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Full Length Article

Interactive Effects of Nitrogen Application and Remained Leaves Numbers on Flue-Cured Tobacco Yield and Quality

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Abstract

The inefficient use of resources always poses risks to tobacco production and results lower quality output. This study was performed to monitor the effects of nitrogen (N) application rates and remained leaves numbers interaction on tobacco leaves. In this 2-year field experiment, tobacco cultivar ‘K326’ was sown using four N application rates (60, 90, 120 and 150 kg N ha^{-1}), keeping 18 and 22 remained leaves numbers on plant. Results revealed that under the combinations of 22 remained leaves numbers and 120 kg N ha^{-1} N application, the flue-cured tobacco observed more flue-cured tobacco leaves (FTLs) yield and quality due to notable expansion in agronomic traits and chlorophyll contents, and chemical components of field fresh tobacco leaves (FFTLs). Besides, the partial least squares path model of FFTLs quality index and FTLs yield and quality was established by using R language. It was shown that agronomic traits, photosynthetic characteristics, sugars and N of FFTLs had a direct impact on the yield and quality of FTLs and were the key characterization factors determining the yield and quality of FTLs. In conclusion, keeping 22 leaves along with 120 kg N ha^{-1} application improved the FTLs yield and flue-curing traits of flue-cured tobacco.

Keywords: Flue-cured tobacco leaves; Nitrogen application rates; Remained leaves number; Yield and quality; Fresh tobacco leaves

Introduction

Flue-cured tobacco (*Nicotiana tabacum* L.) is a special economic crop for leaf using. As an important agronomic measure, controlling N application rates and remained leaves numbers plays an important role in the yield and quality of flue-cured tobacco leaves (FTLs) (Chen *et al.* 2020). Controlling N application rates and remained leaves numbers greatly change quality of field fresh tobacco leaves (FFTLs), while the quality of FFTLs will further affect the curing characteristics and ultimately determine the quality of FTLs (Finch *et al*. 2019). As one of the three elements of nutrition in plant growth, N is not only the material basis of protein, nucleic acid and phosphoric acid, but also the composition of key enzymes of carbon and N metabolism, hormones, and the photosynthetic-pigment chlorophyll (Lin *et al*. 2017; Wei *et al*. 2017; Agami *et al.* 2018). The optimum N application is crucial to harvest better yield and quality of FTLs and is second only to variety. Within a certain range, the yield and quality of FTLs will be greatly improved with the increase of N application rates. However, excessive application of nitrogenous fertilizers will cause soil acidification, the number of water stable aggregates and the diversity of soil microorganisms decreased, and the yield and quality of FTLs decreased (Zou *et al*. 2018; Shen *et al.* 2020).

Increasing the N application rates can improve the leaf area coefficient; more leaf area results more dry matter of FTLs (Craftsbrandner *et al.* 1987). In addition, as N application rates exceed a certain standard, the agronomic traits of flue-cured tobacco increased slowly (Ankica *et al.* 2019). The chlorophyll will increase with the increase of N application rates within a certain N application range, but too high N application rates will lead to the decrease of photosynthesis (Charles and Tommy 1998). There is a positive correlation between N application rates and N compounds in FTLs; especially nicotine is positively regulated by N application rates (Lisuma *et al*. 2019; Chen *et al*. 2019). It is worth mentioning that under the same N application, increasing remained leaves numbers will reduce the nicotine and correspondingly increase the total and reducing sugar contents (Mu *et al*. 2014).

The remained leaves numbers could change the effective illumination of tobacco leaves, and the difference of temperature and humidity between the population, thus affecting the yield and quality of tobacco leaves. With fewer remained leaves numbers, the single leaf can obtain sufficient or even excessive nutrient absorption. Besides, fewer remained leaves numbers can also increase the temperature difference between day and night among plant groups, thus leading to the accumulation of dry matter. With the increase of remained leaves numbers, the plant height will increase, but the weight of single leaf, leaf area per plant, stem circumference and pitch will decrease (King 1986). Meanwhile, more leaves will cause high chlorophyll due to the reduction of light intensity (Zhou *et al*. 2016). Research shown that the remained leaves numbers are negatively related to the total N, protein and nicotine; and are positively correlated with the ratio of total sugar to reducing sugar, and sugar to nicotine in FTLs (Jiang *et al*. 2017).

Nitrogen application rates and remained leaves numbers control many physiological and biochemical characteristics of flue-cured tobacco, it will further affect the carbon and N metabolism process of tobacco plants. At present, there are many studies on the influence of N application rates and remained leaves numbers on the quality of flue-cured tobacco. However, there are few studies on the interaction between these two factors on agronomic traits and chemical composition of FFTLs. Interactive effect of N application rates and remained leaves numbers has great influence on agronomic traits and chemical composition of FFTLs; and the quality index of FFTLs have influence on FTLs. Therefore, this study was conducted to determine the optimum N application rates and the best remained leaves numbers on yield and the coordinated chemical composition of FTLs.

Materials and Methods

Trial site and conditions

This two-year field trail was conducted during the 2017 and 2018 tobacco cropping seasons from middle of April to late August at Majiazhuang village, Jiuxi town, Jiangchuan district of Yuxi city in Yunan, China (elevation 1730 m, 102°38'13″ E, 24°18'14″ N), which is located in the middle of Yunnan Province. This region keeps a temperate climate, subtropical monsoon climate. Meteorological conditions during the tobacco growing period were recorded using weather data collector (CR800, Campbell, U.S.A.) installed in the field and given in Fig. 1. The former crop was *Vicia sepium* L. The soil at the site is red earth, medium fertility, and the nutrient status before seeding consisted of pH 6.68, organic matter 30.2 g kg^{-1}, available N 101.60 mg kg^{-1}, available K 147.9 mg kg^{-1} and available P 24.2 mg kg^{-1}.

Experiment details

Tobacco variety 'K326' (Zhongyan Tobacco Seed Co., Ltd, China) was selected as testing material, which is a high-yield and adequate-quality variety grown widely in China. The experiment was arranged as randomized complete block design under factorial arrangement with three replicates where the N application rates had 4 levels [60 (N_1), 90 (N_2), 120 (N_3) and 150 (N_4) kg N ha^{-1}] and the remained leaves numbers had 2 levels [18 (L_1) and 22 (L_2) leaves]. Each treatment was repeated three times. Dimension of each plot was 10 m × 6.6 m, and the distance of row-row was 1.20 m and distance of plant to plant was 0.55 m which achieved the population density of 16500 plant ha^{-1}. In order to reduce experimental error, guard rows were reasonable set up. Transplanting plantlets under mulch was adopted, and transplanting time was in April 20. The P application rate was 112.5 kg P_2O_2 ha^{-1}, and the K application rate was 281.25 kg K_2O ha^{-1}, and both remained unchanged in different treatment. The fertilizer was strip applied; 50% fertilizer applied before transplanting and the rest applied around 30 days after transplanting. Film-uncovering and earth-up were carried out at 35 days after transplantation. Topping at 65–70 days after transplantation, and chemical bud inhibitor was applied at the time. To determine the yield and quality of flue-cured tobacco, and determine the chemical composition of flue-cured tobacco, the fresh flue-cured tobacco leaves at different N application rates [60 (N_1), 90 (N_2), 120 (N_3) and 150 (N_4) kg N ha^{-1}] and different remained leaves numbers [18 (L_1) and 22 (L_2) leaves] were harvested and cured using the local main technology.

Data recorded

Agronomic characters: At the mature stage of flue-cured tobacco, 10 tobacco plants were randomly selected from each treatment in each plot, and the plant height, leaf area, fresh weight and dry weights were measured. The agronomic characters were determined based on Standard of the People's Republic of China for Investigating Methods of Agronomical Character of Tobacco (YC/142-1998). After decapitation, the height of plants from stem base on the ground to the top of the stem was measured when the growth of stem top had been stabilized. Three replicates were measured. From 1 week to 10 days after topping stage, the length and width of the maximum leaf were measured, and the number of samples was no less than 10. The product of length by width by correction factor (0.6345) was used to represent the leaf area. The fresh weights of the middle leaves were determined immediately after harvest. The middle leaves of tobacco plants were weighed after being killed out at 105°C and dried at 60°C until stabilized.

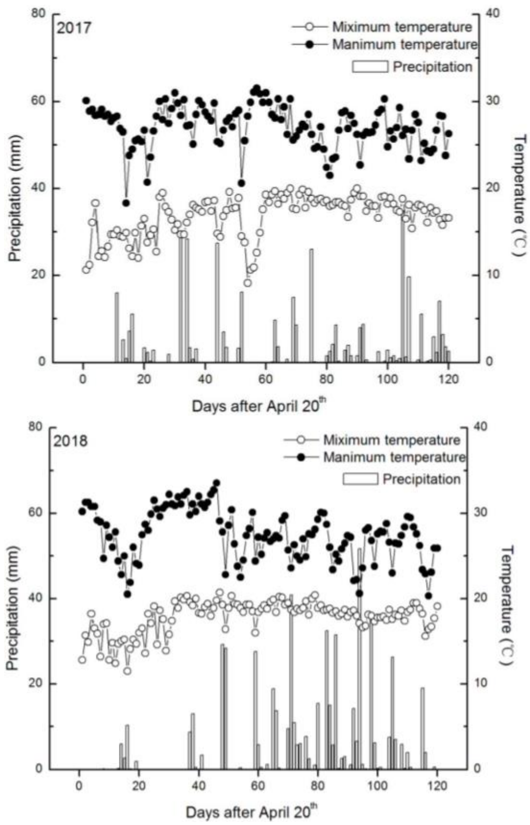

Fig. 1: Maximum temperature, minimum temperature (°C) and precipitation (mm) recorded during the growing seasons in 2017 and 2018

Chlorophyll contents

Chlorophyll contents were expressed by SPAD value. At maturity, 10 tobacco plants were randomly selected from each treatment in each plot, and leaf chlorophyll was estimated with a chlorophyll meter (Minolta SPAD-502 Konica Minolta, Osaka, Japan) at topping (removal of flowers at the top of the plant). Measurements were made three times through nipping the middle of leaves and these data were averaged.

Analyses and methods for indices of economic traits

Based on Standard of the People’s Republic of China for Flue-cured Tobacco (GB2635-1992) and calculated in accordance with procurement price lists of flue-cured tobaccos in 2017 and 2018, the proportions of superior and medium tobacco and average price were calculated. In accordance with dry weight of tobacco leaves at each position, yield of tobacco leaves was calculated.

Analyses of conventional chemical indicators

Three kg of FTLs were random selected in each plot and treatment after curing. And 20 pieces of middle FFTLs were random selected in each plot and treatment at mature period. The contents of starch were determined by spectrophotometry at 660 nm with $HClO_4$ extraction (Kasheva *et al*. 2018). Total sugar, reducing sugar and fructose were determined by rapid colorimetric method with 3, 5-dinitrosalicylic acid (Chen *et al*. 2019). The contents of protein determined with continuous flow analytical method (Wang *et al*. 2015). Total N was determined with elemental analysis method (Haohui *et al.* 2013). Nicotine determined with spectrophotometric method (Rai *et al*. 1994). Polyphenols contents and aroma precursors determined with HPLC coupled with ESI-MS after solid-phase extraction (Li *et al*. 2013).

Statistical analysis

Statistical analysis was performed by two-way analyses of variation (ANOVAs) using S.P.S.S. 22.0 (S.P.S.S. Institute Inc.) and Origin 8.0 (Origin Lab). The significance of differences among treatments was tested using the least significant difference (LSD) method. The partial least squares path modeling (PLSPM) were separately constructed by the indications of agronomic characters, SPAD value, sugar-containing compounds, N-containing compounds, aroma precursors in the FFTLs and yield, yield components, quality parameter in the FTLs, which were used by R software and Amap, Shape, Diagram and software package. PLSPM was also used for analysis of the relationship of subgroup variable data (Tao *et al*. 2017).

Results

Economic characters and chemical composition coordination indexes of FTLs

The yield, average price and sugar-nicotine ratio of K326 flue-cured tobacco varieties under different years (Y), different remained leaves numbers (L) and different N application rates (N) showed significant differences (P < 0.05), and its average price was also significantly affected by the interaction between L × N and Y × L × N (Table 1). From 2017 to 2018, the yield and average price index of flue-cured tobacco were the best with 22 remained leaves numbers in plant. The yield of FTLs was the best when N application rates were 150 kg N ha^{-1}. However, the average price was highest when N application rates were 120 kg N ha^{-1}. The highest treatment for sugar-nicotine ratio was Y2017 × L 2 2 × N60 and the lowest treatment was Y2017×L18×N150 (Table 1).

Agronomic traits and SPAD value in FFTLs at the maturation period

Conduct nonlinear regression fitting of the relationship between main agronomic characters, SPAD value of FFTLs and N application rates under different remained leaves

Table 1: Economic characters and chemical composition coordination indexes of FTLs under four NARs and two RLNs in 2017 and 2018

Year (Y)	Remained leaves numbers (L)	Nitrogen rates (N)	Yield (kg ha^{-1})	Mid-to-high grade leaves (%)	Average price (dollar kg^{-1})	Sugar-nicotine ratio	Nitrogen-nicotine ratio
2017	18	60	1901.34 ± 37.48c	83.91 ± 0.47a	1.73 ± 0.05c	10.06 ± 0.80a	0.63 ± 0.01a
		90	2091.64 ± 40.66bc	87.39 ± 3.78a	2.51 ± 0.16b	7.89 ± 0.43ab	0.58 ± 0.03a
		120	2280.86 ± 56.31ab	81.01 ± 2.04a	3.27 ± 0.17a	8.17 ± 0.41ab	0.64 ± 0.07a
		150	2420.16 ± 33.17a	86.80 ± 1.97a	2.86 ± 0.06ab	5.74 ± 0.32b	0.65 ± 0.04a
	22	60	2033.86 ± 35.53c	86.27 ± 3.14a	1.61 ± 0.12b	11.74 ± 072a	0.61 ± 0.05a
		90	2210.48±151.36bc	90.54 ± 4.21a	2.99 ± 0.2a	9.98 ± 0.55ab	0.59 ± 0.02a
		120	2414.64 ± 25.73ab	87.74 ± 3.82a	3.3 ± 0.12a	8.28 ± 0.28bc	0.61 ± 0.03a
		150	2506.91 ± 34.48a	84.73 ± 2.17a	2.8 ± 0.17a	6.82 ± 0.30c	0.68 ± 0.06a
2018	18	60	1865.21 ± 76.06b	82.74 ± 5.14a	1.98 ± 0.05b	11.50 ± 1.65a	0.65 ± 0.06a
		90	2156.54 ± 96.19a	81.29 ± 2.87a	2.58 ± 0.14b	9.03 ± 0.34ab	0.60 ± 0.02a
		120	2217.33 ± 76.93a	81.82 ± 1.69a	3.42 ± 0.14a	8.10 ± 0.88b	0.68 ± 0.06a
		150	2326.58 ± 47.39a	86.75 ± 3.75a	2.51 ± 0.2b	6.18 ± 0.33b	0.62 ± 0.01a
	22	60	1996.16 ± 128.86b	82.35 ± 3.72a	1.76 ± 0.11c	11.23 ± 2.55a	0.62 ± 0.13a
		90	2149.72 ± 92.31b	83.94 ± 3.45a	3.34 ± 0.24ab	11.42 ± 1.24a	0.62 ± 0.08a
		120	2253.99 ± 144.36a	86.61 ± 4.06a	3.69 ± 0.16a	9.22 ± 0.61b	0.63 ± 0.01a
		150	2507.49 ± 41.51a	83.06 ± 4.49a	2.98 ± 0.13b	8.40 ± 0.65b	0.66 ± 0.07a
	Sources of variation						
	Y		4.3*	6.4*	9.4*	13.8*	0.5ns
	L		19.1***	3.0ns	13.3**	41.4***	0.1ns
	N		80.5***	0.9ns	247.5***	73.8***	2.1ns
	Y× L		0.5ns	0.8ns	2.7ns	0.1ns	0.0ns
	Y× N		1.0ns	2.0ns	2.8ns	0.5ns	0.6ns
	L× N		0.7ns	3.4*	16.5***	2.0ns	1.0ns
	Y× L×N		1.1ns	0.1ns	3.5*	0.0ns	0.0ns

Means with different letters are statistically different from each other at $P \le 0.05$
*= Significant at $P \le 0.05$; *= Significant at $P \le 0.01$; ***= Significant at $P \le 0.001$; ns= Non-significant

numbers in 2017–2018 (Fig. 2), except fresh leaf weight index (determination coefficient r < 0.8), the determination coefficients of other index models are $r^2 \geqslant 0.9$, which indicates that the established equation had high fitting degree with measured values. The plant height index, leaf area per plant, dry leaf weight and SPAD index of FFTLs in these two years was the highest by combining 22 remained leaves numbers with 150 kg N ha^{-1} N application rates.

Sugar-containing and N-containing compounds in FFTLs at maturity

Total sugar, reducing sugar, starch and amino acid indexes of FFTLs at mature stage are significantly different (P < 0.05) under different treatments of remained leaves numbers; and total sugar, reducing sugar, starch, total N, nicotine, protein, amino acid showed significant differences (P < 0.05) in different N application rates (Table 2). Besides, total sugar, starch, total N, and amino acid showed significant differences (P < 0.05) in different remained leaves numbers and N application rates; and only protein showed significant differences N application rates × remained leaves numbers × years, while other index have no significantly differences in different treatment. Total sugar and reducing sugar were highest during 2017 under L22 × N120 combination while the highest starch contents were in 2018 under L18 × N60 combination, and the lowest contents were during 2017 in L22 × N150 combination (Table 2). Protein and amino acid indexes were highest during 2018 under L22 × N120 combination, and the lowest contents were also in 2018 under L18×N90 combination (Table 2).

The aroma precursors in FFTLs at maturity

Neochlorogenic acid, chlorogenic acid and rutin in FFTLs at mature stage showed significant differences (P < 0.05) under different remained leaves numbers; and except the scopoletin other aroma precursors in FFTLs showed significant differences (P < 0.05) under different N application rates. Moreover, only the neochlorogenic acid in FFTLs showed significant differences under different remained leaves numbers and N application rates. Similarly, chlorogenic acid was significantly different (P < 0.05) in different L × N × years combinations while other aroma precursors observed non-significant affected under different treatments. Under the index of polyphenol compounds in flue-cured tobacco, the highest treatment of neochlorogenic acid was Y2017 × L22 × N150 (2.86%), and the lowest was Y2017 × L22 × N60 (1.71%). The highest chlorogenic acid content was Y2017 × L22 × N120 (17.86%), and the lowest was Y2018 × L18 × N90 (9.81%). The highest rutin content was Y2018 × L22 × N120 (15.96%), and the lowest was Y2018 × L18 × N60 (9.30%). Under the index of plastid pigment compounds in flue-cured tobacco, the highest treatment of lutein content was Y2018 × L22 × N120 (0.29%) and the lowest treatment was Y2017 × L18 × N60 (0.14%); the highest treatment of β-carotene content was Y2018 × L22 × N150 (0.46%), The minimum is Y2017 × L18 × N90 (0.23%) (Table 3).

Table 2: Differences of sugar-containing compounds and nitrogen-containing compounds in FFTLs under different NARs and RLNs at the maturation period

Year (Y)	Remaining leaves (R)	Nitrogen fertilization (N)	Total sugar (%)	Reducing sugar (%)	Starch (%)	Fructose (%)	Total nitrogen (%)	Nicotine (%)	Protein (%)	Amino acid (%)
2017	18	60	11.98 ± 0.95b	8.91 ± 0.46b	29 ± 0.38a	1.85 ± 0.09a	1.59 ± 0.08a	2.15 ± 0.07a	9.04 ± 0.80b	18.63 ± 0.70ab
		90	11.06 ± 0.64b	8.5 ± 0.95b	28.09±0.53ab	1.99 ± 0.15a	1.82 ± 0.11a	2.06 ± 0.17a	10.43 ± 1.3ab	17.73 ± 1.68b
		120	15.01 ± 0.85a	11.04 ± 1.34a	25.23 ± 0.61b	2.15 ± 0.05a	2.08 ± 0.02a	2.47 ± 0.46a	11.53 ± 1.04a	20.25 ± 1.10a
		150	14.25 ± 0.92a	10.24 ± 1.12a	21.41 ± 1.40c	2.10 ± 0.01a	2.19 ± 0.07a	2.53 ± 0.24a	11.31 ± 0.74a	19.48 ± 0.85ab
	22	60	10.17 ± 1.07b	7.46 ± 1.77a	31.64 ± 0.41a	2.24 ± 0.11a	1.76 ± 0.17a	2.18 ± 0.17a	9.14 ± 0.52a	20.22 ± 1.48c
		90	13.17 ± 0.43a	9.03 ± 1.10a	27.95 ± 0.94b	2.16 ± 0.17a	1.95 ± 0.11a	2.19 ± 0.05a	9.70 ± 1.31a	21.07 ± 0.40c
		120	13.37 ± 0.67a	9.10 ± 0.64a	23.64 ± 0.98c	2.4 ± 0.12a	1.92 ± 0.03a	2.34 ± 0.27a	9.08 ± 0.88a	24.75 ± 1.12a
		150	11.33 ± 1.11b	7.97 ± 0.63a	24.81 ± 1.33c	2.22 ± 0.07a	2.07 ± 0.17a	2.24 ± 0.10a	11.12 ± 1.42a	23.23 ± 1.03b
2018	18	60	11.19 ± 1.08b	9.27 ± 0.41a	30.23 ± 1.22a	1.61 ± 0.17a	1.6 ± 0.13a	2.18 ± 0.05a	9.1 ± 1b	21.59 ± 0.9ab
		90	10.74 ± 0.67b	9 ± 0.37a	26.93 ± 0.82c	1.66 ± 0.53a	1.8 ± 0.07a	2.22 ± 0.23a	8.71 ± 0.88b	22.42 ± 1.27ab
		120	14.95 ± 1.12a	10.95 ± 0.4a	29.48 ± 1.6b	1.99 ± 0.44a	2 ± 0.11a	2.62 ± 0.52a	9.09 ± 1.55b	23.79 ± 1.72a
		150	12.66±1.75ab	8.94 ± 1.19a	24.57 ± 2.28c	1.65 ± 0.66a	2.13 ± 0.08a	2.25 ± 0.13a	11.67 ± 0.94a	19.91 ± 0.51b
	22	60	10.55 ± 0.89b	6.98 ± 2.00a	31.80 ± 1.25a	1.85 ± 0.10a	1.91 ± 0.17a	1.94 ± 0.15a	9.39 ± 1.39b	20.26 ± 1.72b
		90	10.60 ± 2.48b	7.84 ± 2.86a	28.60 ± 0.61b	1.86 ± 0.76a	1.98 ± 0.12a	2.15 ± 0.15a	11.11±1.33ab	23.78 ± 1.42ab
		120	12.12 ± 0.97a	8.19 ± 0.64a	29.13 ± 1.30b	1.7 ± 0.50a	1.87 ± 0.20a	2.41 ± 0.25a	12.22 ± 0.42a	25.26 ± 1.56a
		150	11.67 ± 1.11a	8.22 ± 1.09a	25.06 ± 1.1c	1.25 ± 0.26a	2.12 ± 0.17a	2.23 ± 0.12a	11.67 ± 1.17a	24.78 ± 1.26ab
	Source of variation									
	Y		4.92*	0.98ns	27.73***	1.4ns	0.01ns	0.09ns	0.41ns	32.99***
	L		11.25**	5.47*	8.33**	0.72ns	1.66ns	2.18ns	1.02ns	46.71***
	N		15.27***	3.82*	69.15***	1.12ns	1.87ns	1.48ns	9.12***	15.21***
	Y×L		0.02ns	0.39ns	0.12ns	2.16ns	1.32ns	0.27ns	13.02**	5.67*
	Y×N		0.61ns	0.09ns	11.17***	0.86ns	0.69ns	0.84ns	0.18ns	2.7ns
	L×N		4.88**	1.45ns	4.54**	1.03ns	1.37ns	0.45ns	0.38ns	5.96**
	Y×L×N		2.21ns	0.93ns	2.67ns	0.49ns	0.13ns	0.78ns	4.27*	1.84ns

Means with different letters are statistically different from each other at $P \leq 0.05$

*= Significant at $P \leq 0.05$; *= Significant at $P \leq 0.01$; ***= Significant at $P \leq 0.001$; ns= Non-significant

Construction of PLSPM with quality indexes of FFTLs, and yield and quality indexes of FTLs

PLSPM results are shown in Fig. 3; the fitting degree of the model is 0.69, which showed that properties and closeness of the relationship among latent variables have good reliability and high accuracy. As show in the model result diagram, the agronomic traits of FFTLs including single leaf weight and leaf area had a significant positive effect on tobacco economic characters (path coefficient =0.99, $P \leqslant 0.01$) and tobacco chemical quality (path coefficient =0.90, $P \leqslant 0.01$). Direct effects of sugars and N on tobacco economic characters and tobacco chemical quality were positive and negative, respectively. The SPAD values had a significant positive effect on tobacco economic characters (path coefficient =2.31, $P \leqslant 0.01$) and tobacco chemical quality (path coefficient =1.40, $P \leqslant 0.01$). Plastid pigments including lutein and beta-carotene content had a negative effect on tobacco economic characters and tobacco chemical quality, but not significant. The indexes of agronomic traits, photosynthetic characteristics, sugar-containing compounds, N-containing compounds and plastid pigment can interact with each other, final jointly improve yield and promote the formation of high-quality tobacco leaves.

Discussion

The interaction of N application rates and remained leaves numbers had significant influence on FTLs' mid-to-high grade leaves, average price, and the content of total sugar, starch, amino acid and neochlorogenic of FFTLs (Tables 1-3). With the decrease of remained leaves numbers per plant, the palisade tissue of leaves will become stronger and the mesophyll tissue ratio will increase, which is one of the main reasons for the increase of single leaf weight and the higher SPAD value. Too many remained leaves will result in the leaves be shielded from each other, which will weaken photosynthesis and increase respiration, thus leading to a small leaf area per plant and a lower leaf weight than those with smaller remained leaves numbers. More remained leaves numbers can weaken the activity of invertase and amylase during carbon metabolism, which will result in higher total sugar content and lower starch content in FFTLs (David and Ramsey 2014). Earlier study showed that FTLs had the highest content of petroleum ether extract, total phenol and chlorogenic acid under the same density, when the remained leaves numbers were 22 (Zhao *et al*. 2008). The reason is probably because that the photosynthetic characteristics of leaves and the acceptable range of effective light radiation may be more in line with the demand of mature tobacco leaves under the treatment of suitable remained leaves numbers, thus making a higher key enzyme activity of polyphenol substances.

Many studies showed that the yield and quality of FTLs could be greatly improved when the N application rates were increased within a certain range (Dale and Gordon 1971). With the increase of N application rates, the sugar-nicotine ratio will decrease as N is an important raw material for nicotine synthesis. Increasing of N application rates will not only enhances the N metabolism intensity of FTLs, but also weakens the carbohydrate transformation

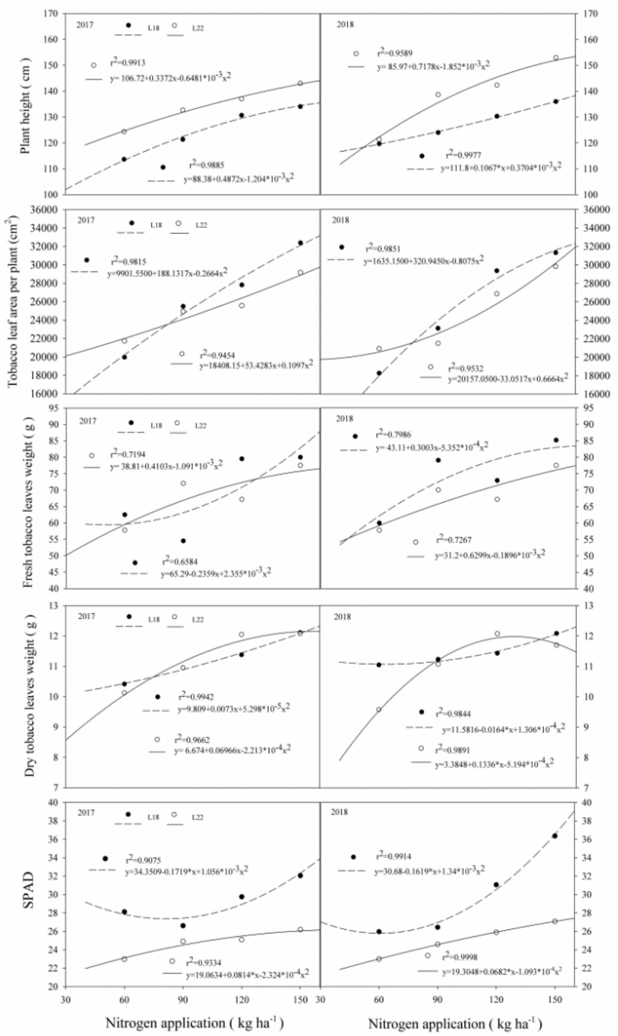

Fig.2: Variation trend of agronomic traits and SPAD in FFTL sunder different N application rates and remained leaves numbers

and accumulation in the carbon metabolism process (Su *et al*. 2020). In the process of N metabolism, the activity of the key enzyme nitrate reductase (NR) will increase significantly with the increase of N application rates, resulting in the increasing of protein and amino acid. However, too higher N application rates will reduce the reducing sugar and starch in mature of FFTLs, and delay starch accumulation as well as weaken carbon accumulation and metabolism (Ankica *et al.* 2019). The increase of N application rates will enhance the activity of phenylalanine ammonia lyase (PAL), the key enzyme and rate limiting enzyme for phenol metabolism. Therefore, N application can promote the synthesis of phenolic compounds. In a certain range, N application rates have a positive correlation with the plant height, single leaf area, single leaf weight and SPAD value of FFTLs. Adequate or excessive supply of N enables photosynthesis to proceed smoothly and to accumulate large amounts of dry matter (Rosa *et al*. 2006).

In the relationship between FTLs and FFTLs, the quality index of FFTLs, including agronomic traits, photosynthetic characteristics, sugars and N have a direct impact on the yield and quality of FTLs. The content of total sugar and reducing sugar of FFTLs have significant correlation with the yield and quality of FTLs. Different remained leaves numbers change the quality index of FFTLs by affecting the population distribution, plant shape and photosynthetic utilization efficiency of tobacco plants, thus affecting the yield and quality of FTLs directly or indirectly. There is a very significant negative correlation between sugar metabolism indexes and N metabolism indexes. Meanwhile, there is a very significant positive correlation between sugar metabolism indexes of FFTLs and chemical composition coordination of FTLs, while N metabolism indexes are opposite. Previous studies found that carbon and N metabolisms are two interdependent transformation processes, N metabolism requires carbon metabolism to provide carbon source and energy, while carbon metabolism requires N metabolism to provide enzyme protein and photosynthetic pigment (Erdal and Turk 2016; Duan *et al*. 2018). The coordination degree of carbon and N metabolism plays an important role in the formation of yield and transformation of chemical components, which is directly or indirectly related to yield and quality of FTLs. Production of high-quality tobacco should make tobacco leaves changed from N metabolism to carbon accumulation metabolism at proper time (Zhang *et al.* 2016). Photosynthetic indexes are positively correlated with plastid pigment and coordination of chemical components, indicating that photosynthesis plays a key role in determining the quality of FTLs. Improvement of photosynthesis is beneficial to growth and development of tobacco plants, accretion and effective transformation of plastid pigment, thereby promoting FTLs quality (Yang *et al*. 2018).

Conclusion

Results of this two-year field study unveiled that application of 120 kg N ha^{-1} and 22 remained leaves numbers are the optimal combination to improve yield and quality of field fresh tobacco leaves, economic returns and chemical composition of flue-cured tobacco leaves. Moreover, agronomic traits, photosynthetic characteristics, sugars and nitrogen of fresh tobacco leaves had a direct impact on the yield and quality of flue-cured tobacco leaves.

Author Contributions

Yi Chen, Qing Yang, Xu Wang and Congming Zou conceived the original research plans. Shubin Sun and Wentao Zhao designed the experiments. Chenggang He and Yun Tang performed the experiments. Junying Li and Ying Lin analyzed the data. Ke Ren, Rui Yang and Conglian He wrote the manuscript. All authors reviewed and approved the final manuscript.

Table 3: Differences of aroma precursors in FFTLs under different NARs and RLNs at the maturation period

Year (Y)	Remaining leaves (R)	Nitrogen fertilization (N)	Neochlorogenic acid (mg/g)	Chlorogenic acid (mg/g)	Caffeic acid (mg/g)	Scopoletin (mg/g)	Rutin (mg/g)	Kaempferol-3-O-rutinoside (mg/g)	Lutein (mg/g)	β-carotene (mg/g)
2017	18	60	1.57±0.02a	11.69±0.93c	0.22±0.04a	0.18±0.02a	10.1±0.86b	0.13±0.02a	0.16±0.02b	0.28±0.01a
		90	2.19±0.1a	14.04±0.42b	0.25±0.03a	0.19±0.02a	11.83±1.34ab	0.15±0.01a	0.18±0.03ab	0.33±0.03a
		120	2.33±0.12a	16.81±0.96a	0.23±0.02a	0.21±0.04a	14.01±1.15a	0.17±0.02a	0.23±0.03ab	0.34±0.04a
		150	2.38±0.14a	13.72±1.69b	0.24±0.03a	0.23±0.02a	13.84±0.82ab	0.16±0.02a	0.24±0.03a	0.3±0.01a
	22	60	2.01±0.03c	12.72±1.17b	0.2±0.02a	0.2±0.02a	9.38±1.19c	0.15±0.02a	0.23±0.02a	0.31±0.02a
		90	2.29±0.09b	11.92±0.41b	0.22±0.03a	0.21±0.04a	12.89±1.01b	0.16±0.02a	0.24±0.06a	0.31±0.01a
		120	2.65±0.19a	15.86±0.73a	0.29±0.03a	0.24±0.05a	15.25±0.5a	0.17±0.02a	0.23±0.02a	0.38±0.03a
		150	2.5±0.16ab	16.78±1.11a	0.21±0.03a	0.23±0.02a	14.25±1.07ab	0.15±0.02a	0.22±0.01a	0.32±0.03a
2018	18	60	2.08±0.08a	11.31±1.08b	0.21±0.04a	0.23±0.03a	9.3±1.16b	0.16±0.02a	0.21±0.06a	0.34±0.03b
		90	2.32±0.15a	9.81±0.9b	0.2±0.03a	0.22±0.02a	12.22±0.97ab	0.19±0.01a	0.17±0.09a	0.29±0.04b
		120	2.34±0.14a	15.57±0.87a	0.25±0.01a	0.21±0.02a	12.42±1.44a	0.2±0.01a	0.3±0.03a	0.37±0.05a
		150	2.28±0.14a	14.97±1.11a	0.26±0.01a	0.18±0.02a	13.28±1.12ab	0.15±0.01a	0.23±0.05a	0.35±0.09ab
	22	60	2.24±0.09a	13.4±0.63c	0.19±0.03a	0.2±0.04a	10.81±1.24b	0.16±0.02a	0.22±0.05a	0.3±0.03b
		90	2.14±0.13b	13.91±1.3c	0.23±0.02a	0.17±0.01a	11.68±0.97b	0.16±0.02a	0.23±0.07a	0.34±0.04ab
		120	2.22±0.12a	16.36±0.5a	0.25±0.02a	0.2±0.03a	15.96±1.66a	0.18±0.02a	0.25±0.03a	0.35±0.08ab
		150	2.42±0.17a	14.93±1.1b	0.24±0.02a	0.22±0.04a	13.57±1.42ab	0.14±0.01a	0.2±0.03a	0.41±0.03a
	Source of variation									
	Y		0.17ns	2.06ns	0.23ns	0.77ns	0.75ns	6.12*	0.6ns	3.48ns
	L		11.46***	12.16***	0.23ns	0.09ns	6.52*	0.98ns	0.94ns	1.55ns
	N		29.28***	46.21***	1.27ns	1.09ns	36.35***	8.08***	3.02*	4.04*
	Y×L		11.46***	6.73*	0.03ns	3.09ns	1.12ns	3.92ns	1.35ns	0.04ns
	Y×N		11.98***	0.85ns	1.6ns	1.97ns	0.39ns	2.2ns	1.18ns	2.02ns
	L×N		3.72*	1.78ns	2.56ns	0.83ns	2.39ns	0.98ns	2.92ns	0.60ns
	Y×L×N		1.76ns	11.18***	2.49ns	2.00ns	2.05ns	0.65ns	0.33ns	2.14ns

Means with different letters are statistically different from each other at p≤*0.05*
*= Significant at p≤0.05; *= Significant at p≤0.01; ***= Significant at p≤0.001; ns= Non-significant

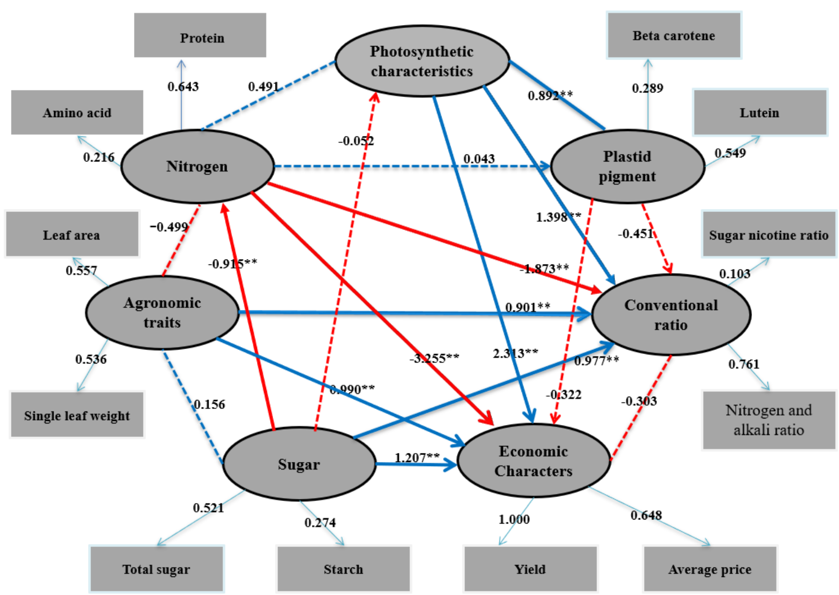

Fig. 3: Partial least squares path modeling of the association of agronomic characters, SPAD value, sugar-containing compounds, nitrogen-containing compounds, aroma precursors in the FFTLs and yield, yield components, quality parameter in the FTLs. Goodness of fit of the modeling is 0.6932. *Blue* and *red arrows* indicate positive and negative path coefficient, respectively, while *solid and dashed lines* indicate significant and non-significant path coefficients, respectively. * and ** indicate significance at the level of $P < 0.05$ and 0.01, respectively

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