Tobacco Growth Monitoring and Variety Identification Based on Electrochemical Fingerprints

Taige Yu[1#], Xuewei Zhang[2#], Song Gao[3#], Mingfeng Cao[4], Xiao Lei[5], Yang Yang[5], Yuan Liu[6], Li Zhu[4], Binqiang Tian[1], Huizhan Gu[5*], Yaoguang Li[7*]*

[1] College of Tobacco Science, Henan Agricultural University, Zhengzhou, Henan Province, China
[2] Raw Materials Supply Centre of China Tobacco Guangdong Industrial Co.Ltd., Guangzhou, Guangdong Province, China
[3] Technology Center of Hubei Xinye Reconstituted Tobacco Development Co., Ltd., Wuhan, Hubei Province, China
[4] Changde Branch of Hunan Tobacco Corporation, Changde, Hunan Province, China
[5] Sichuan of China National Tobacco Corporation, Chengdu, Sichuan Province, China
[6] Nanyang Branch of Henan Tobacco Corporation, Nanyang, Henan Province, China
[7] Technology Center of China Tobacco Henan Industrial Co.Ltd., Zhengzhou, Henan Province, China
[*]E-mail: tianbqtobacco@sina.com; guhuizhan096@163.com; yaoguangli78@163.com

We used electrochemical fingerprinting technique to identify tobacco leaves in this study. Following transplantation, conventional agronomic characteristics and electrochemical fingerprints of four distinct tobacco plants were obtained. The findings indicated that various types of tobacco leaves had distinct electrochemical signatures. Electrochemical fingerprints obtained under the two circumstances may be utilized to generate a two-dimensional density pattern for quick variety identification. Simultaneously, we discovered that electrochemical fingerprinting could be used to monitor tobacco growth.

1. INTRODUCTION

The key of characteristic high quality tobacco is the reflection of tobacco industry on the properties of tobacco raw materials. Characteristic tobacco is closely related to specific ecological conditions and specific cultivation and modulation techniques [1,2]. Specific ecological conditions are difficult to be changed and imitated, which is the ecological basis for the formation of characteristic tobacco. The cultivation and modulation technology can be learned and imitated, but the cultivation and modulation technology of characteristic tobacco leaves is usually properly coordinated with the local

ecological conditions, forming a set of "characteristic" production technology system [3–5]. The determination of tobacco leaf growing appearance in the field and the diagnosis of fresh tobacco maturity are the basic basis for identifying baking characteristics. Field identification is a purposeful assessment activity based on variety. First of all, we should look at the variety. Any variety of tobacco has its own typical characteristics and basic characteristics [6–11]. The second is to look at the overall growth of tobacco looks, to see whether the growth of the variety looks consistent with its typical performance. The best time to judge the appearance of tobacco plants is around the topping. At this stage, we should focus on the whole leaf color and the expansion degree of the upper leaf. If the leaf color is dark and the upper leaf is long, we should leave the leaf late and at most, and take measures to improve carbon metabolism and tobacco quality and baking characteristics [12–15]. For the tobacco fields with light leaf color and short upper leaves, the top should be hit early, less leaves should be left, and a small amount of nitrogen fertilizer should be applied in time, and phosphorus, potassium and other elements should be combined as appropriate, so as to fully develop tobacco and improve its baking characteristics [16–19]. It is of practical application value to judge the curing characteristics of fresh tobacco leaf according to its texture, because it is a comprehensive reflection of moisture, leaf structure and even chemical composition of tobacco leaf. All the fresh tobacco leaves with soft texture, good elasticity and not easy to be broken in the field are easy to be roasted and have good quality after roasting. On the contrary, the tobacco leaves with hard and brittle quality, poor elasticity and easy breakage are difficult to bake, and the quality is poor after baking [20].

Some characteristics of tobacco leaf can be used to determine variety and quality, such as color, soil plant analysis development (SPAD) value and polyphenol oxidase activity. The color parameters of tobacco leaves with different maturity had obvious correlation with their corresponding pigment content during curing. Therefore, color parameters can be used as auxiliary indicators to judge pigment content. The color index of tobacco leaves has significant correlation with the internal chemical composition of tobacco leaves, and can basically reflect the internal quality of tobacco leaves. There were significant differences in color parameters among different producing areas, varieties, parts and absorption types [21–23]. In recent years, SPAD meter is recommended to be used in field nitrogen application in some European and American countries, mainly to measure the relative content of chlorophyll in plant leaves. This is a new type of portable measuring instrument, which is popularized for its convenience in carrying, easy to use and rapid nondestructive testing. SPAD can be used to quantify the maturity of tobacco leaves. The difference of tobacco leaves with different maturity mainly lies in chlorophyll content [24]. A maturity model based on leaf SPAD value can be used to quantify the maturity of fresh tobacco leaves. Polyphenol oxidase (PPO) is a group of plastid metalloenzymes that catalyze the oxidation of polyphenols to quinones. It is a terminal oxidase that is involved in biological oxidation. The substrate is dehydrogenated under the action of PPO, and hydrogen combines with oxygen to form water, but does not produce energy ATP [25]. The activity of polyphenol oxidase decreased with the growth process and leaf maturity of flue-cured tobacco, and was affected by nitrogen application rate, position and number of leaves. All these indexes need to be considered comprehensively before qualitative research on tobacco leaves can be carried out [26].

Electrochemical fingerprinting is a new chemical analysis technique, which has been widely used in plant investigation [27–33]. Electrochemical fingerprinting can be used to detect electrochemically

active substances in plant samples. This fingerprint can be used not only to identify plant species, but also to monitor plant growth. In this work, we first attempted to collect electrochemical fingerprints of different tobacco varieties with this technique. Secondly, we discussed the feasibility of using this technology to monitor tobacco leaf according to the growth status of tobacco leaf and the change of electrochemical fingerprint.

2. EXPERIMENTAL

The experiments were conducted at Henan Agricultural University in 2020-2021. The tested varieties were Yunyan 97, NC52, NC75 and K320. The experimental plot area is 24 hm^2, and the plot area is 1.33 hm^2. The experiments were a single-factor 6-level randomized block design with 3 replicates. After reaching the transplanting standards, the tobacco seedlings were transplanted on April 7. The soil is paddy soil with uniform fertility. Standardized cultivation managements were carried out in all the tested tobacco fields according to the theory of high-quality tobacco production, and the number of leaves left was 21. The middle leaf (9th to 10th position) and upper leaf (15th to 16th position) were used as experimental materials.

From the 50th day of transplanting, samples were taken from the middle and upper leaves of the flue-cured tobacco varieties every 5 days. The agronomic traits, leaf color value, SPAD value, polyphenol oxidase activity and electrochemical fingerprint were determined. The color parameters of the front side of tobacco leaf were measured by HP-C210 portable precision color meter. SPAD value of leaves was measured by SPAD-502 Plus portable chlorophyll meter. The activity of polyphenol oxidase was determined by chemical titration. The assay was performed using a spectrophotometer mod. Catechol was used as a substrate. The final reaction mixture contained 1.5 mL of catechol (40 mM), 2.3 mL of PBS (0.1 M, pH 6.5), and 0.2 mL of crude enzyme. Changes in the absorbance at 420 nm were monitored for 2 min upon oxidation of the substrates catalysed by the enzyme. One unit of enzyme activity (U) was defined as an increase in absorbance of 0.001 min^{-1}. Enzyme activity was measured in duplicate. The electrochemical fingerprint was recorded by CHI1200C electrochemical workstation. A three-electrode system has been used for electrochemical fingerprint recording, where a commercial glassy carbon electrode (GCE, 3 mm), an Ag/AgCl electrode and a Pt electrode were used as the working electrode, reference electrode and counter electrode, respectively. A small amount of leaf (0.01 g) was carefully mixed with 2 mL of solvent. Then, the slurry was sonicated for 5 min for extraction. Then, 2 μL of plant tissue dispersion was drop coated on the working electrode surface and dried naturally. The voltammetric profile (fingerprints) of plant leaf were recorded using differential pulse voltammetry (DPV) in either PBS (pH 7.0, 0.1 M) or ABS (pH 4.5, 0.1 M).

3. RESULTS AND DISCUSSION

According to Figure 1, by comparing the changes of central leaves of all flue-cured tobacco varieties in the ripening process, it can be seen that the variation trends of leaf length and leaf width of

all varieties are consistent. All varieties increased gradually with the increase of transplanting days, but there were significant differences in values among varieties. NC75 had larger leaf length and width than other cultivars [34]. In comparison, NC52 has shorter leaves than other varieties, and its leaf width is second only to K320. However, the length and width of the middle leaf of K320 were both at a lower level, and the leaf was smaller.
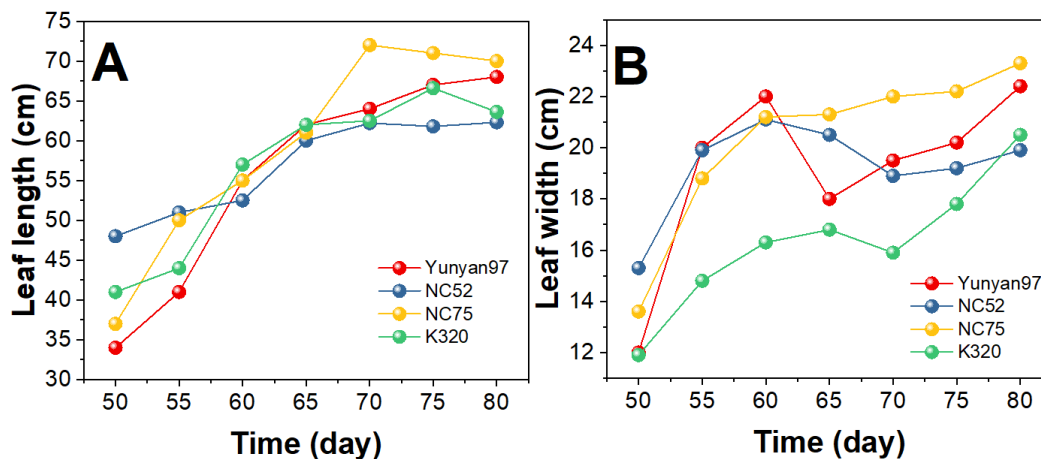
**Figure 1.** (A) Leaf length and (B) leaf width of flue-cured tobacco varieties varied during ripening.

It can be seen from Table 1 that the agronomic traits of all flue-cured tobacco varieties were significantly different when they were harvested at maturity [35]. The height, pitch, waist length and waist width of NC75 were larger than those of the other three varieties. NC52 had lower plant height, stem girth, pitch, waist length and waist width than other cultivars.

Table 1. Main agronomic characters of flue-cured tobacco varieties at maturity (50th day of transplanting).

| Variety	Plant height (cm)	Stem girth (cm)	Pitch (cm)	Waist leaf length (cm)	The waist width (cm)
Yunyan97	114.84	9.35	4.26	70.60	22.39
NC52	93.99	7.87	3.41	64.20	20.05
NC75	112.50	9.17	4.55	72.28	26.05
K320	95.33	8.12	3.91	65.08	20.41

According to Figure 2A and 2B, the activity of polyphenol oxidase in different parts of flue-cured tobacco was in the order of middle leaf > upper leaf. After 50 days of transplanting, the activity of polyphenol oxidase increased gradually with the increase of transplanting days until the maturity of harvest [36]. The polyphenol oxidase activity of NC52 was stronger than that of other flue-cured tobacco

varieties. The polyphenol oxidase activity of K320 was at a low level. The activity of polyphenol oxidase in the middle leaves of Yunyan 97 was higher than that in the upper leaves of Yunyan 97.
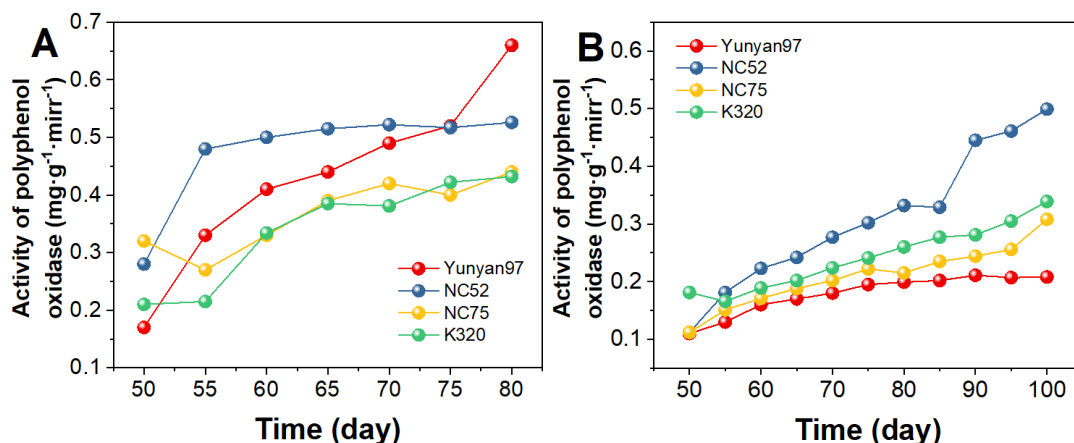

Figure 2. (A) The activity of polyphenol oxidase in the middle leaf of each tobacco variety. (B) the activity of polyphenol oxidase in the upper leaf of each tobacco variety.

SPAD values are used to evaluate the relative content of chlorophyll in the current leaf by measuring the absorption rate of the leaf at two wavelengths (622 nm and 644 nm). As can be seen from Figure 3A and B, chlorophyll relative content of leaves of all flue-cured tobacco varieties increased gradually at first and then decreased gradually from 50 days after transplanting to harvesting [37]. The SPAD value at harvest was lower than that at 50d after transplanting. The SPAD value of middle leaves began to decrease at about 65d after transplanting. The SPAD value of the middle leaf decreased less than that of the upper leaf at about 75 days after transplanting.
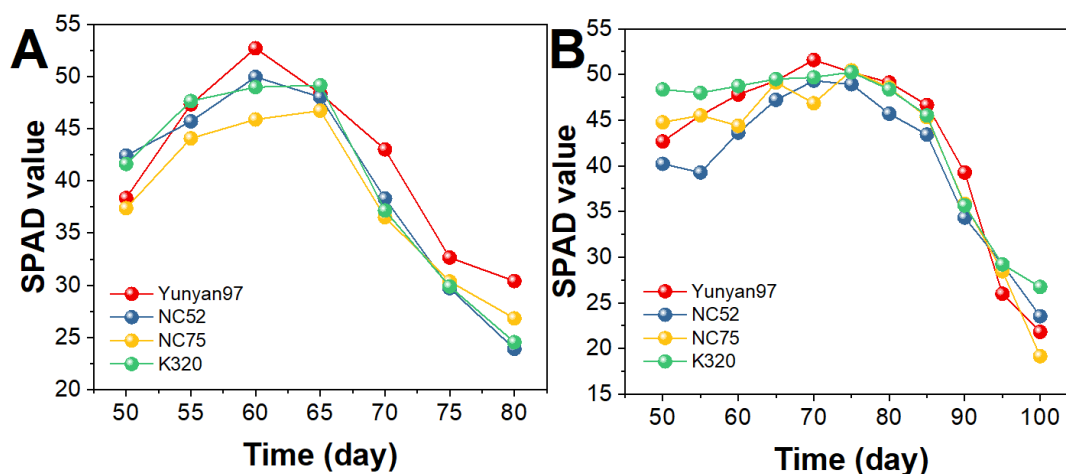
Figure 3. (A) Leaf SPAD value during leaf ripening in the middle of each flue-cured tobacco variety; (B) Leaf SPAD value during leaf ripening in the upper of each flue-cured tobacco variety.
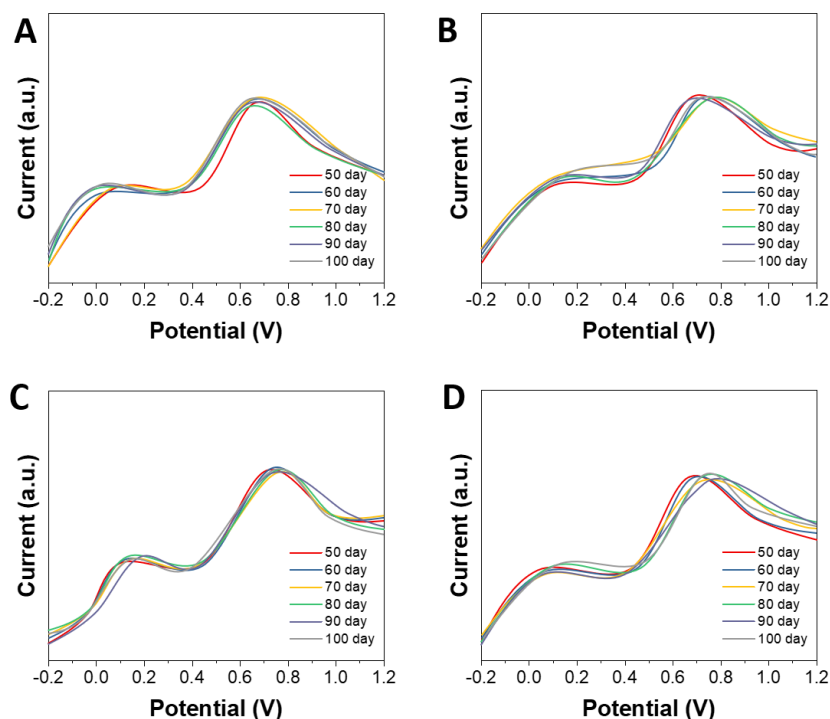
Figure 4 shows the electrochemical fingerprints of four different tobacco leaves over time. The electrochemical fingerprint in this figure was collected under PBS conditions. As can be seen from the figure, all tobacco leaves showed electrochemical activity in the potential window. Some molecules underwent electrochemical oxidation during the scanning process. These may be polyphenols, ascorbic acid and ketones [38,39]. The amount and type of these molecules vary according to the variety of tobacco leaves. As a result, electrochemical fingerprints show different behavior. On the other hand, the fingerprint is not static. The electrochemical fingerprints changed with the time of transplanting. This is due to the change of electrochemically active molecules in different growth stages of tobacco leaves. However, the changes in the electrochemical fingerprints of all samples were not very dramatic. This means that the change of electrochemically active molecules in different varieties of tobacco leaves is not drastic [40]. In this work, these molecules do not need to be identified or quantified because the differences of the total profile are used for plant recognition. Based on these results, electrochemical fingerprinting proved to be a promising technique for tobacco variety identification. The technology could also be used to monitor tobacco leaves.
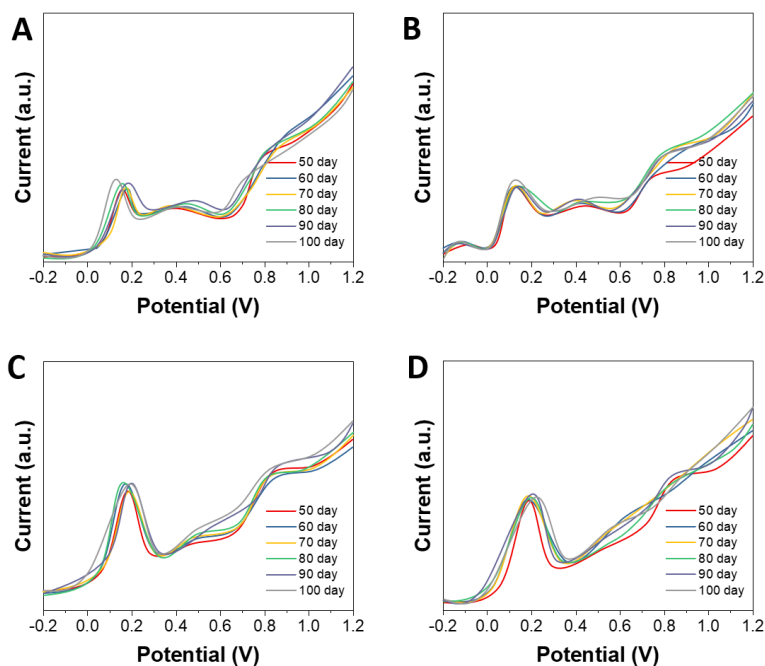
Figure 4. Electrochemical fingerprints of (A) Yunyan 97, (B) NC52, (C) NC75 and (D) K320 recorded under 0.1 M PBS (pH 7.0).

In order to further understand the feasibility of electrochemical fingerprinting technology, DPV curves of four tobacco leaves under ABS were also collected. As shown in Figure 5, the four tobacco leaves also showed electrochemical activity under the condition of acidic buffer solution. Moreover, the electrochemical behavior under such conditions is different from that under PBS. We further conducted MANOVA tests for our data. The p values of the normalized DPV currents recorded for four samples from a same species all larger than 0.05, indicating no significant differences within the samples. However, considerable variables showed p value smaller than 0.05 when comparing different varieties.

Therefore, the electrochemical fingerprints collected under the two conditions can be used for faster variety identification.
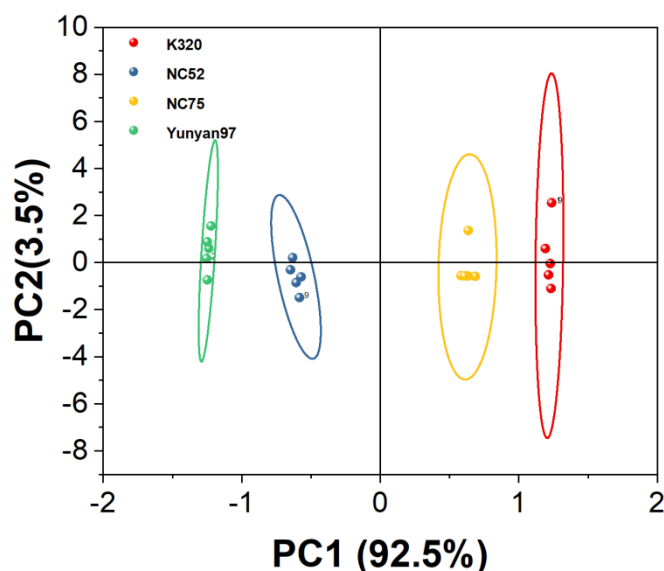

Figure 5. Electrochemical fingerprints of (A) Yunyan 97, (B) NC52, (C) NC75 and (D) K320 recorded under 0.1 M ABS (pH 4.5).

Although it is possible to directly discern species across different varieties or in a small sample size, rapid determination based on multiple electrochemical fingerprints still remains a challenge. Figure 6 shows the 2D density pattern synthesized by using the electrochemical fingerprints collected under the two conditions. It is clear from this pattern that different varieties of tobacco leaf exhibit different hot zones [41]. Therefore, the location of hot zones can be used to identify different varieties of tobacco. However, the human eye cannot accurately recognize from 2D density patterns. For example, there are some similarities between the 2D density patterns of NC52 and NC75. In order to evaluate the feasibility of electrochemical fingerprinting in tobacco identification, five samples were taken from four different tobacco leaves. These data were used for PCA analysis. As shown in Figure 7, the four different tobacco leaves were clustered together separately, and the PCA interpretation was higher than 90%. These results demonstrate that electrochemical fingerprinting is statistically suitable for tobacco identification.
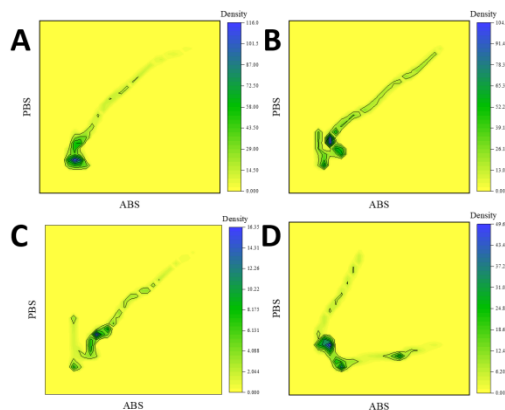
Figure 6. 2D density patterns of (A) Yunyan 97, (B) NC52, (C) NC75 and (D) K320 using data recorded from PBS and ABS conditions.

Figure 7. PCA of electrochemical fingerprints of Yunyan 97, NC52, NC75 and K320 (n=5).

4. CONCLUSION

In this work, we applied electrochemical fingerprinting technology to tobacco leaf identification. Conventional agronomic parameters and electrochemical fingerprints of four different tobacco cultivars were collected after transplanting. The results showed that different kinds of tobacco leaf showed different electrochemical fingerprints. The electrochemical fingerprints collected under the two conditions can be used to synthesize 2D density pattern for rapid variety identification. At the same time, we found that electrochemical fingerprinting can also be potentially used to monitor tobacco growth.

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