida-Muradian, T. Szczesna, Y. Mancebo, C. Frigerio, F. Ferreira, *J. Apic. Res.*, 47 (2008) 154–161.
37. S.A. Heleno, A. Martins, M.J.R. Queiroz, I.C. Ferreira, *Food Chem.*, 173 (2015) 501–513.
38. N. El Hamdani, R. Fdil, M. Tourabi, C. Jama, F. Bentiss, *Appl. Surf. Sci.*, 357 (2015) 1294–1305.
39. J. Samoticha, M.J. Jara-Palacios, J.M. Hernández-Hierro, F.J. Heredia, A. Wojdyło, *Eur. Food Res. Technol.*, 244 (2018) 1933–1943.
40. J. Doebley, *Evol. Int. J. Org. Evol.*, 43 (1989) 1555–1559.
41. M.H. Soleimani, M. Talebi, B.E. Sayedtabatabaei, *Plant Syst. Evol.*, 298 (2012) 1141–1149.
42. F. Zhang, G.E. Yaying, W. Wang, X. Shen, X. Liu, J. Liu, D. Tian, Y.U. Xinying, *Sci. Hortic.*, 141 (2012) 1–6.
43. A. Maksylewicz, R. Baranski, *Acta Biochim. Pol.*, 60 (2013) 753.
44. H. Jian, S. Li, J. Guo, S. Li, Q. Wang, H. Yan, X. Qiu, Y. Zhang, Z. Cai, S. Volis, *Conserv. Genet.*, 19 (2018) 1–16.
45. K. Plenk, K. Bardy, M. Höhn, M. Thiv, M. Kropf, *Ecol. Evol.*, 7 (2017) 6527–6539.
46. M.Q. Zhou, K.G. Zhao, L.Q. Chen, *Sci. Hortic.*, 112 (2007) 331–338.