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Zonal distribution of neutral aroma components in flue-cured tobacco leaves

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ABSTRACT

To understand the regional distributions of neutral aroma components (NACs) of interest in flue-cured tobacco (*Nicotiana tabacum* L.), samples from a single cured leaf lamina were taken at 48 distinct points with the help of a thin transparent plastic board. We investigated 28 NACs belonging to five categories, namely aromatic amino acid cleavage substances (ACS), cembratriendid alkyl degrading products (CADP), carotenoid degradation products (CDP), chlorophyll degradation products (CDPS), and Maillard reaction components (MRC), by comprehensive two-dimensional gas chromatography (GC × GC–TOFMS) analysis. Geostatistical tools and statistical software were subsequently used to analyze the NAC contents. The results showed that the contents of all the five categories had high levels of significance at 0.01. The nugget effect value of the different NACs exceeded 75%, and the Kriging interpolation map showed a continuous spatial structure. Based on the NAC contents, it was clear that only one principal component had a characteristic value greater than 1 (at 4.9), which accounted for 87.81% of the total variation. With the help of the Management Zone Analyst software, the NACs of a single tobacco leaf were accurately divided into six zones after k-means cluster verification. Overall, these results provide valuable information that will improve the use of tobacco leaves in tobacco threshing and redrying. Moreover, the results suggest the approach described herein can also be used for analyzing the distributions of chemicals in leaves with structures similar to tobacco.

1. Introduction

Tobacco is one of the most widely used plant models by the scientific community. Almost 9600 different types of chemicals have been detected so far in both the tobacco plant and its smoke (Rodgman and Perfetti, 2013). It has been reported that a single tobacco leaf contains many characteristic chemicals such as nicotine, solanesol, fraction 1 protein, as well as a large amount of other valuable chemicals that can used as bioactive or nutritious compounds (Hu et al., 2015; Sheen, 1991). To understand the chemical distribution in tobacco leaves more fully, many scholars have carried out regionalization research on tobacco. The distributions of various inorganic compounds at different positions in the tobacco stalk was studied and summarized for the first time by Tso et al. (1966). Jenkins et al. (1987) extracted 36 round (22 mm diameter) fragments from a single leaf of flue-cured tobacco and performed neutron activation tests to determine the distribution patterns of common metal ions, such as Ca, Mg, Mn, Na, K, C1, and Br. Harold et al. (1992) divided a single tobacco leaf of air-cured KY 171 tobacco into 41 distinct segments and measured and analyzed the nitrosamine, nitrate, and alkaloid contents and their correlations. Using the middle-position leaves of cultivar Hongda from fresh flue-cured tobacco leaves as the test material, Zhang et al. (2009) investigated the differences among the contents of non-volatile organic acids, lignin, soluble total sugars, and nicotine in the major vein, as well as in the primary and secondary lateral veins.

The aroma of tobacco leaves implies an integrated response to their smell, taste, and feel, and is an important reason for smoker enjoyment (Dunkle et al., 2016; Lin et al., 2016; Villanti et al., 2013). The neutral aroma components (NACs) of tobacco mainly consist of volatile and semi-volatile aldehydes, ketones, alcohols, esters (lactones), and alkenes, and they play significant roles in determining the tobacco flavor (Sun et al., 2016; Jenkins et al., 2000). Based on the origins of the aroma substances, they can be classified as volatile and semi-volatile substances and further into carotenoid degradation products (CDP), Maillard reaction components (MRC), and cembratriendid alkyl degrading products (CADP) (Yang et al., 2015; Yun et al., 2013). Several research studies have been conducted on NACs in flue-cured tobacco belonging to different ecological cultivation regions and by using different varieties and curing methods (Yu et al., 2009; Weeks et al., 1992; Barrera and Wernsman, 1966). However, very few studies have

Abbreviations: ACS, Aromatic amino acid cleavage substances; CADP, Cembratriendid alkyl degrading products; CDP, Carotenoid degradation products; CDPS, Chlorophyll degradation products; MRC, Maillard reaction components; MZA, Management zone analyst software; NACs, Neutral aroma components; PC, Principal component; PCA, principal component analysis

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investigated the distributions of aroma substances in a single tobacco leaf.

The geostatistics method is used to analyze the changes in the chemical constituents of plants based on a particular region and between sub-regions; this method includes a class of techniques used to analyze and infer values of a variable distributed in space and/or time (Pinheiro et al., 2016; Alemi et al., 1988). The effect of the spatial variability of the soil's physicochemical properties (such as moisture, permeability rate, humidity, and nutrients) on plant growth have been well investigated using geostatistics methods (Jones, 2016; Yang et al., 2013; Xu et al., 2004). The regional variability of the chemical properties of plants has now become an extensively researched topic (Reyes et al., 2017; Wang, 2016). For instance, the Management Zone Analyst (MZA) software program was developed using a fuzzy *c*-means unsupervised clustering algorithm that assigns field information into like classes or potential management zones (Fridgen et al., 2004). An advantage of MZA over other software programs is that it provides concurrent output for a range of cluster numbers so that the user can evaluate how many management zones should be considered (Shukla et al., 2017; Fleming et al., 2000).

In this study, a simulation model of NACs in a single leaf lamina of flue-cured tobacco is developed to identify the NAC distribution data. Specifically, geostatistics and geographic information system software were used to analyze the regional heterogeneities and patterns of aroma substances from different origins. All the data were then imported to MZA to determine the regional partitioning of NACs in a single tobacco leaf lamina.

2. Results

2.1. Identification of NACs of interest in tobacco

Twenty-eight NACs of interest belonging to five categories, namely, CDP, MRC, CADP, chlorophyll degradation products (CDPS), and aromatic amino acid cleavage substances (ACS), were determined (Croissant et al., 2009; Moßhammer et al., 2006; Martins et al., 2000; Shi and Liu, 1998) (Table 1). With 16 constituents, CDP is the largest class. The types of chemicals detected in our study were similar to those reported previously (Yang et al., 2015; Ding et al., 2013).

2.2. Statistical characteristics of NACs among sampling points

The characteristics of various NACs and their statistical values are listed in Table 2. The variation coefficients of the five types of aroma substances were between 44.94% (ACS) and 66.76% (CADP). The skewness and kurtosis data showed that the different aroma substances had similar contents. They all showed identical peak states; however, CADP had the highest kurtosis. The double-tailed asymptotic probability p in the Kolmogorov-Smirnov normality test was greater than 0.05, indicating that these data obey or approximately obey a normal distribution.

Table 1
Types and categories of NAC tested.

Types	Categories
ACS	benzaldehyde; benzylalcohol; phenylacetaldehyde; phenethyl alcohol
MRC	furfural; furfuryl alcohol; 2-acetyl furan; 5-methylfurfural; 3,4-dimethyl-2,5-furandione; 2-acetyl pyrrole
CDPS	neophytadiene
CADP	solanone
CDP	4-acetyl-3-methoxyphenol; 6-methyl-5-hepten-2-ol; 6-methyl-5-hepten-2-one; linalool; isophorone oxide; (Z)-1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-buten-1-one; dihydrodamascenone; dihydroactinidiolide; megastigmatrienone 1; megastigmatrienone 2; megastigmatrienone 3; megastigmatrienone 4; 3-hydroxy-β-damascone; solavetivone; geranylacetone; farnesyl acetone

Table 3 shows the correlation coefficients among the different NAC types. The correlation coefficient between ACS and CDP was the highest at 0.95, whereas that between CDPS and CADP was the lowest at 0.70. Correlations between all variables had high levels of significance ($P < 0.01$); therefore, PCA could be used to analyze the origin of the variables, thus reducing the dimensionality and hence the complexity of the data.

2.3. NAC semivariance analysis of leaf sampling points

The geostatistical method was used for further analysis of the NAC regional distribution of the tobacco leaf sampling points (Table 4). To ascertain the best model, the semivariance function was used to determine the contents of various NACs based on the rule of the maximum determinant coefficient and minimum residual. Different NACs of a single flue-cured tobacco leaf lamina showed clear spatial variations and good semivariance functions; the nugget effect values of the five types of aroma substances exceeded 75%, showing weak spatial correlations. The different NACs types had inconsistent variation values, ranging from 16.18 cm for CDPS to 54.80 cm for ACS, although they were greater than the maximum sampling spacing variation of 7.0 cm (Fig. 1). Therefore, the positive sill values of the various sampling points indicate that positive base effects resulted from sampling errors, short-range variations, and random as well as inherent variations.

2.4. Regional distribution of various NACs

The Kriging interpolation map of various NAC contents is shown in Fig. 2a–e. As seen, the contents of all NACs were higher in the petiole and lower in the blade tip lengthwise, and they decreased from high values near the main vein to lower values near the leaf edge breadthwise. The entire leaf showed obvious regional variability.

2.5. Analysis of principal components and clustering partition

Because some NACs had higher correlations (Table 3), PCA was used to analyze the clustering conditions of all variables (Table 5). At 4.39, the characteristic value of PC1 was the only PC value that exceeded 1, which accounted for 87.81% of the total variable variance. Given the small values of all other PCA, PC1 can be taken as the dominant result of all PCA.

The statistical results obtained with MZA are shown in Fig. 3. If the curves of both the fuzziness performance index and the normalized classification entropy reached their lowest points, then those numbers can be taken as the final sorting results (Fridgen et al., 2004). This result means that it is reasonable to divide the leaf into six zones. Although MZA is normally applied to soil fertilization it has been successfully used to identify distribution zones of neutral aroma compounds in tobacco leaves. However, further testing must be performed to verify whether or not it is also reasonable for tobacco leaves in this study.

2.6. Rationality of distribution zone classification

The dimensions of all indexes in these tests are expressed as percentages; therefore, no standardization is required for the PCA. The MZA analysis result showed that there are six classification zones; the 48 sampling points of the single tobacco leaf were therefore classified into six different data zones. Using variance analysis, the classification number in the k-means cluster analysis using SPSS 17.0 was also set at six. Table 6 shows that the NACs at the six sampling points had a significance level of 0.05, suggesting that it is feasible to apply the MZA software to the zonal division of NACs in a single tobacco leaf. Zone 1 contained the highest contents of ACS, MRC, CADP, CDPS, and CDP, at 16.44, 24.08, 73.1, 1457.16, and 19.28 μg/g, respectively. The contents of all the constituents showed a decreasing tendency from zone 1 to