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Effects of Environmental Factors on the Growth and Quality of the Tobacco Variety Zhusha 2: A Case Study in Yunan, China

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The geographical environment can significantly impact the growth and quality of tobacco leaves. Therefore, the objective of this study was to assess the response of a new tobacco variety (Zhusha 2), to different geographical environments and to identify the best site for its cultivation. Field experiments were conducted in 17 different counties in Yunnan using Zhusha 2. The physical properties, chemical components, and quality of the tobacco leaves were determined and assessed. Soil nutrients, pH, organic matter, altitude, precipitation, and monthly average temperature were shown to significantly affect tobacco quality. The physical properties of the tobacco leaves varied significantly between sites, and the chemical components of the tobacco leaves also differed markedly across locations. No significant relationships were found between environmental factors and the sensory quality of tobacco leaves. Soil available nutrient content was significantly and negatively related to appearance quality, but soil pH showed a notable positive relationship with appearance quality. Climatic factors and altitude did not significantly affect appearance quality. Based on the Chinese standard (YC/T 138-1998), the tobacco leaves from Yongren (County) and Gejiu scored the highest with 134.9 out of 150 points for appearance and sensory qualities, followed by those from Shidian, but the leaves from Longyang scored the lowest with 125.8. However, the scores did not differ significantly among the 17 sites. Considering nicotine content, tobacco leaf from the 17 sites were further classified into three clusters: low nicotine leaf, < 0.5 g/kg (Luliang, Shilin, Chuxiong, Yongren), middle nicotine leaf, >0.5 g/kg & < 2 g/kg (Shuangbai, Mile, Shidian, Chengjiang, Longling, Gejiu, Jianshui, Malong, Xuanwei, Shizong), and high nicotine leaf, >2 g/kg (Changning, Tengchong, Longyang). Therefore, based on quality and toxic nicotine content scores, Luliang, Shilin, Chuxiong, and Yongren were identified as more suitable locations for cultivating Zhusha 2.
Keywords: Altitude; Ecological conditions; *Nicotiana tabacum* L; Precipitation; Soil nutrient

INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is believed to be native to South America (Musk and Klerk, 2003) and has spread rapidly worldwide since its discovery. Tobacco is an important economic crop in China (Zheng *et al*., 2020) which has become the leading country in cigarette consumption and production globally (Hu *et al*., 2006; Martins-da-Silva *et al*., 2022). Although smoking is harmful to health (Hecht, 2003; Mackay *et al*., 2013; Smith and Jackson, 2018), cigarettes are still produced and sold worldwide owing to their enormous demand. Therefore, it is necessary to breed better tobacco varieties to reduce toxicity in humans. Additionally, because environmental conditions can affect the synthesis of secondary metabolites, it is possible to reduce toxic matter in tobacco leaves by improving cultivation.

Tobacco is a highly adaptable cash crop that can be grown across a wide area from 60° N to 45° N (Xu *et al*., 2008). Yunnan Province is the most important tobacco production area in China, accounting for approximately 50% of the country's total tobacco leaf yield in China (Tang *et al*., 2020). Owing to its unique climate and geographical environment, Yunnan Province can produce high-quality flue-cured tobacco with a golden color, fragrant aroma, mild effects, and a pure taste (Li *et al*., 2019). To date, the most widely cultivated tobacco varieties include K326、Yunyan87, RG11, and others. During the production of Yunyan 87, researchers discovered a rare cinnabar variant strain, and subsequently developed a new strain, Zhusha 2, using molecular technology (Zhao *et al*., 2023). Zhusha 2 quickly gained popularity due to its high appearance and sensory quality, unique "glutinous rice fragrance" and significant market value. However, varying environmental conditions can significantly affect the growth, yield, and quality of tobacco

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(Tang *et al*., 2020; Zheng *et al*., 2020; Gao *et al*., 2022; Teng *et al*., 2024). For example, the use of potassium-solubilizing bacteria can enhance tobacco growth by increasing the availability of soil potassium (Zhang and Kong, 2014). Nitrogen deficiency can result in the visible pallid coloration of cured leaves; however, excessive nitrogen is detrimental to leaf quality (Soares *et al*., 2020). Precipitation and temperature can significantly affect the quality of tobacco leaves (Tang *et al*., 2020). Variations in altitude can also affect tobacco growth and quality by changing climatic conditions and soil properties (Thomas, 1993; Gao *et al*., 2022).

Only under suitable conditions (climate, soil, and altitude) can a crop achieve its optimal performance. Therefore, for a new tobacco variety, it is essential to conduct multi-point experiments to identify the most suitable production area for achieving high tobacco yield with higher quality. Hence, multipoint field experiments are urgently needed to determine the most suitable location for this special tobacco variety. In this study, Zhusha 2 was cultivated at 17 different sites in Yunnan Province. At harvest, we measured the physical properties and main chemical components (aroma, elements, plant alkaloids, etc.) of the tobacco leaves, and assessed their appearance and sensory qualities. The aim was to determine the effects of environmental factors on tobacco leaf quality and identify the most suitable sites for Zhusha 2 cultivation.

MATERIALS AND METHODS

Experimental sites and tobacco variety: Field experiments were conducted in 17 counties (Luliang, Shilin, Chuxiong, Yongren, Shuangbai, Mile, Shidian, Chengjiang, Longling, Gejiu, Jianshui, Malong, Xuanwei, Shizong, Changning, Tengchong, and Longyang) in Yunnan Province, China, at altitudes ranging from 1270 to 2070 m (Figure 1). The geographical and climatic conditions of each site are listed in Table 1. The basic physicochemical properties of the soil varied significantly across the experimental fields, with the following ranges: organic matter (OM) [17.2, 48.5], available nitrogen (AN) [49.4, 179], available phosphorus (AP) [12.7, 109], available potassium (AK) [93.5, 360], and pH [5.42, 7.32] (Table 2).

Figure 1. The 17 sites of experimental sites for tobacco in Yunnan Province, 2021. Each four-pointed star refers to an experimental site.

Table 1. Climatic and geographic information related to the experimental sites.

Site	Annual precipi-tation (mm)	Monthly lowest temperature (℃)	Monthly highest temperature (℃)	Altitude (m)	North latitude	East longitude
Luliang	1190	15.5	24.2	1850	24°44′~25°18′	103°23′~104°02′
Xuanwei	955	15.0	24.3	2070	25°53′~26°44′	103°35′~104°40′
Malong	1322	14.3	24.0	1860	25°08′~25°37′	103°16′~103°45′
Shizong	850	14.8	24.0	1830	24°20′~25°00′	103°42′~104°34′
Shilin	786	16.8	26.7	1920	24°30'~25°0'	103°9'~103°40'
Chengjiang	1182	21.0	28.5	1730	24°29'~24°55'	102°47'~103°04'
Mile	895	18.0	28.2	1450	23°50′~24°39′	103°04′~103°49′
Gejiu	1138	16.8	22.7	1770	23°01′~23°36′	102°54′~103°25′
Jianshui	768	19.2	27.2	1450	23°12'~24°10'	102°37'~103°11'
Chuxiong	515	17.5	26.5	1910	24°30′~25°15′	100°35′~101°48′
Yongren	850	18.0	28.7	1570	25°51′~26°30′	101°14′~101°49′
Shuangbai	541	16.2	24.2	1460	24°13′~24°55′	101°03′~102°02′
Longyang	1351	17.5	25.8	1700	24°46′~25°38′	98°43′~99°26
Shidian	789	18.2	27.3	1740	24°16'~24°59'	98°54'~99°22'
Tengchong	736	15.2	24.2	1620	24°38'~25°52'	98°05'~98°45'
Longling	973	16.3	23.8	1830	24°07′~24°50′	98°25′~99°11′
Changning	469	15.2	25.2	1270	20°14′~25°12′	99°16′~100°12′
Maximum	1351	21.0	28.7	2070		
Minimum	469	14.3	22.7	1270		
Average	902	16.9	25.6	1704		

Table 2. Basic soil properties for the experimental sites including soil organic matter (OM), available nitrogen (AN), available phosphorus (AP), available potassium (AK), and soil pH.

Site	OM (g/kg)	AN (mg/kg)	AP (mg/kg)	AK (mg/kg)	pH
Luliang	19.8	179.0	109.7	360.0	5.63
Xuanwei	46.3	150.9	23.2	319.8	6.37
Malong	34.4	123.8	31.7	151.3	5.42
Shizong	32.9	118.7	29.9	301.7	6.66
Shilin	17.2	80.9	20.5	197.3	6.48
Chengjiang	21.9	121.1	37.8	294.2	6.21
Mile	30.6	67.8	12.7	144.2	6.51
Gejiu	34.6	49.4	17.3	159.7	7.01
Jianshui	23.7	101.3	25.6	255.4	6.41
Chuxiong	33.9	147.0	30.1	236.0	6.09
Yongren	18.6	93.8	20.7	97.9	6.05
Shuangbai	28.6	142.0	24.6	192.6	6.28
Longyang	26.1	106.0	22.2	197.5	7.32
Shidian	33.4	129.7	26.7	263.8	7.11
Tengchong	48.5	168.5	34.6	93.5	5.79
Longling	32.8	124.2	33.8	193.3	6.18
Changning	28.8	107.3	36.3	191.3	6.09
Maximum	48.5	179.0	109.7	360.0	7.32
Minimum	17.2	49.4	12.7	93.5	5.42
Average	30.4	117.9	34.7	216.0	6.33

The tobacco variety, Zhusha 2, used in the multipoint field experiment, was bred by the Yunnan Academy of Tobacco Agricultural Sciences. This variety was developed through molecular selection of the Yunyan 87 natural mutant strain and was genetically stable. of Zhusha 2 is characterized by a unique "glutinous rice fragrance" minimal harness and irritation, and a pleasant aftertaste.

***Experimental design and management*:** Tobacco seedlings were raised in greenhouses near the experimental sites before the field experiments began. In late March 2020, the field soil was ridged with a line spacing of 120 cm before transplanting. Planting holes (diameter: 30 cm; depth: 20 cm) were dug with a row spacing of 55 cm for transplantation on the ridges in early April. Basic fertilizer (compound fertilizer, N:P_2O_5: K_2O = 12:8:24) was then applied into the hole at a rate of 450 kg/ha. After fertilization, a 10 cm soil layer was placed onto the fertilizer and the tobacco seedlings were transplanted into the holes. Irrigation and pest control were conducted as required.

***Sampling and measurement*:** Tobacco leaf samples were collected prior to harvesting. During the sampling process, ten typical tobacco plants at each site were collected and brought to the laboratory for further analysis. Measurements of the physical properties of the tobacco leaves, such as leaf thickness, leaf density, tensile breaking strength, filling value, balanced moisture content, percentage of the stem, and single leaf weight, were conducted following the method described by Yin et al. (2009). The chloride content of the tobacco leaves was determined using a titration method (Darvishzadeh and Alavi, 2011). The potassium and nitrogen levels in the tobacco leaves were determined using methods described in a previous study (Lu, 1999). Total sugar-reduced sugar and total alkaloid content in tobacco leaf samples were determined using the methods described by Peng et al. (2022). The nicotine, nornicotine, myosmine, neonicotine, and anabazyna levels were determined according to the Tobacco Industry Standards of China (YC-T 383-2010). The aroma content of tobacco leaves was determined following the methods described by Qin et al. (2021).

The appearance and sensory quality of the tobacco leaves were assessed according to the tobacco industry standards of China (YC/T 138-1998). For the appearance quality, ten experts from the China Tobacco Technology Centre in Yunnan Province were invited to assess and score the tobacco leaves sampled from 17 experimental sites. The appearance of tobacco leaves was assessed based on six criteria: color, maturity, oil content, identity, leaf structure, and chroma each rated out of 10 points. Similarly, sensory quality of tobacco leaves was evaluated by 10 experts based on five aspects: aroma quality (25 points), aroma quantity (25 points), miscellaneous smell (15 points), irritation (15 points), and aftertaste (20 points).

***Data statistics*:** Data were managed using Microsoft Excel 2016. Single-factor analysis of variance for different treatments was performed with SPSS 26.0. The graphs were generated using Origin 2021 and R. Duncan's method was employed for multiple comparisons.

RESULTS

***Physical properties of the tobacco leaves*:** The Zhusha 2 tobacco variety exhibited varying performances across the 17 experimental sites, indicating that geographical and climatic conditions significantly impacted tobacco growth (Figure 2). The single-leaf weight ranged from 10.2 g in Shuangbai County to 20.2 g in Shidian County, with an average of 14.6 g. The percentage stems varied from 17.9% in Longling County to 33.2% in Malong County, with an average of 26.6%. Leaf thickness ranged from the 17 sites varied from 0.11 mm (Yongren County) to 0.21 mm in Luliang County with an average of 0.15 mm. The leaf density ranged from 0.72 g/cm³ (Luliang County) to 1.60 g/cm³ (Xuanwei County) with an average of 1.12 g/cm³. Balanced moisture content, varied from 13.4% in Longling County to 16.5 % in Luling County and averaged 14.9 % across 17 sites. The highest tensible breaking strength was 4.02 N at Luliang County, while the lowest one was 2.82 N at Jianshui County, with an average of 3.16 N across the 17 sites. The filling values of tobacco leaf were within the ranges between 2.29 cm³/g (Chuxiong County) and 3.51 cm³/g (Longling County) with an average of 2.76 cm³/g.

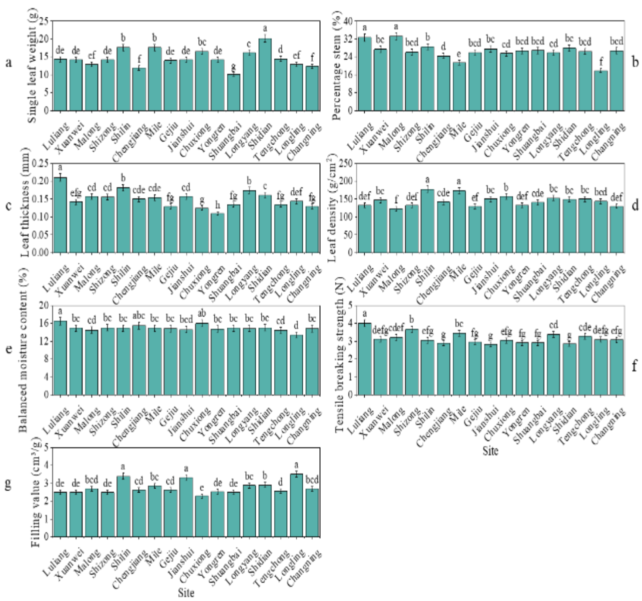

Figure 2. Physical properties of tobacco leaf from 17 experimental sites (a) single leaf weight, (b) percentage stem, (c) leaf thickness, (d) leaf density, (e) balanced moisture content, (f) tensile breaking strength, (g) filling value. Columns labeled with different alphabets indicate significant differences based on the Duncan test at a 5% probability.

***Part of the chemical compositions of the tobacco leaves*:** Total sugar, reducing sugar, total plant alkaloids, nitrogen, potassium, and chlorine in the leaves were significantly different among the 17 sites (**Figure 3**). The total sugar content was highest in tobacco leaves at Yongren (367 g/kg) and lowest at Jianshui (209 g/kg), with an average of 278 g/kg. In contrast, the reducing sugar content ranged from 123 g/kg at Jianshui to 252 g/kg at Gejiu at an average of 197 g/kg for the 17 sites. The total plant alkaloids varied between sites with a range between 6.91 g/kg (Shuangbai) and 17.5 g/kg (Longyang). The nitrogen content in tobacco leaves was highest at Jinashui (28.7 g/kg) but lowest at Yongren (18.9 g/kg) with an average of 24.3 g/kg. Potassium and chlorine contents ranged from 10.2 g/kg (Chengjiang) to 22.3 g/kg (Shidian) and from 0.87 g/kg (Shilin) to 11.2 g/kg (Gejiu), respectively.

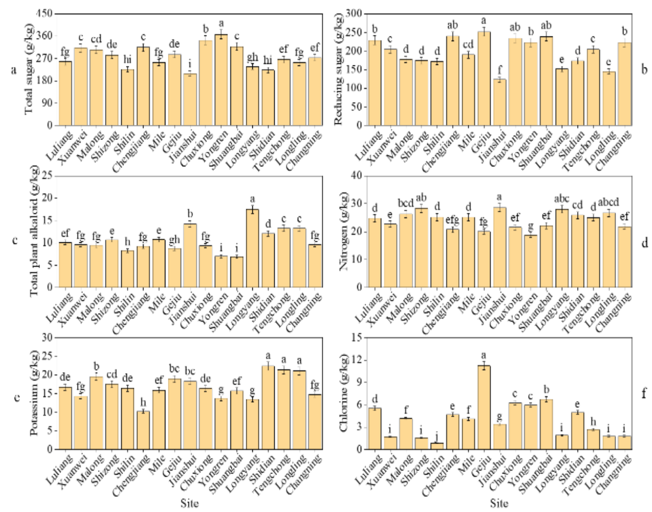

Figure 3. Chemical compositions of the tobacco leaf from 17 experimental sites, including (a) total sugar, (b) reducing sugar, (c) total plant alkaloid, (d) nitrogen, (e) potassium, and (f) chloride. Columns labeled with different alphabets show significant differences according to the Duncan test at 5% probability.

***Aroma contents of the tobacco leaves*:** Aroma content is crucial for tobacco leaf quality. Six key aroma components were analyzed (Figure 4). The neophytadiene content of tobacco leaves was highest at Yongren (1787 μg/g) and lowest at Jianshui (843 μg/g). Caryophyllene oxide ranged from 6.26 μg/g to 153 μg/g with an average of 90.5 μg/g across the 17 sites. Solanone contents in tobacco leaves for 17 sites varied between 6.25 μg/g and 55.2 μg/g, averaging 30.6 μg/g. The highest hegastigmatrienone content was found at Mile (73.3 μg/g) and the lowest was determined in the leaves collected from Shizong (27.9 μg/g). The content ranges of β-Damascone ranged from 1.61 μg/g (Changning) to 28.4 μg/g at Longling and phytol ranged from 1.74 μg/g (Changning) to 20.6 μg/g at Longling.

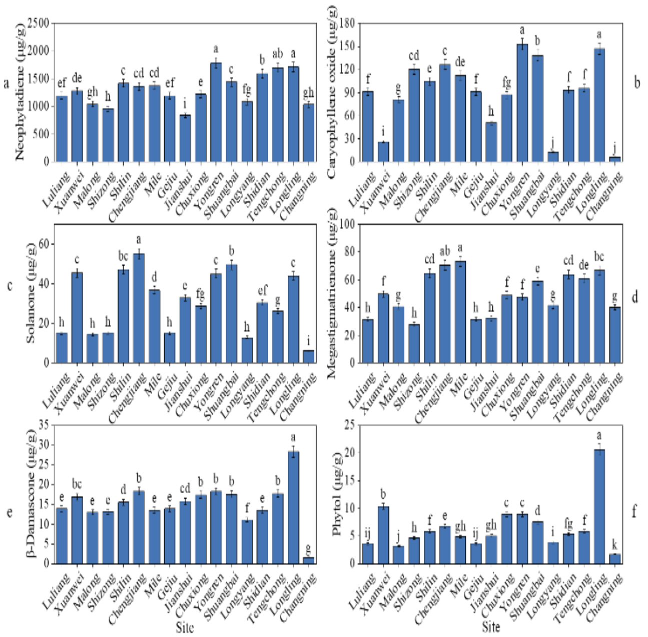

Figure 4. Aroma contents of the tobacco leaf from 17 experimental sites including (a) neophytadiene, (b) caryophyllene oxide, (c) solanone, (d) hegastigmatrienone, (e) β-Damascone, (f) phytol. Columns labeled with different alphabets show significant differences according to the Duncan test at 5% probability.

Toxic components of the tobacco leaves**:** Tobacco quality is influenced not only by favorable components but also by toxic components. Five types of toxic matter present in tobacco leaves from 17 sites were analyzed (Figure 5). Nicotine, the most well-known toxic matter in tobacco, ranged from 0.21 g/kg (Chuxiong) to 4.03 g/kg (Longyang) with an average of 1.19 g/kg. Nornicotine in the tobacco leaves varied from 3.18 g/kg (Chengjiang) to 9.03 g/kg (Jianshui) with an average of 6.03 g/kg. The myosmine of tobacco leaves for 17 sites varied from 0.06 g/kg (Changning) to 0.12 g/kg (Longling) with an average of 0.09 g/kg. Neonicotine and anabazyna in tobacco leaves ranged from 0.23 g/kg (Chengjiang) to 0.85 g/kg (Shidian) and from 0.05 g/kg (Chengjiang) to 0.17 g/kg (Shidian), respectively.

Scores of the tobacco leaves**:** The appearance and sensory qualities were assessed and scored by the experts (Figure 6). For the appearance, the highest score was 55.0 points for the tobacco leaves from Shizong while the lowest score was 47.0 points for Changning and Luliang and the average point for the appearance quality of tobacco leaves was 51.2 points. The sensory quality scores ranged from 78.0 points (Shuangbai, Tengchong, and Longling) to 81.0 points (Chuxiong), with an average of 79.4 points. Summing these two scores they were awarded to Shizong and Chuxiong, received the highest total points, whereas Changning received the lowest score.

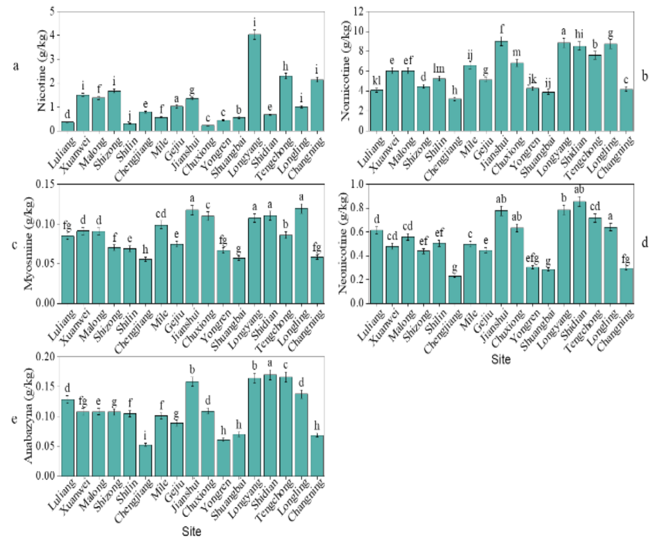

Figure 5. Toxic components of the tobacco leaf from 17 experimental sites including (a) nicotine, (b) nornicotine, (c) myosmine, (d) neonicotine, (e) anabazyna. Columns labeled with different alphabets show significant differences according to the Duncan test at 5% probability.

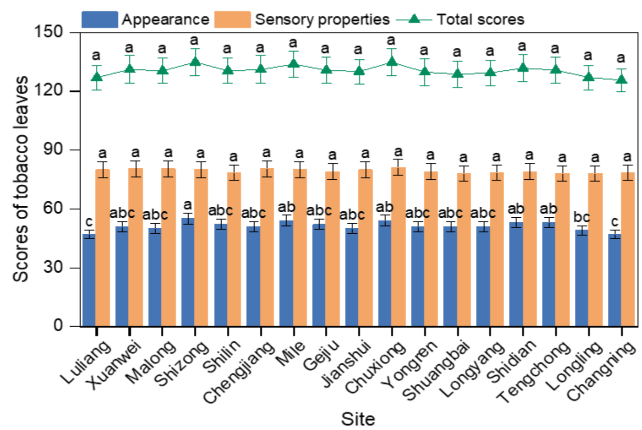

Figure 6. Scores for the appearance and sensory properties of the tobacco leaf from 17 experimental sites. Different alphabets indicate significant differences according to the Duncan test at 5% probability.

DISCUSSION

Effects of climatic and geographical conditions on tobacco quality**:** Environmental conditions are crucial for plant growth and reproduction (Gao *et al*., 2022). Altitude changes can lead to differences in climatic conditions and soil properties (Hauggaard-Nielsen *et al*., 2009; Parra-Coronado *et al*., 2015) which can affect tobacco growth (Liao *et al.*, 2024). In the present study, soil nutrients and organic matter

(OM) showed a positive correlation with altitude (Figure 7), which is in agreement with a previous report (Wang and Song, 2013). Higher OM and nutrient contents can usually benefit the growth of tobacco. This may be caused by the lower temperatures, which decrease the decomposition speed of biological residues by the microbiome and reduce the uptake of nutrients by plants. Precipitation was also positively correlated with altitude, whereas the monthly lowest/highest temperatures were negatively correlated with altitude. The rainfall required for tobacco growth is approximately 400 mm-600 mm (Xie *et al*., 2019). All 17 sites with rainfall ranging from 469 mm to 1351 mm, are suitable for tobacco cultivation suitable and can benefit tobacco leaf quality.

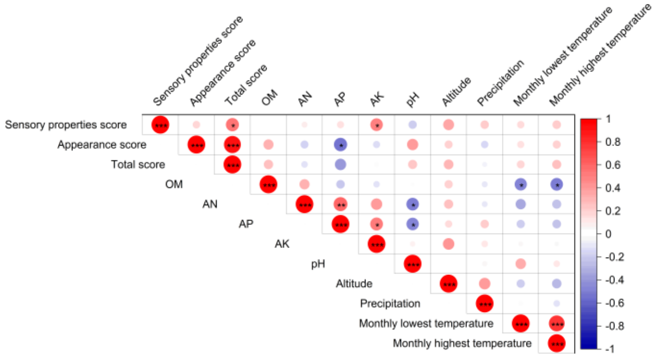

Figure 7. Correlation analysis of environmental factors with tobacco leaf scores. OM, AN, AP, and AK refer to organic matter, available nitrogen, available phosphorus, and available potassium, respectively. Blue circles indicate negative correlations and red circles indicate positive correlations. The larger circles mean stronger correlations; *, ** and * refer to significance at 0.05, 0.01, and 0.001 levels, respectively.**

Because nutrient supply, soil structure, temperature, and precipitation are critical factors influencing crop growth (Yang *et al*., 2022; Yuan *et al*., 2022; Zhao *et al*., 2022), these factors collectively affected tobacco leaf quality at the 17 sites in this study. For example, potassium is essential for both plant growth and quality (Zörb *et al*., 2014; Zhao *et al*., 2019), and the high potassium (K) concentration in tobacco leaves can alleviate the appearance and burning properties, thereby improving market value and farmers' incomes (Lu *et al.*, 2017). Given the tobacco leaf scores, higher available nitrogen (AN), available phosphorus (AP), and available potassium (AK) contents might be harmful to the quality of tobacco appearance. Conversely, higher levels of AK and latitude are associated with improved sensory quality of tobacco leaves.

The physical properties of the tobacco leaves are closely related to their quality. Factors such as filling value, balanced moisture content, and stem percentage can also influence the cigarette manufacturing process, product style, cost, and other economic factors (Yin *et al.*, 2009). For instance, a higher filling value can reduce the number of tobacco leaves needed per cigarette box, leading to improved combustion and less tar production. Thus, it can lower the cost, suggesting that a higher filling value has dual economic and safety values. Natural environmental conditions form the ecological basis for the production of high-quality tobacco leaves (Tang *et al*., 2022). Correlation analysis revealed that the appearance of tobacco leaves was significantly related to soil and climatic conditions (Figure 8). Single-leaf weight was negatively correlated with AN and AP, but positively correlated to soil pH, altitude, and monthly highest and lowest temperatures. Conversely, the percentage of stems was positively related to AN, AP, AK, altitude, and precipitation but negatively related to soil pH and monthly lowest/highest temperatures. There was a positive relationship between leaf thickness and AP, AK, altitude, and precipitation, while it was negatively related to leaf thickness, soil OM, and the lowest annual temperatures. Leaf density values were positively correlated with OM but negatively correlated with AP, AK, altitude, and precipitation. Soil AN, AP, and AK were positively correlated with balanced moisture content. Tensible breaking strength was positively related to AN, AP, AK, altitude, and precipitation, but negatively related to soil pH and monthly highest and lowest temperatures. The filling value was negatively related to OM, AN, AP, and AK, but positively related to soil pH and the highest and lowest monthly temperatures.

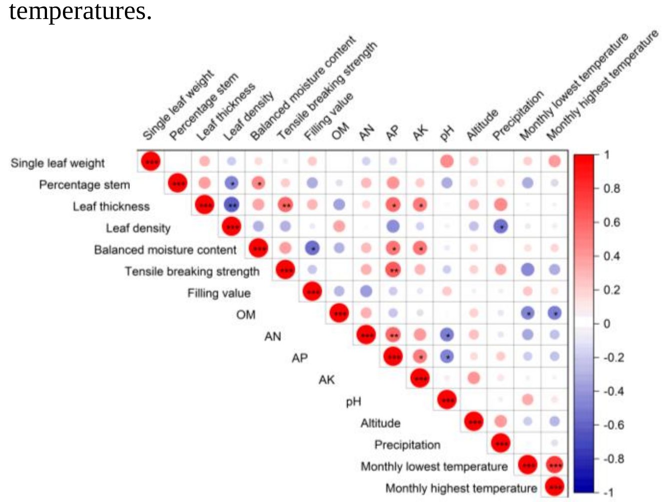

Figure 8. Correlation analysis of environmental factors with physical properties of tobacco leaf. OM, AN, AP, and AK refer to organic matter, available nitrogen, available phosphorus, and available potassium, respectively. The blue circle indicates negative correlations and the red circle indicates positive correlations. The larger circles mean stronger correlations; *, ** and * refer to significance at 0.05, 0.01, and 0.001 levels, respectively.**

Soil OM was negatively correlated with caryophyllene oxide and solanone, but positively correlated with potassium in tobacco leaves, nicotine, nornicotine, myosmine, neonicotine, and anabazyna (Figure 9). AN showed no significant correlation with the chemical components of the tobacco leaves. Soil AP was negatively correlated with megastigmatricenone, and soil AK was negatively correlated with neophytadiene and megastigmatricenone. Soil pH was negatively related to caryophyllene oxide, total sugar, and reducing sugar but positively related to nitrogen in tobacco leaves, total plant alkaloids, nicotine, nornicotine, myosmine, neonicotine, and anabazyna. Similar to AN, altitude showed no significant correlation with the chemical components of tobacco leaves. Precipitation was negatively correlated with neophytadiene, caryophyllene oxide, solanone, and megastigmatrienone but positively correlated with nitrogen, total plant alkaloids, and nicotine in tobacco leaves. The lowest and highest temperatures were significantly positively correlated with solanone and megastigmatricenone levels but were negatively correlated with total potassium in tobacco leaves.

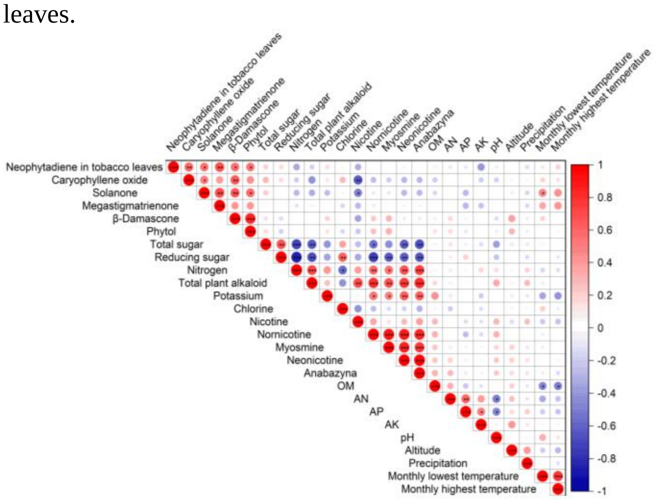

Figure 9. Correlation analysis of environmental factors with the physical properties of tobacco leaf. OM, AN, AP, and AK refer to organic matter, available nitrogen, available phosphorus, and available potassium, respectively. The blue circle indicates negative correlations and the red circle refers to positive correlation. The larger circles mean stronger correlations; *, ** and * refer to significance at 0.05, 0.01, and 0.001 levels, respectively.**

***The most suitable site to produce Zhusha 2*:** High-quality tobacco production generally requires soil with the following characteristics: pH ranges from 5.6 to 6.5 (or from 5.0 to 7.0), OM ranges from 10 to 20 g/kg, AN ranges from 45 to 135 mg/kg, AP ranges from 10 to 35 mg/kg, AK ranges from 120 to 200 mg/kg (Hu et al., 2014; Que *et al*., 2019). Therefore, the soil pH at the experimental sites was not a limiting factor for tobacco at most of the 17 sites. Soil OM is one of the most important indices of soil fertility; it is favorable for improving the soil's physical, chemical, and biological properties (Yang *et al.*, 2019; Jiang *et al*., 2022). The soil OM of the experimental sites was within the suitable range or even higher; hence, the test soils were more fertile than those required by tobacco. Nitrogen, phosphorus, and potassium are necessary macronutrients for plants (Li *et al*., 2022; Song *et al*., 2022; Tang *et al*., 2022; Yuan *et al*., 2022; Zhao *et al*., 2022). Similar to OM, AN, and AP at all sites, tobacco growth was satisfied. Apart from the AK in Yongren and Tengchong, the AK at the other sites was also within the suitable range. The above analysis suggests that the soil at all experimental sites can be used for tobacco production.

Sufficient water and suitable temperatures are crucial for tobacco growth and development (Xu *et al*., 2008). Both excessively low and high temperatures can lead to yield loss and reduced quality of tobacco leaves (Blümel *et al*., 2015; Tang *et al*., 2020). The optimal temperature for flue-cured tobacco production ranges from 18℃ to 28℃ throughout the growing period. However, the ideal temperature for the transplanting period can vary between locations. For instance, Xu *et al.* (2008) found that 15℃ is suitable for transplanting in regions such as Xianfeng, Xiangyang, and Zaoyang, in Hubei Province. In this study, the transplanting temperature ranged from 14.3℃ to 21℃, which was also not a limiting factor affecting tobacco growth. Precipitation is a crucial environmental factor that affects tobacco growth (Wu *et al*., 2013), with both leaf yield and quality being closely related to this factor (Tang *et al*., 2019). The diverse climatic and topographical conditions lead to great variations in precipitation at different sites in Yunnan Province (Thomas, 1993). Annual precipitation at the 17 sites varied enormously, indicating that moisture is an important factor influencing tobacco growth and leaf quality. Correlation analysis revealed a positive relationship between the sensory quality of tobacco leaves and precipitation. Variations in altitude can lead to significant differences in light, temperature, water, and heat resources, as well as changes in soil types and physicochemical properties, all of which impact the quality of tobacco leaves (Wang and Song, 2013; Gao *et al*., 2022). In the present study, as altitude increased, precipitation, and soil nutrients, OM increased, whereas the monthly average temperature decreased. Therefore, the altitude can affect the quality of tobacco leaves by altering other environmental conditions.

Tobacco leaves are mainly used to produce cigarettes, and it is well known that cigarette smoking is harmful to health" (Hecht, 2003; Mackay *et al*., 2013). Therefore, it is crucial to decrease the potentially toxic effects of cigarettes, such as nicotine, nornicotine, myosmine, neonicotine, and anabazyna. Clustering analysis divided the tobacco leaves from the 17 sites into three categories based on nicotine content: low nicotine tobacco leaf, < 0.5 g/kg (Luliang, Shilin, Chuxiong, Yongren), middle nicotine tobacco leaf, >0.5 g/kg & < 2 g/kg (Shuangbai, Mile, Shidian, Chengjiang, Longling, Gejiu, Jianshui, Malong, Xuanwei, Shizong), and high nicotine tobacco leaf, >2 g/kg (Changning, Tengchong, Longyang). Based on comprehensive analysis, Luliang, Shilin, Chuxiong, and Yongren identified the most suitable sites for cultivating Zhusha2.

***Conclusions*:** This study primarily focused on the effects of environmental factors on tobacco leaf quality and the suitability of 17 experimental sites for cultivating the new tobacco variety Zhusha2. Based on the results of this study, it can be concluded that altitude was the most influential factor affecting tobacco leaves as it impacted precipitation and temperature. Soil properties showed no significant differences in the tobacco leaves across the experimental sites. Based on the appearance and sensory qualities, as well as the content of the most toxic matter—nicotine, Luliang, Shilin, Chuxiong, and Yongren–were identified as the most suitable sites for cultivating Zhusha2.

Conflict of Interest: The Authors declare that there is no conflict of interest.

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REFERENCES

Blümel, M., N. Dally, C. Jung. 2015. Flowering time regulation in crops-what did we learn from Arabidopsis? Current Opinion in Biotechnology 32:121-129.

Darvishzadeh, R., R. Alavi. 2011. Genetic analysis of chloride concentration in oriental tobacco genotypes. Journal of Plant Nutrition 34:1070-1078.

Gao, L., J.M. Gao, R. Wang, X.H. Ren, J. Fan, G.M. Shen. 2022. Effect of altitude on the release of metabolites in the tobacco rhizosphere soil. Eurasian Soil Science 55: 1786-1793.

Hauggaard-Nielsen, H., M. Gooding, P. Ambus, G. Corre-Hellou, Y. Crozat, C. Dahlmann, A. Dibet, P. von Fragstein, A. Pristeri, M. Monti, E.S. Jensen. 2009. Pea–barley intercropping for efficient symbiotic N_2-fixation, soil N acquisition and use of other nutrients in European organic cropping systems. Field Crops Research 113: 64-71.

Hecht, S.S. 2003. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. Nature Reviews Cancer 3: 733-744.

Hu, L., L. Zhou, J. Wang, X. Zhang, T. Wang, Y. Pan, X. Shan. 2014. Comprehensive evaluation of soil fertility in tobacco-growing areas in Yunnan Province. Journal of Henan Agricultural Sciences 43: 52-59 (in Chinese).

Hu, T.W., Z. Mao, M. Ong, E. Tong, M. Tao, H. Jiang, K. Hammond, K.R. Smith, J. de Beyer, A. Yurekli. 2006. China at the crossroads: the economics of tobacco and health. Tobacco Control 15: i37-i41.

Jiang, Y., R. Zhang, C. Zhang, J. Su, W. Cong, X. Deng. 2022. Long-term organic fertilizer additions elevate soil extracellular enzyme activities and tobacco quality in a tobacco-maize rotation. Frontiers in Plant Science 13: 973639.

Li, H., X. Du, C. Yuan, C. Zhou, Q. Yang, J. Chen, Z. Gan. 2022. Effects of potassium fertilizer reduction combined with supplement of polyaspartic acid on soil physicochemical properties, potassium absorption and utilization of flue-cured tobacco. Tobacco Science & Technology 1-15 (in Chinese).

Li, Y., K. Ren, C. Zou, J. Xie, X. He, Y. Chen, B. Hu, J. Shen, X. Hu, J. Chen, Z. Xia, Y. Wu, Y. Jin. 2019. Effects of ferrous iron toxicity on agronomic, physiological and quality indices of flue-cured tobacco. Agronomy Journal 111: 2193-2206.

Liao, R., Z. Liu, W. Dongchen, X. Deng, E. Ma, N. Manzoor, C. Lin, S. Zhou, W. Tong, M. Zhou, J. Li, Z. Mao. 2024. Integrated metabolomic and metagenomic strategies shed light on interactions among planting environments, rhizosphere microbiota and metabolites of tobacco in Yunnan, China. Frontiers in Microbiology 15: 1386150.

Lu, D., W. Huan, T. Yan, Y. Wang, C. Jiang, X. Zhang, X. Chen, H. Wang, C. Zu, J. Zhou. 2017. A heterogeneous potassium supply enhances the leaf potassium concentration of ridge-cultivated tobacco grown in calcareous soil. Journal of Plant Nutrition and Soil Science 180: 659-666.

Lu, R. 1999. Analytical methods of soil agrochemistry. China Agricultural Science and Technology Press, Beijing, China (in Chinese).

Mackay, J., B. Ritthiphakdee, K.S. Reddy. 2013. Tobacco control in Asia. Lancet 381: 1581-1587.

Martins-da-Silva, A.S., J. Torales, R.F.V. Becker, H.F. Moura, M.W. Campos, T.M. Fidalgo, A. Ventriglio, J.M. Castaldelli-Maia. 2022. Tobacco growing and tobacco use. International Review of Psychiatry 34: 51-58.

Musk, A.W., N.H.D. Klerk. 2003. History of tobacco and health. Respirology 8: 286-290.

Parra-Coronado, A., G. Fischer, J.H. Camacho-Tamayo. 2015. Development and quality of pineapple guava fruit in two locations with different altitudes in Cundinamarca, Colombia. Bragantia 74:359-366.

Peng, Y., Y. Bi, L. Dai, H. Li, D. Cao, Q. Qi, F. Liao, K. Zhang, Y. Shen, F. Du, H. Wang. 2022. Quantitative analysis of routine chemical constituents of tobacco based on thermogravimetric analysis. ACS Omega 7: 26407-26415.

Qin, G., G. Zhao, C. Ouyang, J. Liu. 2021. Aroma components of tobacco powder from different producing areas based on gas chromatography ion mobility spectrometry. Open Chemistry 19:442-450.

Que, J., Z. Tang, X. Li, Z. Chen, L. Chen, Z. Ren, B. Wang, F. Ni, Q. Lu, Y. Fu, K. Huang, Z. Li, T. Xia. 2019. Analysis of soil pH and organic matter of tobacco-planting soil in Mile City in Honghe Prefecture. Southwest China Journal of Agricultural Sciences 32: 1633-1638 (in Chinese).

Smith, M.A., A. Jackson. 2018. Tobacco use, tobacco cessation and musculoskeletal health. Orthopaedic Nursing 37: 280-284.

Soares, T.d.M., F.S. Coelho, V.B. de Oliveira, O. Pontes, P.S. Pavinato. 2020. Soil nitrogen dynamics under tobacco with different fertilizer management in southern Brazil. Geoderma Regional 21: e00282.

Song, H., X. Chen, L. Cheng, D. Lu, H. Wang. 2022. Negligible ammonia volatilization loss with one-time root-zone targeted application of common nitrogen, phosphorus and potassium fertilizers in transplanted rice cultivation. Agriculture, Ecosystems & Environment 338: 108072.

Tang, F., Y. Yao, J. Song, C. Wang, Y. Liu. 2022. Interactive influence of soil erosion and cropland revegetation on soil enzyme activities and microbial nutrient limitations in the Loess Hilly-Gully Region of China. Agronomy 12: 2796.

Tang, H., J. Wang, X. Li, Z. Zeng, L. Liu, L. Liu, H. Yan. 2019. The effect of water-nitrogen coupling on growth, yield and quality of flue-cured tobacco. Journal of Irrigation and Drainage 38: 17-24 (in Chinese).

Tang, Z., L. Chen, Z. Chen, Y. Fu, X. Sun, B. Wang, T. Xia. 2020. Climatic factors determine the yield and quality of Honghe flue-cured tobacco. Scientific Reports 10: 19868.

Teng, L., G. Jiang, Z. Ding, Y. Wang, T. Liang, J. Zhang, H. Dai, F. Cao. 2024. Evaluation of tobacco-planting soil quality using multiple distinct scoring methods and soil quality indices. Journal of Cleaner Production 441: 140883.

Thomas, A. 1993. The onset of the rainy season in Yunnan province, PR China and its significance for agricultural operations. International Journal of Biometeorology 37: 170-176.

Wang, E., W. Song. 2013. Analysis on soil nutrients difference at different altitudinal in tobacco soil. Modern Agricultural Science and Technology 226-227, 231 (in Chinese).

Wu, W., X. Tang, C. Yang, H. Liu, N. Guo. 2013. Investigation of ecological factors controlling quality of flue-cured tobacco (*Nicotiana tabacum* L.) using classification methods. Ecological Informatics 16: 53-61.

Xie, Z., X. Shao, W. Duan, H. Li, H. Shi. 2019. Research and application advances in fertigation technology in flue-cured tobacco production. Soils 51: 235–242.

Xu, Z., Y. Li, Q. Bi, J. He, J. Li. 2008. Evaluation on climatic feasibility of tobacco-growing areas in Hubei and its similarity to that of foreign tobacco-growing areas. Acta Ecologica Sinica 28: 3832-3838 (in Chinese).

Yang, P., P.J. Drohan, H. Long, M. Yang, Y. Bian, E. Ma. 2022. Water use efficiency, yield and quality of tobacco (*Nicotiana tabacum* L.) using negative pressure irrigation. Industrial Crops and Products 178: 114552.

Yang, X., X. Chen, X. Yang. 2019. Effect of organic matter on phosphorus adsorption and desorption in a black soil from Northeast China. Soil and Tillage Research 187: 85-91.

Yin, Q., Y. Zhang, C. Xue, G. Wang, J. Xi. 2009. Physical characteristics and their regional variation in China's flue-cured tobacco leaf. Acta Tabacaria Sinica 15: 33-38 (in Chinese).

Yuan, Y., S. Gai, C. Tang, Y. Jin, K. Cheng, M. Antonietti, F. Yang. 2022. Artificial humic acid improves maize growth and soil phosphorus utilization efficiency. Applied Soil Ecology 179: 104587.

Zhang, C., F. Kong. 2014. Isolation and identification of potassium-solubilizing bacteria from tobacco rhizospheric soil and their effect on tobacco plants. Applied Soil Ecology 82: 18-25.

Zhao, X., S. Gao, D. Lu, X. Chen, G. Yuan, H. Wang. 2022. Grain yield and soil potassium fertility changes arising from different potassium-bearing materials in rice–wheat rotation. Nutrient Cycling in Agroecosystems 124:117-129.

Zhao, X., S. Gao, D. Lu, H. Wang, X. Chen, J. Zhou, L. Zhang. 2019. Can potassium silicate mineral products replace conventional potassium fertilizers in rice–wheat rotation? Agronomy Journal 111:2075-2083.

Zhao, X., M. Luan, Y. Zhang. 2023. Analysis of chemical composition difference between Yunyan 87 and Zhusha 2. Plant Fiber Sciences in China 45:281-286.

Zheng, X., W. Song, E. Guan, Y. Wang, X. Hu, H. Liang, J. Dong. 2020. Response in physicochemical properties of tobacco-growing soils and N/P/K accumulation in tobacco plant to tobacco straw biochar. Journal of Soil Science and Plant Nutrition 20:293-305.

Zörb, C., M. Senbayram, E. Peiter. 2014. Potassium in agriculture--status and perspectives. Journal of Plant Physiology 171:656-669.