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Development of an electrochemical biosensor for phylogenetic analysis of Amaryllidaceae based on the enhanced electrochemical fingerprint recorded from plant tissue

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ABSTRACT:

A biosensor has been developed based on disposable screen-printed electrode for recording the electrochemical fingerprint of plant leaf tissue. A thin layer of polydopamine functionalized graphene sheets was coated on the plant tissue modified electrode for signal enhancement. The voltammetric data recorded under different buffer solutions can be derived as patterns for species identification. As the distribution of electrochemical active compounds in plants is controlled by genes, these fingerprints can reflect differences at the genetic level between species. Therefore, the electrochemical fingerprint of plant tissues can be used for phylogenetic research without qualitative analysis. 19 species of Amaryllidaceae including *A. africanus, Clivia miniata, Clivia nobilis, Crinum firmifolium, Crinum latifolium, Crinum moorei, Curculiga gracilis, Cyrtanthus breviflorus, Habranthus robustus, Haemanthus albiflos, Haemathus multiflorus, Hippeastrum rutilum, Hymenocallis littoralis, Leucojum aestivum, Sprekelia formosissima, Tulbaghia violacea, Zephyranthes grandiflora, Zephyranthes macrosiphon* and *Zephyranthes minima* have been selected deliberately. The dendrogram deduced from the electrochemical fingerprint was compared with the molecular phylogenetics. The results indicate the electrochemical fingerprint-based phylogenetic study is a persuasive methodology for plant phylogenetic analysis.

1. Introduction

The morphological features of plants used to predominate in the study of evolution and phylogenetics. However, the morphological features of plants are easily influenced by the environment, and there are general phenomena of convergence and parallel evolution, which makes it difficult to determine the evolutionary status of many taxa. With the popularity of molecular techniques such as DNA sequencing and PCR, plant phylogenetics has shifted from a single morphological study to the stage of combining molecular evidence with morphological features. For example, For example, Verbenaceae is a family of plants under the Lamiales. In recent years, molecular evidence has shown that many genera that were once classified as Verbenaceae should be reclassified as Lamiaceae (Marx et al., 2010; Ryding, 1995; Santos Tozin et al., 2016). However, the molecular evidence is also influenced by the chosen molecular marker, so there is still much debate in the phylogenetics of plants (Benzoni et al., 2007; Li et al., 2016; Meyer and Zardoya, 2003).

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Except the classical taxonomy and molecular taxonomy, some other taxonomic technique, other taxonomic techniques are also widely used in phylogenetic studies of plants, such as cytotaxonomy (Almeida et al., 2017; de Moraes et al., 2017), karyosystematics (Barbosa et al., 2017; Di-Nizo et al., 2017), chemotaxonomy (Wink et al., 2018; Xu et al., 2016) and palynology (McGlone et al., 2017; Sinopoli et al., 2018). Among them, chemotaxonomy is often used as an assistive technique to provide evidence for phylogenesis in plants. This is because different plants produce different chemicals, such as alkaloids, glycosides and terpenes, depending on the type of metabolism. The inheritance and variation of these chemical components in plants are closely related to the phylogenetic position of the plant. Plant species with close relatives often have similar physiological and biochemical characteristics due to their close genetic relationship. For example, the largest group of indole alkaloids is Plumerane type indole alkaloids. These alkaloids are found only in the subfamily Plumerioideae of Apocynaceae (Reina et al., 2011). Chromatography, mass spectrum and NMR are the main techniques used for the analysis of chemical components in the plant. The target molecules have been further used for chemotaxonomy purposes. However, these instruments need a sophisticated operation process with long-time analysis. So far, chemotaxonomy is not a preferred method for plant phylogenetic analysis.

Electroanalysis is a widely studied analytical technique for the detection and identification of analytes *in vivo* or *in vitro* (Alwarappan et al., 2007; Bojdi et al., 2014a; Hosseini et al., 2014; Kalate Bojdi et al., 2015a; Kesavan et al., 2019; Rahmani et al., 2018; Sukumaran et al., 2017; Tashkhourian et al., 2016; Wightman et al., 1988; Wightman, 2006; Wu et al., 2017; Yadav et al., 2014). Using a range of signal amplification techniques, electrochemical sensors can detect very low concentrations of the target analyte. So far, it has been widely used for environmental pollutant monitoring (Ramnani et al., 2016), pharmaceutical compound detection (Beitollahi and Mostafavi, 2014; Bojdi et al., 2014b) and food analysis (Bojdi et al., 2016b, 2016a; Kalate Bojdi et al., 2015b; Zeng et al., 2016). In addition to the detection of a single substance, electroanalytical techniques can also scan for electrochemical active compounds in complex systems (Fu et al., 2020; Li et al., 2019; Zhang et al., 2019). It has been used for plant identification based on fingerprinting of electrochemical active compounds in plant tissue (Doménech-Carbó et al., 2015a; Domínguez and Doménech-Carbó, 2015). Later on, these fingerprint has been correlated to the phylogenetic position of the plant species and showed convincing results (Doménech-Carbó et al., 2015b; Doménech-Carbó et al., 2017a, b).

Our previous works also demonstrated the feasibility of the electroanalysis of plant tissue to the phylogenetic position of the plant (Fu et al., 2019b, 2019a). Species from *Lycoris* have been selected as samples for species identification and infrageneric relationship investigation. In this study, we extend the methodology towards plant family level analysis for the first time. 19 species of Amaryllidaceae were selected deliberately. Amaryllidaceae is a family of Asparagales due to their complicated relationship. For example, an earlier taxonomy system (Cronquist system) placed the Amaryllidaceae in the Liliales. In 2003, the genetic relatability-based APG II system was proposed to merge it with the Allioideae and Agapanthoideae (Ii, 2003; Kamenetsky and Okubo, 2012). We modified the plant tissue immobilization process for giving a better signal. In addition, we proposed a new pattern recognition mode for quick species identification. Finally, the phylogenetic tree deduced from the electrochemical fingerprint has been compared with molecular phylogenetical results.

2. Experimental

2.1. Sample collection and chemicals

Leaves of *Agapanthus africanus, A. africanus, Clivia miniata, Clivia nobilis, Crinum firmifolium, Crinum latifolium, Crinum moorei, Curculiga gracilis, Cyrtanthus breviflorus*, *Habranthus robustus, Haemanthus albiflos, Haemathus multiflorus, Hippeastrum rutilum, Hymenocallis littoralis, Leucojum aestivum, Sprekelia formosissima, Tulbaghia violacea, Zephyranthes grandiflora, Zephyranthes macrosiphon* and *Zephyranthes minima* were collected from Hangzhou Botanic Garden (Zhejiang, China) and Nanjing Botanical Garden Mem. Sun Yat-Sen (Nanjing, China) in August 2019. Healthy leaves of each species were carefully collected and stored at −20 °C before analysis. KH_2PO_4, Na_2HPO_4, sodium acetate, acetic acid and Tris powder were purchased from Macklin Co., Ltd. All other chemicals were analytical-grade reagents and were used without further purification. Milli-Q water (18.2 MΩ/cm) was used throughout the experiments.

2.2. Preparation of polydopamine functionalized graphene dispersion

Preparation of polydopamine functionalized graphene dispersion was based on our previous work with some modifications (Fu et al., 2016, 2015). Typically, 10 mg of graphene oxide was dispersed in 30 mL Tris-buffer (10 mM, pH 8.5) and sonicated for 1 h. 10 mg of dopamine was then added into the dispersion and sonicated for another 10 min. The resulting mixture was kept stirring for 24 h. The polydopamine functionalized graphene was collected and washed three times by water using centrifugation.

2.3. Extract preparation and electrode surface modification

Dimethyl sulfoxide (DMSO) was used as a solvent for plant leaf extract preparation. Typically, 1 mL of DMSO was added into 0.05 g chopped plant leaf by 1 min grinding using a microdismembrator (MY-10, Shanghai Jingxin, Co. Ltd.). Then, the slurry was sonicated using a bath sonicator for 5 min to form a dispersion. 5 μL of the extract was drop coated on the working electrode of a screen-printed electrode (SPE, Nanjing Youyun Technology Co., Ltd.) After drying in the room temperature, 1 μL of polydopamine functionalized graphene dispersion (0.1 mg/mL) was further drop coated on the working electrode. After drying in the room temperature, 50 μL of buffer solution was dropped on the electrode surface to cover the three-electrode system. 0.1 M of ABS (pH = 4.5), PBS (pH = 7.0) and Tris (pH = 9.0) were used as electrolyte for electrochemical fingerprint recording. All electrochemical fingerprints were recorded using a CHI660C workstation with a homemade adapter constructed by Noguchi socket and DuPont line. Differential pulse voltammetry was used for recording the electrochemical fingerprints of all plant tissue between −0.1 and 1.5 V.

3. Results and discussion

Leaves are one of the vegetative organs of vascular plants. Its function is to synthesize organic matter through photosynthesis and to provide the root system with the power to absorb water and mineral nutrients through transpiration. Each plant has a unique leaf shape, and even plants of the same family have different leaf shapes. Information about the shape of the leaves is stored in the DNA. For example, the split-leaf of the *Cardamine hirsuta* is attributed to a unique homeobox gene, which inhibits the proliferation and growth of cells in the phyllodes, causing them to separate from each other (Vlad et al., 2014). *Arabidopsis thaliana* does not have this gene, so its leaves are not completely divided. In this study, the leaves of 19 species of Amaryllidaceae were deliberately chosen for reflecting whether the difference between their electrochemical active compounds can be used for phylogenetical analysis. Fig. 1A shows a schematic diagram of the proposed electrochemical fingerprint recording process. A simple plant tissue extraction process was carried out using DMSO. We noticed a superior extraction process compared with our previous work using water, ethanol, glycol and DMF as solvent (Fu et al., 2019b, 2019a, 2018). The immobilization of the plant tissue on the electrode surface is crucial for fingerprint recording. Normally, film-forming materials such as Nafion or chitosan can be coated after the plant tissue immobilization (Mauritz and Moore, 2004).

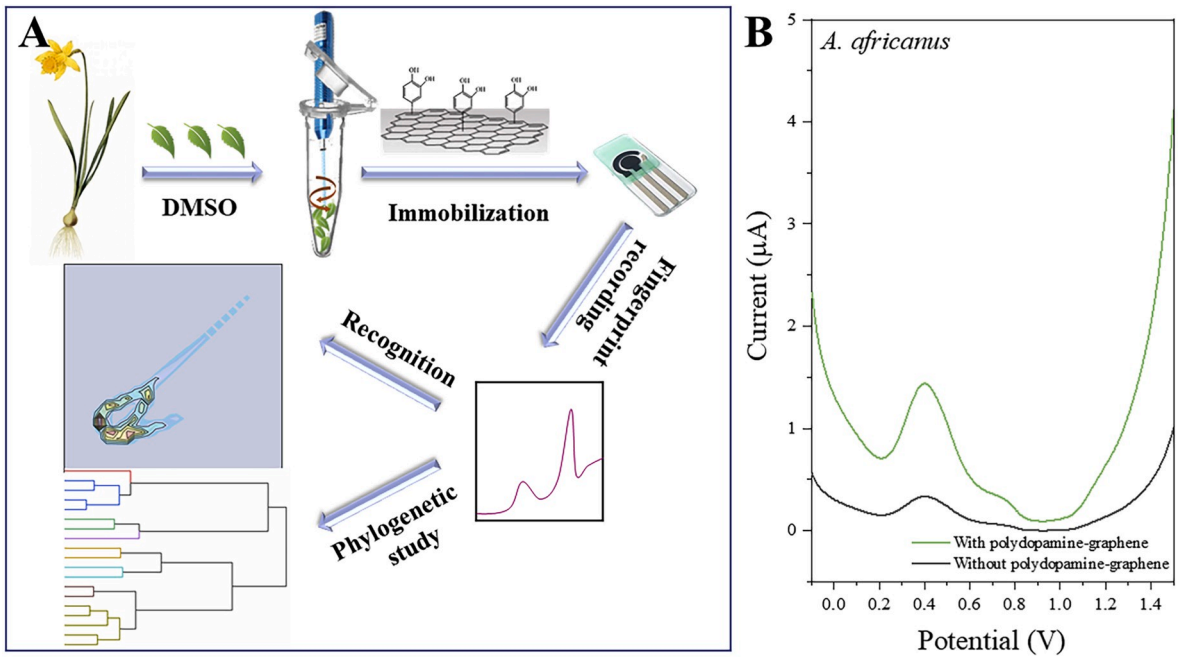

Fig. 1. (A) Schematic diagram of electrochemical biosensor for plant fingerprint recording. (B) DPV curve comparison of *A.s africanus* recorded on SPE with or without graphene addition.

However, these film-forming materials could affect the conductivity of the electrode surface and consequently reduce the signal sensitivity. Therefore, we added an extra thin layer polydopamine functionalized graphene sheets on the plant tissue surface for improving the immobilization as well as conductivity. Fig. S1 shows the SEM image of the plant tissue immobilized SPE. It can be seen that the plant tissue has been covered on the polydopamine functionalized graphene sheets. The electroactive surface area (EASA) was measured using Randles–Sevcik equation (I_p=(2.69 × 10^5)$n^{3/2}AD^{1/2}Cv^{1/2}$). The EASA value of the bare plant tissue modified electrode was calculated to be 0.0422. Leaves of Amaryllidaceae *spp.* contains a large number of long-chain molecules of cellulose with poor electroconductivity. Either bare SPE, graphene modified SPE, polydopamine modified SPE and polydopamine functionalized graphene sheets modified SPE (without plant tissue) showed no noticeable peak during the potential range from −0.2 to 1.5 V, suggesting either polydopamine and graphene showed no oxidation process between the scan window. Fig. 1B shows the DPV curves of the *A. africanus* in the presence and absence of a thin layer of polydopamine functionalized graphene sheets. It is pertinent to note that the addition of polydopamine functionalized graphene sheets significantly enhance the electrochemical signal of the plant tissue. It can be also noticed that the peak potential of two curves only showed imperceptible shift, suggesting the thin layer of polydopamine functionalized graphene sheets does not seem to have affected the electrochemical behavior of compounds extracted from plant tissue. Fig. S2 shows DPV curves of the *A. africanus* for five individual SPEs, indicating the acceptable steady performance.

Fig. 2 shows DPV curves of 19 species of Amaryllidaceae recorded on SPE in 0.1 M PBS (DPV curves of each species recorded in 0.1 ABS and Tris are shown in Figs. S3 and S4). As shown in the figure, several peaks can be noticed between −0.1 V and 1.5 V correspond to the oxidation of electrochemical active compounds oxidizable compounds. These compounds could be flavonols (Liu et al., 2020), phenolic acids (Irakli et al., 2018), procyanidins (Ramsay and Mueller-Harvey, 2016), alkaloids (Marín-Sáez et al., 2019) and pigments (Chou et al., 2020). We did not include the negative scan because it contains much less information compared with that of the positive scan. In addition, the dissolved oxygen could produce a distinct peak during the negative scan if we extend the potential window. Therefore, we only include a positive scan for representing the information of plant tissue. The methodology of plant classification is based on the statistical analysis of character information rather than the quantitative detection of a particular component. Therefore, plant phylogenetic studies based on the electrochemical fingerprint do not need to identify oxidation peaks one by one. In fact, for complex systems, such as plant tissue, direct electrochemical sensing cannot achieve a qualitative and quantitative analysis of all responding compounds. However, the electrochemical oxidation reactions in which each of the responding compounds is involved contribute more or less to the whole profile. The resulting electrochemical fingerprint represents the profile of electrochemical active compounds from plant tissue participated in electrochemical oxidation under buffer condition. It is pertinent to note that the obvious differences of the electrochemical fingerprint can be noticed between species, indicating the variation of the oxidized compounds during the DPV scan.

Fig. 3 shows the parallel coordinate plot of the normalized current of *A. africanus, C. nobilis* and *C. latifolium* recorded under three different buffer solutions as examples. The parallel coordinate plot is a common method to analyze and display multivariate data. To better demonstrate the differences in electrochemical fingerprints among different species, we divided 19 species into three groups. The electrochemical fingerprint of each species showed different tendencies, indicating different species show discernible differences recorded in the different buffer solutions. Therefore, the electrochemical profile of the plant tissue showed the potential of using the voltammetric data for species discrimination. The rapid identification of plant species is important in agriculture, horticulture and plant-based medical materials production. Although the species with different genus may have different morphological features, correct identification still is a challenge for people unfamiliar with botany. Because the leaves of different species contain different electrochemical active compounds, these electrochemical fingerprints can be used for rapid identification of species.

Direct species identification based on the electrochemical fingerprint is not an efficient way. The electrochemical fingerprints of some of these species share some similar characteristics, such as *H. littoralis and L. aestivum* (Fig. 2). Therefore, pattern recognition based on multiple electrochemical fingerprints can be used for species identification more

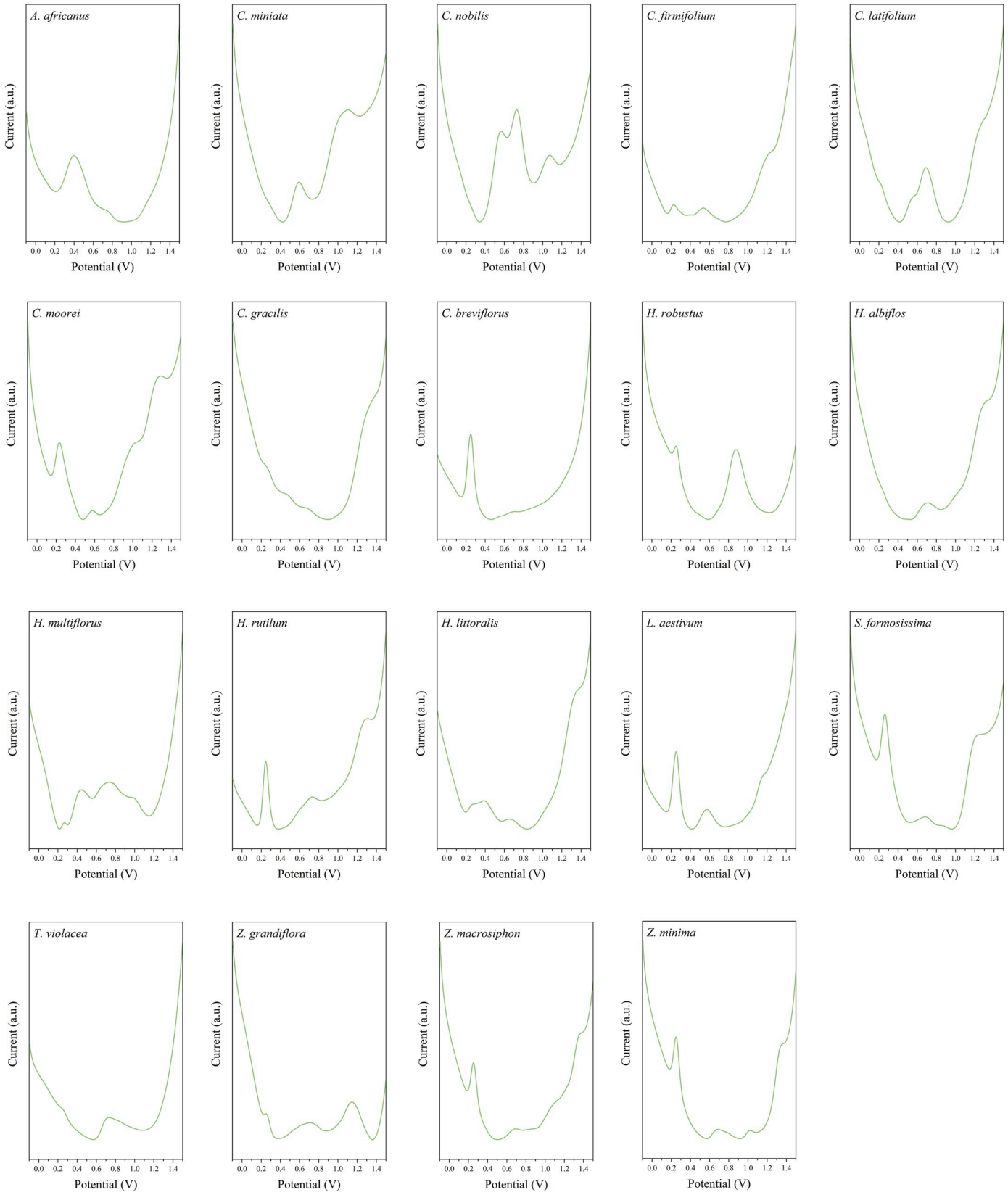

Fig. 2. DPV curves of 19 species of Amaryllidaceae recorded on SPE in 0.1 M PBS. Additional DPV curves of each species recorded in 0.1 ABS and Tris are shown in Figs. S3 and S4.

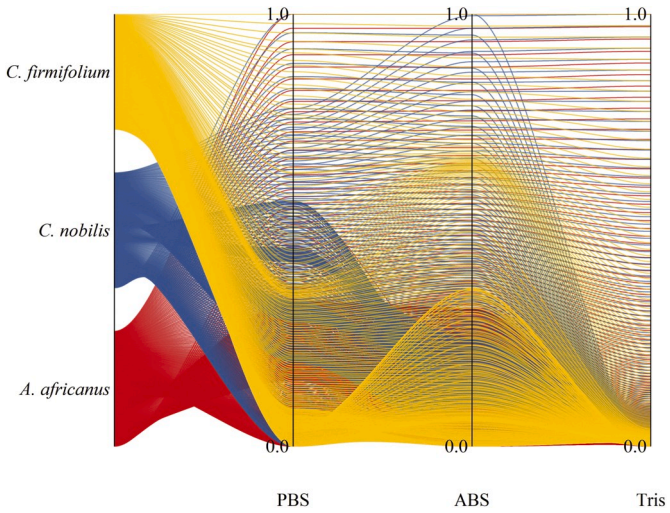

Fig. 3. Parallel coordinate plot of normalized currents of *A. africanus, C. nobilis* and *C. latifolium* recorded in 0.1 M PBS, ABS and Tris.

efficiently. Fig. 4A shows a series of scatter patterns of *A. africanus* deduced from the fingerprint recorded under ABS, PBS and Tris conditions (Figs. S5–22 show the scatter patterns of *C. miniata, C. nobilis, C. firmifolium, C. latifolium, C. moorei, C. gracilis, C. breviflorus, H. robustus, H. albiflos, H. multiflorus, H. rutilum, H. littoralis, L. aestivum, S. formosissima, T. violacea, Z. grandiflora, Z. macrosiphon* and *Z. minima,* respectively). Without a doubt, the scatter pattern is easier to identify species than the direct electrochemical fingerprint. However, the scatter recognition mode only presents two sets of data. Each point has the same property weight, so it still needs to calculate the number of scattered points at different locations for species recognition. Therefore, we proposed an additional pattern recognition model based on the density functional theory. Fig. 4B shows the three 2D density plots of *A. africanus* using the normalized current data from 0.1 M PBS vs. 0.1 M ABS, 0.1 M ABS vs. 0.1 M Tris and 0.1 M Tris vs. 0.1 M PBS (Figs. S3–20 show the scatter patterns of *C. miniata, C. nobilis, C. firmifolium, C. latifolium, C. moorei, C. gracilis, C. breviflorus, H. robustus, H. albiflos, H. multiflorus, H. rutilum, H. littoralis, L. aestivum, S. formosissima, T. violacea, Z. grandiflora, Z. macrosiphon* and *Z. minima,* respectively). In this recognition model with the assistance of well-established image recognition methods (A. K. Jain et al., 2000), such as voxel similarity measurement and image segmentation-based similarity measurement (M. Holden et al., 2000), unknown species can be identified by matching patterns or even by locating high-density areas with the database.

Principal component analysis (PCA) is often used for dimensionality reduction of high dimensional variables. The 3D PCA grouping result showed *H. multiflorus, H. albiflos, C. miniata, C. nobilis, Z. grandiflora, Z. macrosiphon, Z. minima, C. gracilis* and *L. aestivum* were grouped in a close spatial position (Fig. 5). *A. africanus, T. violacea, C. firmifolium, C. latifolium* and *C. moorei* were grouped in a close spatial position. In addition, *C. breviflorus, H. rutilum* and *H. littoralis* can be considered as outliers among the species. PCA data showed a certain clustering trend, confirms the obvious differences in electrochemical active compounds among plant species, reflecting that there may also be significant differences at the gene level. However, the three extracted factors could only explain 73% of the data, suggesting that PCA analysis of electrochemical fingerprint data does not have a high interpretative capability.

As discussed above, there is a reasonable assumption that the noticed differences in electrochemical fingerprint reflect the genetic diversities of samples. In this work, we attempted to use the electrochemistry-based chemotaxonomic methodology for the phylogenetic study in family level. Fig. 6 shows the dendrogram of 19 species deduced from the electrochemical fingerprint recorded using three buffer solutions. The phylogenetic tree was divided into four main principal infrageneric clades. The first clade consisted of the species *C. breviflorus, H. multiflorus, H. albiflos, C. miniate* and *C. nobilis*. The second clade consisted of

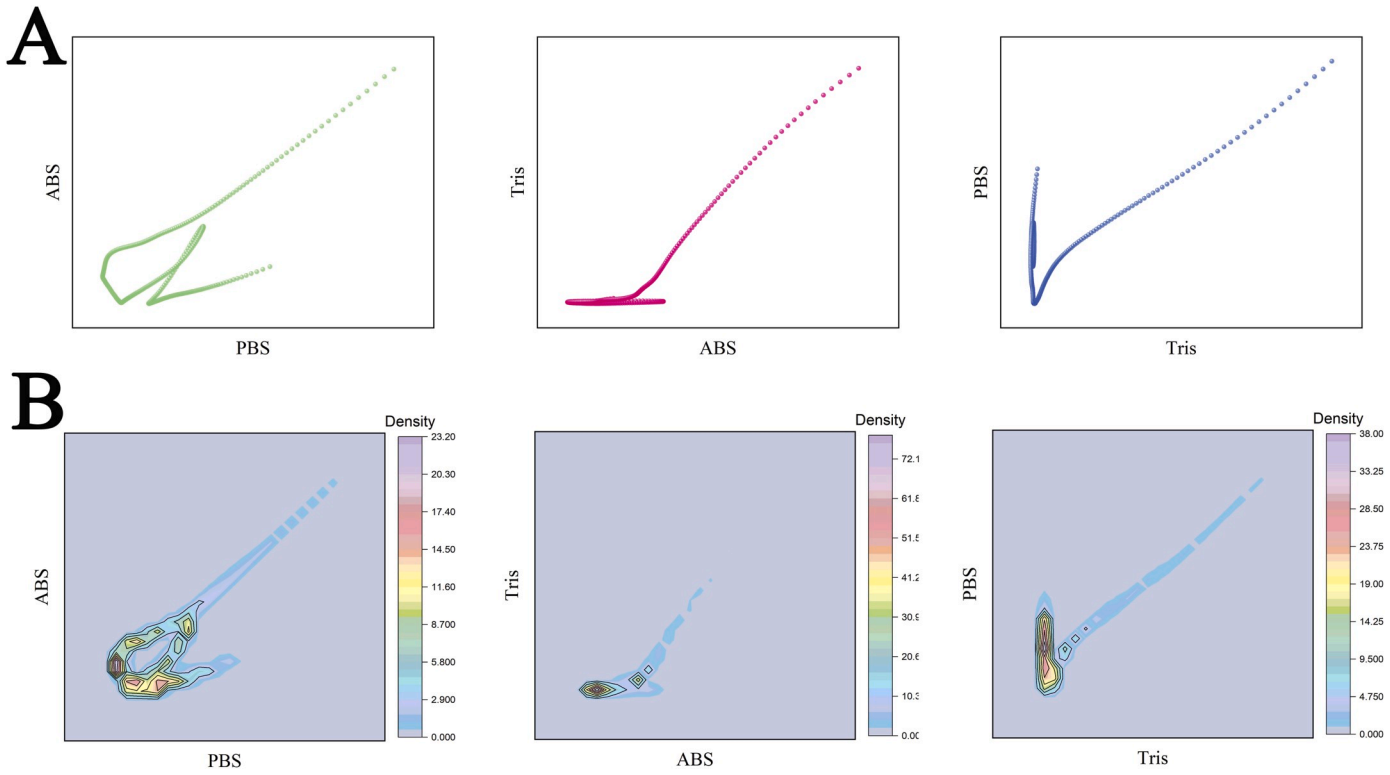

Fig. 4. (A) 2D scatter patterns and (B) 2D density patterns of *A. africanus* based on normalized fingerprint recorded from 0.1 M PBS vs. 0.1 M ABS, 0.1 M ABS vs. 0.1 M Tris and 0.1 M Tris vs. 0.1 M PBS. Additional 2D scatter patterns and 2D density patterns of each species are shown in Figs. S5–S22.

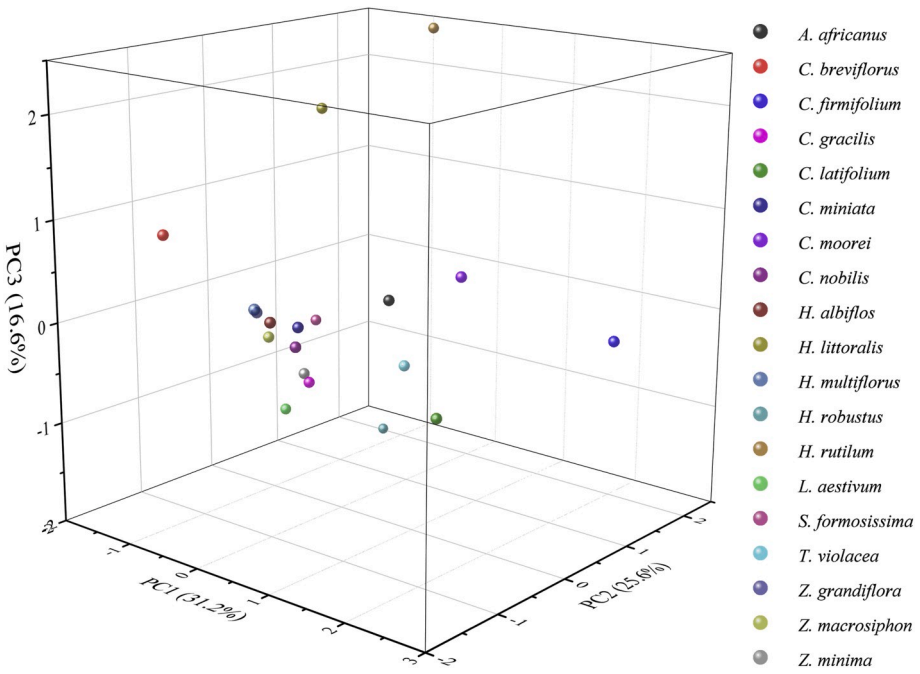

Fig. 5. 3D PCA analysis of *A. africanus*, C. miniata, *C. nobilis*, C. firmifolium, *C. latifolium*, C. moorei, *C. gracilis*, C. breviflorus, H. robustus, H. albiflos, H. multiflorus, H. rutilum, H. littoralis, L. aestivum, S. formosissima, *T. violacea*, Z. grandiflora Z. macrosiphon and Z. minima using normalized current recorded after five solvents extractions.

the species *C. firmifolium, C. latifolium* and *C. moorei*. The third group consisted of the species *H. rutilum, H. littoralis, H. robustus* and *S. formosissima*. The last clade consisted of the species *Z. grandiflora, Z. macrosiphon, Z. minima, C. gracilis* and *L. aestivum*.

The intergeneric relationships within the Amaryllidaceae are a very complex topic that faces many challenges even using molecular sequencing (Graham and Barrett, 2004; Meerow et al., 2000). RAPD, ITS, ISSR, and SCoT were used for the analysis of certain genus within Amaryllidaceae (Akhavan et al., 2015; Jiang et al., 2013; Meerow et al., 2000; Ran et al., 2001). The previous report claimed the chemical diversity of Amaryllidaceae significantly correlated with phylogeny (Rønsted et al., 2012). The dendrogram deduced from electrochemical fingerprint indicates the *C. miniate* and *C. nobilis* were in a strongly supported cluster, while the *H. robustus* and *S. formosissima* have a close relationship. These results are consistent with ITS analysis (Bay-Smidt et al., 2011; Rønsted et al., 2012). The close relationship of genera *Clivia* and *Haemathus* from our results is consistent with biogeography because of they both origin from South Africa as well as the *matK* sequencing result (Ito et al., 1999). The biogeographic fact also reveals the deduced relationship between *C. latifolium* and *C. moorei* (Meerow et al., 2003).

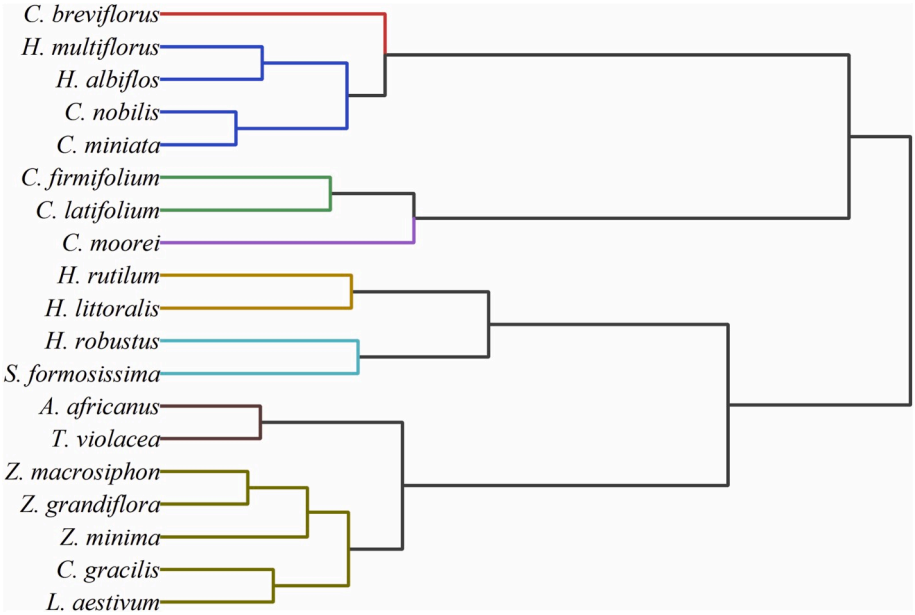

Fig. 6. Dendrogram of *A. africanus, C. miniata, C. nobilis, C. firmifolium, C. latifolium, C. moorei, C. gracilis, C. breviflorus, H. robustus, H. albiflos, H. multiflorus, H. rutilum, H. littoralis, L. aestivum, S. formosissima, T. violacea, Z. grandiflora Z. macrosiphon* and *Z. minima* based on the electrochemical fingerprints recorded in three buffer solutions.

The *C. breviflorus* in the first clade showed a close relationship with the genus *Clivia*. Although there is no specific study of the phylogenetic position of *Cyrtanthus* among the Amaryllidaceae, the floral and macroecological study within *Cyrtanthus* still revealed the relatively close relationship of the *Cyrtanthus* with *Clivia* (Snijman and Meerow, 2010). Both *Hippeastrum* and *Habranthus* were in a tribe of plants belonging to the subfamily Amaryllidoideae (Strydom, 2005). The dendrogram deduced from the electrochemical fingerprint also showed them in one clade. Species of *Tulbaghia* is popular in horticulture, however, the species delimitation still faces challenges. In 2016, the first report analyzed the infrageneric relationship within *Tulbaghia* using ITS and plastid-encoded trnL-F and ndhF (Stafford et al., 2016). Our results provide a possible interfamily status of the *Tulbaghia* within Amaryllidoideae. In addition, the *L. aestivum* has been widely studied due to its medical application (Gentili et al., 2018; Ptak et al., 2017). To the best of our knowledge, however, its phylogenetic relationship has not been studied so far. Our results indicate the *L. aestivum* was closely related to the *Curculiga* and *Zephyranthes*. We believe these results not only provide evidence to the phylogenetic assumption proposed by botanist, but it can light the way of the research direction.

4. Conclusion

In summary, the electrochemical fingerprint of leaf tissue has been used for species identification and phylogenetic investigation. The immobilization of plant tissue has been overcame by drop coating a thin layer polydopamine functionalized graphene. The fingerprint of plant tissue successfully recorded under different buffer solutions. These fingerprints have been used for generating the pattern recognition modes for species identification. Moreover, this work extended the methodology to the family level for phylogenetic analysis. The dendrogram deduced from the electrochemical fingerprints of 19 Amaryllidoideae species gave a persuasive taxonomical result compared with molecular studies. The dendrogram indicates the following:

(1) Agreed with the ITS study, *C. miniate* and *C. nobilis* show a notably close relationship while the *H. robustus* and *S. formosissima* have a close relationship.
(2) Observed a close relationship between genera *Clivia* and *Haemathus* due to the same biogeographic origin.
(3) *Cyrtanthus* and *Clivia* show a relatively distant relationship.
(4) *Hippeastrum* and *Habranthus* show a close relationship because they belong to a tribe of subfamily Amaryllidoideae.
(5) *L. aestivum* closely relate to the *Curculiga* and *Zephyranthes*

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Li Fu: Conceptualization, Methodology, Project administration, Writing - original draft. **Yuhong Zheng:** Methodology, Validation. **Pengcong Zhang:** Methodology, Investigation. **Haoyang Zhang:** Investigation. **Yuting Xu:** Investigation, Formal analysis. **Jingtao Zhou:** Investigation, Formal analysis. **Huaiwei Zhang:** Software. **Hassan Karimi-Maleh:** Software, Writing - review & editing. **Guosong Lai:** Writing - review & editing. **Shichao Zhao:** Project administration. **Weitao Su:** Visualization, Supervision. **Jinhong Yu:** Supervision, Writing - review & editing. **Cheng-Te Lin:** Methodology, Supervision, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2020.112212.

References

Akhavan, A., Saeidi, H., Rahiminejad, M.R., Zarre, S., Blattner, F.R., 2015. Interspecific relationships in Allium subgenus Melanocrommyum sections Acanthoprason and Asteroprason (Amaryllidaceae) revealed using ISSR markers. Syst. Bot. 40, 706–715.
Almeida, L.A., Nunes, L.A., Bitencourt, J.A., Molina, W.F., Affonso, P.R., 2017. Chromosomal evolution and cytotaxonomy in wrasses (Perciformes; Labridae). J. Hered. 108, 239–253.
Alwarappan, S., Butcher, K.S.A., Wong, D.K., 2007. Evaluation of hydrogenated physically small carbon electrodes in resisting fouling during voltammetric detection of dopamine. Sensor. Actuator. B Chem. 128, 299–305.
Barbosa, P., Pucci, M.B., Nogaroto, V., Almeida, M.C., Artoni, R.F., Vicari, M.R., 2017. Karyotype analysis of three species of Corydoras (Siluriformes: callichthyidae) from southern Brazil: rearranged karyotypes and cytotaxonomy. Neotrop. Ichthyol. 15, e160056.
Bay-Smidt, M.G.K., Jäger, A.K., Krydsfeldt, K., Meerow, A.W., Stafford, G.I., Van Staden, J., Rønsted, N., 2011. Phylogenetic selection of target species in Amaryllidaceae tribe Haemantheae for acetylcholinesterase inhibition and affinity to the serotonin reuptake transport protein. South Afr. J. Bot. 77, 175–183.
Beitollahi, H., Mostafavi, M., 2014. Nanostructured base electrochemical sensor for simultaneous quantification and voltammetric studies of levodopa and carbidopa in pharmaceutical products and biological samples. Electroanalysis 26, 1090–1098.
Benzoni, F., Stefani, F., Stolarski, J., Pichon, M., Mitta, G., Galli, P., 2007. Debating phylogenetic relationships of the scleractinian Psammocora: molecular and morphological evidences. Contrib. Zool. 76, 35–54.
Bojdi, M.K., Behbahani, M., Sahragard, A., Amin, B.G., Fakhari, A., Bagheri, A., 2014a. A palladium imprinted polymer for highly selective and sensitive electrochemical determination of ultra-trace of palladium ions. Electrochim. Acta 149, 108–116.
Bojdi, M.K., Mashhadizadeh, M.H., Behbahani, M., Farahani, A., Davarani, S.S.H., Bagheri, A., 2014b. Synthesis, characterization and application of novel lead imprinted polymer nanoparticles as a high selective electrochemical sensor for ultra-trace determination of lead ions in complex matrixes. Electrochim. Acta 136, 59–65.
Bojdi, M.K., Behbahani, M., Hesam, G., Mashhadizadeh, M.H., 2016a. Application of magnetic lamotrigine-imprinted polymer nanoparticles as an electrochemical sensor for trace determination of lamotrigine in biological samples. RSC Adv. 6, 32374–32380.
Bojdi, M.K., Behbahani, M., Omidi, F., Hesam, G., 2016b. Application of a novel electrochemical sensor based on modified siliceous mesocellular foam for electrochemical detection of ultra-trace amounts of mercury ions. New J. Chem. 40, 4519–4527.
Chou, S., Chen, B., Chen, J., Wang, M., Wang, S., Croft, H., Shi, Q., 2020. Estimation of leaf photosynthetic capacity from the photochemical reflectance index and leaf pigments. Ecol. Indicat. 110, 105867.
de Moraes, R.L.R., Bertollo, L.A.C., Marinho, M.M.F., Yano, C.F., Hatanaka, T., Barby, F. F., Troy, W.P., Cioffi, M. de B., 2017. Evolutionary relationships and cytotaxonomy considerations in the genus Pyrrhulina (Characiformes, Lebiasinidae). Zebrafish 14, 536–546.
Di-Nizo, C.B., da Silva Banci, K.R., Sato-Kuwabara, Y., Silva, M.J. de J., 2017. Advances in cytogenetics of Brazilian rodents: cytotaxonomy, chromosome evolution and new karyotypic data. Comp. Cytogenet. 11, 833.
Doménech-Carbó, A., Domínguez, I., Hernández-Muñoz, P., Gavara, R., 2015a. Electrochemical tomato (Solanum lycopersicum L.) characterisation using contact probe in situ voltammetry. Food Chem. 172, 318–325.
Doménech-Carbó, A., Ibars, A.M., Prieto-Mossi, J., Estrelles, E., Scholz, F., Cebrián-Torrejón, G., Martini, M., 2015b. Electrochemistry-based chemotaxonomy in plants using the voltammetry of microparticles methodology. New J. Chem. 39, 7421–7428.
Doménech-Carbó, A., Doménech-Carbó, M.T., Ferragud-Adam, X., Ortiz-Miranda, A.S., Montoya, N., Pasíes-Oviedo, T., Peiró-Ronda, M.A., Vives-Ferrándiz, J., Carrión Marco, Y., 2017a. Identification of vegetal species in wooden objects using in situ microextraction-assisted voltammetry of microparticles. Anal. Methods 9, 2041–2048.
Doménech-Carbó, A., Ibars, A.M., Prieto-Mossi, J., Estrelles, E., Doménech-Carbó, M.T., Ortiz-Miranda, A.S., Martini, M., Lee, Y., 2017b. Access to phylogeny from voltammetric fingerprints of seeds: the Asparagus case. Electroanalysis 29, 643–650.
Domínguez, I., Doménech-Carbó, A., 2015. Screening and authentication of tea varieties based on microextraction-assisted voltammetry of microparticles. Sensor. Actuator. B Chem. 210, 491–499.
Fu, L., Lai, G., Jia, B., Yu, A., 2015. Preparation and electrocatalytic properties of polydopamine functionalized reduced graphene oxide-silver nanocomposites. Electrocatalysis 6, 72–76.

Fu, L., Lai, G., Zhu, D., Jia, B., Malherbe, F., Yu, A., 2016. Advanced catalytic and electrocatalytic performances of polydopamine-functionalized reduced graphene oxide-palladium nanocomposites. ChemCatChem 8, 2975–2980.
Fu, L., Zheng, Y., Zhang, P., Zhang, Haoyang, Zhuang, W., Zhang, Huaiwei, Wang, A., Su, W., Yu, J., Lin, C.-T., 2018. Enhanced electrochemical voltammetric fingerprints for plant taxonomic sensing. Biosens. Bioelectron. 120, 102–107.
Fu, L., Wu, M., Zheng, Y., Zhang, P., Ye, C., Zhang, H., Wang, K., Su, W., Chen, F., Yu, J., Yu, A., Cai, W., Lin, C.-T., 2019a. Lycoris species identification and infrageneric relationship investigation via graphene enhanced electrochemical fingerprinting of pollen. Sensor. Actuator. B Chem. 298, 126836.
Fu, L., Zheng, Y., Zhang, P., Zhang, Haoyang, Wu, M., Zhang, Huaiwei, Wang, A., Su, W., Chen, F., Yu, J., Cai, W., Lin, C.-T., 2019b. An electrochemical method for plant species determination and classification based on fingerprinting petal tissue. Bioelectrochemistry 129, 199–205.
Fu, L., Zhang, H.Y., Zheng, Y., Zhang, H.W., Liu, Q., 2020. An electroanalytical method for brewing vinegar authentic identification. Rev. Mex. Ingen. Quím. 19, 803–812.
Gentili, R., Abeli, T., Parolo, G., Ciappetta, S., Montagnani, C., Müller, J.V., Rossi, G., Citterio, S., 2018. Genetic structure of Leucojum aestivum L. in the Po Valley (N-Italy) drives conservation management actions. Conserv. Genet. 19, 827–838.
Graham, S.W., Barrett, S.C., 2004. Phylogenetic reconstruction of the evolution of stylar polymorphisms in Narcissus (Amaryllidaceae). Am. J. Bot. 91, 1007–1021.
Holden, M., Hill, D.L.G., Denton, E.R.E., Jarosz, J.M., Cox, T.C.S., Rohlfing, T., Goodey, J., Hawkes, D.J., 2000. Voxel similarity measures for 3-D serial MR brain image registration. IEEE Trans. Med. Imag. 19, 94–102.
Hosseini, H., Behbahani, M., Mahyari, M., Kazerooni, H., Bagheri, A., Shaabani, A., 2014. Ordered carbohydrate-derived porous carbons immobilized gold nanoparticles as a new electrode material for electrocatalytical oxidation and determination of nicotinamide adenine dinucleotide. Biosens. Bioelectron. 59, 412–417.
Ii, A., 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Bot. J. Linn. Soc. 141, 399–436.
Irakli, M., Chatzopoulou, P., Ekateriniadou, L., 2018. Optimization of ultrasound-assisted extraction of phenolic compounds: oleuropein, phenolic acids, phenolic alcohols and flavonoids from olive leaves and evaluation of its antioxidant activities. Ind. Crop. Prod. 124, 382–388.
Ito, M., Kawamoto, A., Kita, Y., Yukawa, T., Kurita, S., 1999. Phylogenetic relationships of Amaryllidaceae based on matK sequence data. J. Plant Res. 112, 207–216.
Jain, A.K., Duin, R.P.W., Mao, Jianchang, 2000. Statistical pattern recognition: a review. IEEE Trans. Pattern Anal. Mach. Intell. 22, 4–37.
Jiang, X., Gao, Y., Tong, Z., Huang, C., 2013. Establishing and optimizing a SCoT-PCR system for Lycoris. J. Zhejiang A&F Univ. 30, 444–452.
Kalate Bojdi, M., Behbahani, M., Mashhadizadeh, M.H., Bagheri, A., Hosseiny Davarani, S.S., Farahani, A., 2015a. Mercapto-ordered carbohydrate-derived porous carbon electrode as a novel electrochemical sensor for simple and sensitive ultra-trace detection of omeprazole in biological samples. Mater. Sci. Eng. C 48, 213–219.
Kalate Bojdi, M., Behbahani, M., Najafi, M., Bagheri, A., Omidi, F., Salimi, S., 2015b. Selective and sensitive determination of uranyl ions in complex matrices by ion imprinted polymers-based electrochemical sensor. Electroanalysis 27, 2458–2467.
Kamenetsky, R., Okubo, H., 2012. Ornamental Geophytes: from Basic Science to Sustainable Production. CRC press.
Kesavan, S., Gowthaman, N., Alwarappan, S., John, S.A., 2019. Real time detection of adenosine and theophylline in urine and blood samples using graphene modified electrode. Sensor. Actuator. B Chem. 278, 46–54.
Li, X., Li, S., Kong, R., Li, R., 2016. Molecular separation of two long taxonomically debated cyanobacterial genera Cylindrospermopsis and Raphidiopsis (Nostocales) based on the ITS-L phylogeny. Harmful Algae 57, 88–97.
Li, Z., Zhang, X., Zhou, J., Zhang, H., Zheng, Y., Liu, Q., Fu, L., 2019. Authentical screening of tea beverages based on voltammetric profile. Int. J. Electrochem. Sci 14, 10531–10537.
Liu, Z., Bruins, M.E., de Bruijn, W.J.C., Vincken, J.-P., 2020. A comparison of the phenolic composition of old and young tea leaves reveals a decrease in flavanols and phenolic acids and an increase in flavonols upon tea leaf maturation. J. Food Compos. Anal. 86, 103385.
Marín-Sáez, J., Romero-González, R., Garrido Frenich, A., 2019. Effect of tea making and boiling processes on the degradation of tropane alkaloids in tea and pasta samples contaminated with Solanaceae seeds and coca leaf. Food Chem. 287, 265–272.
Marx, H.E., O'Leary, N., Yuan, Y., Lu-Irving, P., Tank, D.C., Múlgura, M.E., Olmstead, R. G., 2010. A molecular phylogeny and classification of Verbenaceae. Am. J. Bot. 97, 1647–1663.
Mauritz, K.A., Moore, R.B., 2004. State of understanding of nafion. Chem. Rev. 104, 4535–4586.
McGlone, M.S., Richardson, S.J., Burge, O.R., Perry, G.L., Wilmshurst, J.M., 2017. Palynology and the ecology of the New Zealand conifers. Front. Earth Sci. 5, 94.
Meerow, A.W., Guy, C.L., Li, Q.-B., Yang, S.-L., 2000. Phylogeny of the American Amaryllidaceae based on nrDNA ITS sequences. Syst. Bot. 708–726.
Meerow, A.W., Lehmiller, D.J., Clayton, J.R., 2003. Phylogeny and biogeography of Crinum L. (Amaryllidaceae) inferred from nuclear and limited plastid non-coding DNA sequences. Bot. J. Linn. Soc. 141, 349–363.
Meyer, A., Zardoya, R., 2003. Recent advances in the (molecular) phylogeny of vertebrates. Annu. Rev. Ecol. Evol. Syst. 34, 311–338.
Ptak, A., Morańska, E., Saliba, S., Zieliński, A., Simlat, M., Laurain-Mattar, D., 2017. Elicitation of galanthamine and lycorine biosynthesis by Leucojum aestivum L. and L. aestivum 'Gravety Giant'plants cultured in bioreactor RITA®. Plant Cell Tissue Organ Cult. 128, 335–345.
Rahmani, T., Bagheri, H., Behbahani, M., Hajian, A., Afkhami, A., 2018. Modified 3D graphene-Au as a novel sensing layer for direct and sensitive electrochemical determination of carbaryl pesticide in fruit, vegetable, and water samples. Food Anal. Methods 11, 3005–3014.
Ramnani, P., Saucedo, N.M., Mulchandani, A., 2016. Carbon nanomaterial-based electrochemical biosensors for label-free sensing of environmental pollutants. Chemosphere 143, 85–98.
Ramsay, A., Mueller-Harvey, I., 2016. Procyanidins from Averrhoa bilimbi fruits and leaves. J. Food Compos. Anal. 47, 16–20.
Ran, Y., Hammett, K.R., Murray, B.G., 2001. Phylogenetic analysis and karyotype evolution in the genus Clivia (Amaryllidaceae). Ann. Bot. 87, 823–830.
Reina, M., Ruiz-Mesia, W., Ruiz-Mesia, L., Martínez-Díaz, R., González-Coloma, A., 2011. Indole alkaloids from aspidosperma rigidum and A. Schultesii and their antiparasitic effects. Z. Naturforsch. C Biosci. 66, 225–234.
Rønsted, N., Symonds, M.R., Birkholm, T., Christensen, S.B., Meerow, A.W., Molander, M., Mølgaard, P., Petersen, G., Rasmussen, N., Van Staden, J., 2012. Can phylogeny predict chemical diversity and potential medicinal activity of plants? A case study of Amaryllidaceae. BMC Evol. Biol. 12, 182.
Ryding, O., 1995. Pericarp structure and phylogeny of theLamiaceae-Verbenaceae-complex. Plant Systemat. Evol. 198, 101–141.
Santos Tozin, L.R. dos, de Melo Silva, S.C., Rodrigues, T.M., 2016. Non-glandular trichomes in Lamiaceae and Verbenaceae species: morphological and histochemical features indicate more than physical protection. N. Z. J. Bot. 54, 446–457.
Sinopoli, G., Masi, A., Regattieri, E., Wagner, B., Francke, A., Peyron, O., Sadori, L., 2018. Palynology of the last interglacial complex at lake ohrid: palaeoenvironmental and palaeoclimatic inferences. Quat. Sci. Rev. 180, 177–192.
Snijman, D.A., Meerow, A.W., 2010. Floral and macroecological evolution within Cyrtanthus (Amaryllidaceae): inferences from combined analyses of plastid ndhF and nrDNA ITS sequences. South Afr. J. Bot. 76, 217–238.
Stafford, G.I., Wikkelsø, M.J., Nancke, L., Jäger, A.K., Möller, M., Rønsted, N., 2016. The first phylogenetic hypothesis for the southern African endemic genus Tulbaghia (Amaryllidaceae, Allioideae) based on plastid and nuclear DNA sequences. Bot. J. Linn. Soc. 181, 156–170.
Strydom, A., 2005. Phylogenetic Relationships in the Family Amaryllidaceae.
Sukumaran, P., Veettil, V.T., Rajappa, S., Li, C.-Z., Alwarappan, S., 2017. Ionic liquid modified N-doped graphene as a potential platform for the electrochemical discrimination of DNA sequences. Sensor. Actuator. B Chem. 247, 556–563.
Tashkhourian, J., Daneshi, M., Nami-Ana, F., Behbahani, M., Bagheri, A., 2016. Simultaneous determination of hydroquinone and catechol at gold nanoparticles mesoporous silica modified carbon paste electrode. J. Hazard Mater. 318, 117–124.
Vlad, D., Kierzkowski, D., Rast, M.I., Vuolo, F., Dello Ioio, R., Galinha, C., Gan, X., Hajheidari, M., Hay, A., Smith, R.S., Huijser, P., Bailey, C.D., Tsiantis, M., 2014. Leaf shape evolution through duplication, regulatory diversification, and loss of a homeobox gene. Science 343, 780.
Wightman, R.M., 2006. Probing cellular chemistry in biological systems with microelectrodes. Science 311, 1570–1574.
Wightman, R.M., May, L.J., Michael, A.C., 1988. Detection of dopamine dynamics in the brain. Anal. Chem. 60, 769A–793A.
Wink, M., Botschen, F., Gosmann, C., Schäfer, H., Waterman, P.G., 2018. Chemotaxonomy seen from a phylogenetic perspective and evolution of secondary metabolism. Annu. Plant Rev. Online 364–433.
Wu, F., Yu, P., Mao, L., 2017. Bioelectrochemistry for in vivo analysis: interface engineering toward implantable electrochemical biosensors. Curr. Opin. Electrochem. 5, 152–157.
Xu, M., Heidmarsson, S., Olafsdottir, E.S., Buonfiglio, R., Kogej, T., Omarsdottir, S., 2016. Secondary metabolites from cetrarioid lichens: chemotaxonomy, biological activities and pharmaceutical potential. Phytomedicine 23, 441–459.
Yadav, S.K., Agrawal, B., Chandra, P., Goyal, R.N., 2014. In vitro chloramphenicol detection in a Haemophilus influenza model using an aptamer-polymer based electrochemical biosensor. Biosens. Bioelectron. 55, 337–342.
Zeng, Y., Zhu, Z., Du, D., Lin, Y., 2016. Nanomaterial-based electrochemical biosensors for food safety. J. Electroanal. Chem. 781, 147–154.
Zhang, X., Zhou, J., Li, Z., Qin, Y., Yu, R., Zhang, H., Zheng, Y., Zhu, J., Zhang, D., Fu, L., 2019. The qualitative electrochemical determination of multiple components in seaweed fertilizer. Int. J. Electrochem. Sci 14, 6283–6291.