Postprint: Effects of Meteorological Factors on the Content of Major Aroma Precursors in High-Aroma Flue-Cured Tobacco Varieties

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Abstract

To investigate the effects of meteorological factors on the content of main aroma precursor substances in high-aroma flue-cured tobacco varieties, using the flue-cured tobacco variety 'Yunyan 87' as a control, the contents of main aroma precursor substances in the high-aroma flue-cured tobacco variety 'Yuyan 11' at five different locations were compared, and grey correlation analyses were conducted between aroma precursor substances and meteorological factors for the two varieties respectively. The research results indicated that the content of main aroma precursor substances in 'Yuyan 11' was significantly higher than that of 'Yunyan 87' at each location. The content of tobacco leaf carotenoids in low-latitude areas was significantly lower than that in high-latitude areas; the content of polyphenolic substances gradually increased with decreasing altitude and increasing sunshine hours during the maturation period; the content of leaf glandular trichome secretions was significantly higher in high-latitude areas than in low-latitude areas overall, with the highest content found in Lushi, Henan. The carotenoid content in tobacco leaves of the two varieties was greatly influenced by light and temperature factors such as sunshine hours during the maturation period and average temperature during the vigorous growth period, while the duration of sunshine hours during the field growth period had a significant impact on the content of polyphenolic substances. The two varieties showed substantial differences in the grey correlation trend between tobacco leaf petroleum ether extract and glandular trichome secretion contents and climatic factors; the petroleum ether extract content in 'Yuyan 11' leaves was greatly affected by sunshine hours during the tobacco growth period, whereas that in 'Yunyan 87' was more influenced by average temperature. The glandular trichome secretion content in 'Yuyan 11' leaves was greatly affected by sunshine hours during the maturation period and average temperature during the vigorous growth period, whereas that in 'Yunyan 87' was more influenced by average temperature during the root extension period, evaporation during the maturation period, and average temperature during the maturation period. When selecting planting regions for the high-aroma flue-cured tobacco variety 'Yuyan 11' , the effects of sunshine hours during the maturation period and average temperature during the vigorous growth period on quality should be fully considered.

Full Text

Effects of Meteorological Factors on Aroma Precursor Contents in High-Aroma Flue-Cured Tobacco Varieties

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Abstract
To investigate the effects of meteorological factors on the main aroma precursor contents in high-aroma flue-cured tobacco varieties, we compared the aroma precursor contents of the high-aroma variety 'Yuyan 11' with those of the conventional variety 'Yunyan 87' at five different locations, and conducted grey correlation analysis between aroma precursors and meteorological factors for each variety. The results showed that the main aroma precursor contents of 'Yuyan 11' were significantly higher than those of 'Yunyan 87' at every location. Carotenoid contents in tobacco leaves were significantly lower in low-latitude regions than in high-latitude regions. Polyphenol contents gradually increased with decreasing altitude and increasing sunshine hours during the maturity stage. Leaf trichome exudates were significantly higher in high-latitude regions overall, with the highest content observed in Lushi, Henan. Carotenoid contents in both varieties were strongly influenced by light and temperature factors such as sunshine hours during the maturity stage and average temperature during the vigorous growth stage, while polyphenol contents were primarily affected by sunshine duration during the field growth period. The grey correlation trends between petroleum ether extract and trichome exudate contents and climatic factors differed substantially between the two varieties. The petroleum ether extract content of 'Yuyan 11' was more strongly influenced by sunshine hours during the growth period, whereas that of 'Yunyan 87' was more affected by average temperature. The trichome exudate content of 'Yuyan 11' was more strongly influenced by sunshine hours during the maturity stage and average temperature during the vigorous growth stage, while that of 'Yunyan 87' was more affected by average temperature during the rooting stage, evaporation during the maturity stage, and average temperature during the maturity stage. When selecting planting areas for the high-aroma flue-cured tobacco variety

'Yuyan 11' , the effects of sunshine hours during the maturity stage and average temperature during the vigorous growth stage on quality should be fully considered.

Keywords: Flue-cured tobacco; High-aroma variety; Meteorological factor; Aroma precursor; Grey correlation

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The content of tobacco aroma substances significantly influences the intrinsic quality of tobacco leaves and determines their aroma characteristics. Most tobacco aroma compounds are secondary metabolites, and their synthesis and accumulation are affected by ecological conditions, variety, and cultivation practices [1-4]. Variety is an intrinsic factor that determines tobacco leaf style and quality; the expression patterns and levels of genes within a variety are influenced by environmental conditions. During tobacco growth, ecological conditions and cultivation measures greatly affect the expression of genes related to carbon and nitrogen metabolism, ultimately influencing tobacco leaf chemical composition and the content and ratio of major secondary metabolites, thereby significantly impacting leaf quality. Extensive research has long been conducted on the relationships between ecological environment [5-7], variety [8], cultivation measures [9], curing [10], and tobacco quality. Among ecological factors, climatic factors have a substantial impact on tobacco quality [11-14], and numerous studies have examined the relationship between climatic factors and tobacco aroma substances, though most have been limited to the relationship between a single climatic factor and aroma compounds in a specific region.

Henan Agricultural University has focused on tobacco leaf trichome secretions as a breakthrough for breeding high-aroma varieties by increasing the content of leaf surface aroma substances [15-17]. With the breeding objectives of high quality, disease resistance, and strong aroma, they developed the high-aroma flue-cured tobacco hybrid 'Yuyan 11' , characterized by abundant aroma and high aroma substance content, though its high-aroma characteristics vary under different ecological conditions. 'Yunyan 87' is currently the most widely planted flue-cured tobacco variety in China' s major tobacco-growing regions, with broad adaptability and excellent leaf quality. Using 'Yuyan 11' and 'Yunyan 87' as materials, this study investigated differences in main aroma precursor contents between the two varieties at five locations with different latitudes, longitudes, and altitudes, and analyzed the grey correlation between meteorological conditions and aroma precursors to provide a basis for matching suitable varieties, environments, and cultivation practices in the production of high-aroma flue-cured tobacco 'Yuyan 11' .

1.1 Experimental Materials

The tested flue-cured tobacco varieties were 'Yuyan 11' and 'Yunyan 87' . 'Yuyan 11' is a high-aroma flue-cured tobacco hybrid bred by Henan Agricultural University and approved by the National Tobacco Variety Approval Committee in 2013. 'Yunyan 87' is currently the main flue-cured tobacco variety cultivated in China' s tobacco-growing regions.

1.2 Experimental Design

The experiment was conducted in 2011 at five tobacco-growing counties with significantly different meteorological conditions. Altitude, latitude, and longitude of each experimental site were measured using a handheld GPS. Geographic information for each site was: Weining County, Bijie, Guizhou (altitude 2,119 m, 103°59 E, 26°45 N); Shibing County, Qiandongnan, Guizhou (altitude 823 m, 108°09 E, 27°09 N); Lushi County, Sanmenxia, Henan (altitude 799 m, 111°09 E, 34°09 N); Neixiang County, Nanyang, Henan (altitude 356 m, 111°94 E, 33°03 N); and Yuzhou City, Xuchang, Henan (altitude 140 m, 113°46 E, 34°20 N).

Soil types and fertility status were determined by sampling before transplanting. Weining had silt loam soil with organic matter 14.18 g·kg ^{1}, alkali-hydrolyzable nitrogen 118.95 mg·kg ^{1}, available phosphorus 45.65 mg·kg ^{1}, and available potassium 244.44 mg·kg ^{1}. Shibing had reddish-brown soil with organic matter 33.00 g·kg ^{1}, alkali-hydrolyzable nitrogen 155.50 mg·kg ^{1}, available phosphorus 44.85 mg·kg ^{1}, and available potassium 233.38 mg·kg ^{1}. Lushi had loess soil with organic matter 25.43 g·kg ^{1}, alkali-hydrolyzable nitrogen 105.43 mg·kg ^{1}, available phosphorus 34.56 mg·kg ^{1}, and available potassium 225.36 mg·kg ^{1}. Neixiang had yellow-cinnamon soil with organic matter 15.58 g·kg ^{1}, alkali-hydrolyzable nitrogen 158.51 mg·kg ^{1}, available phosphorus 35.86 mg·kg ^{1}, and available potassium 158.66 mg·kg ^{1}. Yuzhou had cinnamon soil with organic matter 20.80 g·kg ^{1}, alkali-hydrolyzable nitrogen 130.32 mg·kg ^{1}, available phosphorus 27.43 mg·kg ^{1}, and available potassium 129.53 mg·kg ^{1}.

A large-plot comparative design was used in the field, with each variety planted on at least 667 m^2 at each site at a density of 16,530 plants·hm ^{2}. Pure nitrogen application was 90 kg·hm ^{2} with an N:P O :K O ratio of 1:1.5:3. All fertilizers were inorganic (tobacco-specific compound fertilizer, calcium superphosphate, and potassium sulfate), with calcium superphosphate and potassium sulfate used to supplement phosphorus and potassium deficiencies. Seventy percent of fertilizer was applied as base fertilizer and 30% as top dressing. After harvest and curing using the three-stage curing process, representative plants were selected after the rosette stage, and leaves 1–22 from bottom to top were tagged (fewer leaves were tagged if the plant had fewer). Samples were taken 20 days after topping, with middle leaves (positions 13 and 14) selected for analysis. Some samples were used to determine chlorophyll and leaf surface secretion contents, while others were killed-green for determination of polyphenols, carotenoids, and other compounds.

1.3 Meteorological Data Collection

The selected meteorological factors included average temperature, relative humidity, sunshine duration, rainfall, evaporation, and cloudiness. Ecological factor data were provided by meteorological bureaus of the counties (cities) where the five experimental sites were located (see Table 1). The tobacco growth period from transplanting to leaf maturity was divided into three developmental stages at each site: rooting stage (recovery to rosette), vigorous growth stage (rosette to budding), and maturity stage (budding to middle leaf maturity). The statistical periods for meteorological elements during the rooting stage were late April to mid-May for Shibing, mid-May to early June for Weining, Lushi, and Yuzhou, and early to late May for Neixiang. For the vigorous growth stage, periods were late May to mid-June for Shibing, mid-June to early July for Weining and Lushi, and early to late June for Neixiang and Yuzhou. For the middle leaf maturity stage, periods were late June to mid-July for Shibing, mid-July to early August for Weining and Lushi, and early to late July for Neixiang and Yuzhou.

1.4 Measurement Methods

Chlorophyll and carotenoid contents were determined by spectrophotometry after ethanol extraction [18]. Petroleum ether extracts were measured using the residual method [8]. Extraction, concentration, and quantitative determination of leaf surface secretions followed the methods of Han Jinfeng et al. [19] and Yang Tiezhao et al. [15]. Specifically, 15 circular pieces (10 cm diameter) were cut from the fresh tobacco leaves at symmetrical positions on both sides of the main vein, at the leaf base, middle, and tip. These pieces were extracted three times in 1,000 mL dichloromethane for 2 s each time, with 1 mL internal standard (containing n-heptadecanol) added. The extract was filtered, concentrated using a rotary evaporator, dried with a nitrogen blower, silylated, and then analyzed by GC/MS with NIST library searching for qualitative identification [15].

1.5 Data Analysis

Main aroma precursor content results are presented as means of three replicates. Data were organized using Microsoft Excel 2000 software. DPS software was used for T-tests to analyze differences between varieties and ANOVA for differences among locations, with Duncan' s new multiple range test for multiple comparisons at $P<0.05$ significance level. According to Deng Julong' s grey system theory [20], the 18 meteorological factors (six factors across three growth stages) and main aroma precursor content indicators were treated as the same grey system. Meteorological factors served as comparative sequences, while aroma precursor contents served as reference sequences. Data were standardized, and grey correlation analysis was performed with a resolution coefficient of 0.5 using DPS software [21].

2.1 Differences in Meteorological Factors Among Experimental Sites During Tobacco Growth

The five experimental sites showed regular variation in meteorological factors with altitude and latitude. Altitudes were Weining (2,119 m), Shibing (823 m), Lushi (799 m), Neixiang (356 m), and Yuzhou (140 m). Longitude changed little but increased slightly with decreasing altitude (from 103°59 E to 111°94 E). Latitude was similar between Weining (26°45 N) and Shibing (27°09 N), and among Lushi (34°09 N), Neixiang (33°03 N), and Yuzhou (34°20 N). As shown in Table 1, average temperature and sunshine duration during the rooting, vigorous growth, and maturity stages increased with decreasing altitude and were significantly lower at low-latitude sites than at high-latitude sites. Rainfall and cloudiness decreased with decreasing altitude, while rainfall was higher at low-latitude sites than at high-latitude sites. Average temperature during the vigorous growth stage was below 24°C at Weining and Shibing but above 24°C at Lushi, Neixiang, and Yuzhou. Average temperature during the maturity stage was 17.63°C at Weining and above 26°C at the other four sites. Meteorological factors at the five sites showed regular patterns of variation with altitude and latitude.

Differences in main aroma precursor and trichome exudate contents between the two varieties at different locations are shown in Table 2 . Plastid pigments (chlorophyll and carotenoids) are major components affecting tobacco leaf quality and usability, determining not only the color of cured leaves but also contributing degradation products closely related to aroma quality and quantity [1]. Under the same cultivation conditions, 20 days after topping, chlorophyll and carotenoid contents in middle leaves of 'Yuyan 11' at all five locations were significantly higher than those of 'Yunyan 87' .

Carotenoid contents in both varieties were significantly lower in low-latitude regions (Weining, Shibing) than in high-latitude regions (Lushi, Neixiang, Yuzhou). Polyphenol content is positively correlated with tobacco leaf quality. Polyphenol contents in middle leaves of 'Yuyan 11' were significantly higher than those of 'Yunyan 87' across all five locations, showing a consistent trend of increasing with decreasing altitude and increasing sunshine hours during the maturity stage. Petroleum ether extracts in tobacco contain volatile and non-volatile essential oils, resins, aliphatic hydrocarbons, paraffins, and other substances considered important sources of tobacco aroma, closely related to leaf fragrance and harmony [1]. Except at the Shibing site, petroleum ether extract contents of 'Yuyan 11' were significantly higher than those of 'Yunyan 87' , with the highest content observed in Lushi, Henan.

The main components of tobacco leaf trichome secretions are cembranoid compounds, which are important sources of tobacco aroma [17]. Under the same cultivation conditions, 20 days after topping, GC/MS analysis of the main components of trichome secretions from middle leaves showed that cembratrienediols accounted for over 95% of detected substances. Overall, contents were higher in high-latitude regions than in low-latitude regions, with the highest content in Lushi, Henan, possibly due to higher temperatures during the vigorous growth stage in high-latitude regions. Total trichome exudate content of 'Yuyan 11' was significantly higher than that of 'Yunyan 87', with greater differences in high-latitude regions than in low-latitude regions. Particularly in Lushi, the content in 'Yuyan 11' was 3.8 times that in 'Yunyan 87', consistent with findings from Wang Dong et al. [22–23].

2.3.1 Grey Correlation Analysis Between Carotenoid Content and Meteorological Factors at Different Growth Stages

The grey correlation trends between carotenoid contents and meteorological factors were similar for 'Yuyan 11' and 'Yunyan 87' (Table 3). Based on grey correlation magnitude, the main influencing factors were sunshine hours during the maturity stage, average temperature during the vigorous growth stage, sunshine hours during the rooting stage, evaporation during the maturity stage, average temperature during the maturity stage, evaporation during the vigorous growth stage, sunshine hours during the vigorous growth stage, and average temperature during the rooting stage, all with correlation degrees above 0.71. Light and temperature factors were thus the main causes of carotenoid content differences among regions. Average temperature and sunshine duration during the field growth period were significantly higher at high-latitude sites than at low-latitude sites (Table 1), and carotenoid contents in both varieties were correspondingly significantly higher at high-latitude sites.

2.3.2 Grey Correlation Analysis Between Polyphenol Content and Meteorological Factors at Different Growth Stages

The grey correlation trends between polyphenol contents and meteorological factors were consistent for both varieties (Table 3). Based on grey correlation magnitude, the main influencing factors were sunshine hours during the vigorous growth stage, sunshine hours during the rooting stage, evaporation during the vigorous growth stage, evaporation during the rooting stage, sunshine hours during the maturity stage, with correlation degrees above 0.52. Sunshine duration during the field growth period was thus the main cause of polyphenol content differences, consistent with the observed trend of increasing polyphenol content with decreasing altitude and increasing sunshine hours during the maturity stage.

2.3.3 Grey Correlation Analysis Between Petroleum Ether Extract Content and Meteorological Factors at Different Growth Stages

The grey correlations between petroleum ether extract contents and meteorological factors differed substantially between the two varieties (Table 4). For 'Yuyan 11', the main meteorological factors influencing petroleum ether extract content were, in order of correlation magnitude: evaporation during the rooting stage, sunshine hours during the rooting stage, sunshine hours during the vigorous growth stage, evaporation during the vigorous growth stage, sunshine hours during the maturity stage, average temperature during the vigorous growth stage, and average temperature during the maturity stage. For 'Yunyan 87' , the main climatic factors were: average temperature during the maturity stage, average temperature during the rooting stage, average temperature during the vigorous growth stage, evaporation during the maturity stage, sunshine hours during the maturity stage, cloudiness during the maturity stage, sunshine hours during the rooting stage, and sunshine hours during the vigorous growth stage. Thus, petroleum ether extract content in 'Yuyan 11' was more strongly influenced by sunshine hours during the growth period, whereas in 'Yunyan 87' it was more strongly influenced by average temperature.

2.3.4 Grey Correlation Analysis Between Trichome Exudate Content and Meteorological Factors at Different Growth Stages

The grey correlations between trichome exudate contents and meteorological factors also differed substantially between varieties (Table 4). For 'Yuyan 11' , the main climatic factors were, in order of correlation magnitude: sunshine hours during the maturity stage, average temperature during the vigorous growth stage, sunshine hours during the rooting stage, evaporation during the vigorous growth stage, sunshine hours during the vigorous growth stage, average temperature during the maturity stage, evaporation during the rooting stage, and average temperature during the rooting stage. For 'Yunyan 87' , the main factors were: average temperature during the rooting stage, evaporation during the maturity stage, average temperature during the maturity stage, sunshine hours during the maturity stage, average temperature during the vigorous growth stage, evaporation during the vigorous growth stage, sunshine hours during the vigorous growth stage, and evaporation during the rooting stage. Thus, trichome exudate content in 'Yuyan 11' was more strongly influenced by sunshine hours during the maturity stage and average temperature during the vigorous growth stage, whereas in 'Yunyan 87' it was more strongly influenced by average temperature during the rooting stage, evaporation during the maturity stage, and average temperature during the maturity stage.

3 Conclusions and Discussion

This study investigated differences in main aroma precursor contents between 'Yuyan 11' and 'Yunyan 87' at five locations with different altitudes. Meteorological factors during the tobacco growth period showed regular patterns of variation with altitude and latitude. Carotenoid contents were significantly lower in low-latitude regions than in high-latitude regions. Polyphenol contents gradually increased with decreasing altitude and increasing sunshine hours during the maturity stage. Trichome exudate contents were significantly higher in high-latitude regions overall, with the highest content in Lushi, Henan. The high-aroma variety 'Yuyan 11' had higher main aroma precursor contents than

'Yunyan 87' under all meteorological conditions. These results demonstrate that climatic factors are fundamental conditions for forming tobacco quality characteristics and regional style, representing one of the external determinants of leaf quality. In addition to the direct influence of genetic background on leaf quality, variety factors also indirectly affect quality by influencing the expression patterns and levels of related genes under different ecological conditions during growth and development [2], ultimately affecting tobacco leaf chemical composition and the content and ratio of major secondary metabolites.

The grey correlation trends for carotenoid and polyphenol contents with climatic factors were consistent between 'Yuyan 11' and 'Yunyan 87' , whereas those for petroleum ether extract and trichome exudate contents differed substantially. Carotenoid contents were strongly influenced by light and temperature factors such as sunshine hours during the maturity stage and average temperature during the vigorous growth stage, while polyphenol contents were primarily affected by sunshine duration during the field growth period. Carotenoids are closely related to light energy transfer in photosynthesis and are easily affected by combined factors including light intensity, light quality, temperature, and moisture [24–26]. Numerous studies have confirmed that polyphenol contents increase with light intensity and duration [27]. Petroleum ether extracts and trichome exudates are major leaf surface chemical substances and important sources of tobacco aroma that directly affect leaf quality, with multiple influencing factors [1]. This study found that petroleum ether extract content in 'Yuyan 11' was more strongly influenced by sunshine hours during the growth period, whereas in 'Yunyan 87' it was more influenced by average temperature. Trichome exudate content in 'Yuyan 11' was more strongly influenced by sunshine hours during the maturity stage and average temperature during the vigorous growth stage, whereas in 'Yunyan 87' it was more influenced by average temperature during the rooting stage, evaporation during the maturity stage, and average temperature during the maturity stage. These results indicate that variety-environment interactions substantially affect leaf surface chemical substances. The higher trichome exudate content in 'Yuyan 11' compared to 'Yunyan 87' , particularly the 3.8-fold difference in Lushi, was confirmed by subsequent studies to result from upregulated expression of the key cembratriene-diol synthesis genes CYC-1 and CYP71D16 in 'Yuyan 11' [23,28], with larger diurnal temperature differences and higher light intensity promoting upregulation of CYC-1 and CYP71D16, thereby enabling sufficient synthesis and accumulation of cembratriene-diols [22].

The main aroma precursor contents of the high-aroma flue-cured tobacco variety 'Yuyan 11' were significantly higher than those of the main cultivated variety 'Yunyan 87' under different meteorological conditions. The petroleum ether extract content of 'Yuyan 11' was strongly influenced by sunshine hours during the growth period, while its trichome exudate content was strongly influenced by sunshine hours during the maturity stage and average temperature during the vigorous growth stage. Therefore, when selecting planting areas for 'Yuyan 11' , the effects of these light and temperature factors on main aroma precursor contents and leaf quality should be fully considered.

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Note: Figure translations are in progress. See original paper for figures.