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Nitrogen Metabolism and Curing Characteristics of Flue-cured Tobacco Variety NC71

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Abstract [**Objectives**] To explore the growth characteristics, nitrogen metabolism and curing characteristics of the new variety NC71 in strongly aromatic tobacco leaves production area. [**Methods**] With Yunyan 87 as the control and NC71 as the experimental material, the experiment was carried out in Nanyang tobacco area for two consecutive years to study the growth characteristics of tobacco plants, the activity of key enzymes in nitrogen metabolism, the content of secondary metabolites, and the differences of tobacco-related water content, PPO enzyme activity and total phenol content. [**Results**] NC71 plants had lower height, better appearance quality and lower nicotine, total sugar and chlorine content. NC71 high nitrogen treatment and Yunyan 87 medium nitrogen treatment had similar key enzyme activity of nitrogen metabolism, content of secondary metabolites; Yunyan 87 and NC71 were easy to cure and resistant to curing. [**Conclusions**] This study provides a reference for measuring the appropriate nitrogen application rate and optimizing the curing process from the activities of the key enzymes NR and GS in nitrogen metabolism at the mature stage of the variety.

Key words Variety, Nitrogen metabolism, Curing characteristics

1 Introduction

Nitrogen plays an important role in the growth and development of plants. Nitrogen level exerts an important influence on metabolism of plant internal matters, physiological and biochemical processes, as well as the product quality. In tobacco, the activities of nitrogen metabolism-related enzymes such as nitrate reductase (NR), glutamine synthetase (GS), glutamate synthase (GOGAT), glutamate dehydrogenase (GDH), *etc.* directly reflects the level of nitrogen assimilation and absorption by tobacco[1]. According to findings of Zhou Jianfei *et al.* [2], increasing the nitrogen application amount can delay the peak of GS activity and the GS activity varies with varieties. Zhang Shengjie[3] found that the NR activity of flue-cured tobacco leaves of different genotypes reached extremely significant differences, which was similar to the findings of of Liu Weiqun[4]. The GDH activity is different for flue-cured tobacco varieties with different nitrogen efficiency, and the GDH activity of nitrogen-efficient varieties is lower, and the increase of nitrogen dosage is not favorable for the maturing and yellowing of flue-cured tobacco[5]. Besides, nitrogen metabolism also affects the secondary metabolism of tobacco leaves. Carotenoids and phenolic compounds are the main secondary metabolites of tobacco plants and also important aroma precursors of tobacco leaves[6-8]. Findings of Wang Xinfa[9] indicate that carotenoid content is different in different genotypes and nitrogen dosages. The total amount of phenolic compounds is significantly different in different tobaccos[10], and the degradation products after modulation can increase the amount of aroma and improve the aftertaste.

The curing characteristics of tobacco leaves reflect the different quality levels of fresh tobacco leaves, while the curing scheme of tobacco leaves and the determination of the curing operation details mainly depend on the curing characteristics of the tobacco leaves[11], so it is very important to study the curing characteristics of the tobacco leaves. The moisture content in the fresh tobacco leaves influences the transformation of physiological and biochemical activities within the tobacco leaves. The yellowing of tobacco leaves at the early stage should be done when the tobacco leaves have a certain moisture content, and the yellow color of tobacco leaves must be maintained through the drying and water loss stage[12]. Polyphenol oxidase is very important in the curing process of tobacco leaves. Its activity is directly related to the curing characteristics of flue-cured tobacco varieties. Studies have found that the content of polyphenol oxidase varies with varieties and the proportion of the variegated smoke that appears after curing is not the same, showing a significant positive correlation, and the curing resistance of tobacco leaves is also related to the content of polyphenol oxidase during color fixation[13]. Through studying the changes of pigment and water content, Sun Fushan *et al.* [14] concluded that the curing characteristics of Honghua Dajinyuan had general curing characteristics, Cuibi 1 had poor curing characteristics, and Yunyan 85 had better curing characteristics. Through the study of pigment, moisture, PPO activity and

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chemical components, Xiao Zhijun *et al.* [15] found that Yunyan 87 had better curing characteristics, K326 had medium curing characteristics, and G80 had poor curing characteristics.

At present, studies about nitrogen metabolism and curing characteristics of Yunyan 87 are mature, but there are few reports on the nitrogen metabolism and curing characteristics of the new variety NC71. In view of this, taking Yunyan 87 and NC71 in Nanyang tobacco area as materials, we studied the differences in nitrogen metabolism and secondary metabolism between flue-cured tobacco varieties, to reveal the internal relationship between the formation of some aroma precursors and the process of nitrogen metabolism in flue-cured tobacco and provide a theoretical basis for choosing the appropriate amount of nitrogen for different varieties. We also discussed the changes in moisture content and polyphenol oxidase activity during the curing process, to optimize and improve the curing process of various varieties, and provide a theoretical basis for the development of strongly aromatic tobacco in Henan Province.

2 Materials and methods

2.1 Experimental design The experimental flue-cured tobacco varieties were NC71 and Yunyan 87, and the middle leaves of the tobacco plant (12 – 14 leaves, counted from the bottom) were selected and 5 repetitions were set for each experiment. Agronomic traits, disease resistance, appearance quality and intrinsic quality of raw tobacco leaves were tested in Fangcheng County, Neixiang County, and Xichuan County of Nanyang City in 2018 – 2019. The planting row spacing was 120 cm, and the plant spacing was 55 cm. The tobacco seedlings were transplanted on April 28, 2018 and May 3, 2019. Other management measures were carried out in accordance with the local high-quality tobacco production requirements.

Nitrogen metabolism and curing characteristics tests were carried out in Jinye Tea Garden, Fangcheng County of Nanyang City in 2018 – 2019. The soil type of the test site was yellow loam, the soil pH was 7.48, the organic matter content was 11.45 g/kg, the alkaline nitrogen content was 55.00 mg/kg, the available phosphorus content was 15.01 mg/kg, and the available potassium content was 135.00 mg/kg. The tobacco seedlings were transplanted on April 28, 2018 and May 3, 2019.

Nitrogen metabolism experiment adopts split plot design, in which varieties were the main plot and the nitrogen application amount was the sub plot. For the nitrogen application amount, we set three treatments, namely, low nitrogen N1 (30 kg/ha), medium nitrogen N2 (45 kg/ha), and high nitrogen N3 (60 kg/ha), and samples were taken 110 d after transplanting. For the curing characteristics, we set three treatments according to the different maturity of tobacco leaves (Table 1). After harvesting, tobacco leaves were cured according to the conventional three-stage curing process, and samples were taken at 0, 24, 48, 60, 72, 96, and 120 h during the curing. The planting row spacing was 120 cm, and the plant spacing was 55 cm. Other management measures were carried out in accordance with the requirements of local high-quality tobacco production.

Table 1 Classification criteria for tobacco leaves at different maturity levels

Treatment	Leaf features	Leaf vein	Trichome	Leaf tip and margin
Unripe	Light green leaves	1/2 main veins white and branch veins blue	Few fell off	Tip slightly hooked down
Ripe	Mainly yellow, 60% – 80% leaf area became yellow	All main veins white and bright and 2/3 branch veins white	Many fell off	Tip and margin curled down
Overmature	Nearly all leaf surfaces were yellow with mature spots	All main and branch veins white and bright	Most fell off	Tip withered and margin burnt

2.2 Measurement items and methods

2.2.1 Agronomic traits. We investigated the agronomic traits in accordance with *Investigating and Measuring Methods of Agronomical Character of Tobacco* (YC/T 142-2010). We measured and recorded the plant height, stem girth, internodes space, maximum leaf length and maximum leaf width, and selected 5 representative plants for each treatment. Max. leaf area = Max. leaf length × Max. leaf width × 0.634 5.

2.2.2 Investigation of disease resistance. We investigated the disease resistance in accordance with *Grade and Investigation Method of Tobacco Diseases and Insect Pests* (GB/T 23222-2008). Incidence rate of tobacco plants (%) = number of diseased plants /Total number of investigated plants × 100.

2.2.3 Appearance quality. In accordance with China national standard *Flue-cured Tobacco* (GB2635-1992), experts from Henan Branch of China National Tobacco Corporation evaluated color, maturity, leaf structure, identity, oil content, chroma, leaf tissue features, suppleness, and luster.

2.2.4 Chemical quality. We measured the content of nicotine, total sugar, reducing sugar, chlorine and potassium in tobacco leaves in accordance with *Tobacco and Tobacco Products – Determination of Water Soluble Sugars – Continuous Flow Method* (YC/T 159-162-2002).

2.2.5 Sensory evaluation. The tobacco leaves got damp to a moisture content of 17.0% – 21.0%, with a cutting width of 0.8 mm. Adjustment was made under conditions specified in *Tobacco and Tobacco Products—Atmosphere for Conditioning and Testing* (GB/T 16447-1996), to make the moisture content of the cut tobacco reach the rolling standard and then rolled into a single-material cigarette. The test cigarettes were adjusted for 72 h under the conditions specified in standard GB/T 16447-1996 and then evaluated by experts from Henan Branch of China National Tobacco Corporation.

2.2.6 Enzyme activity determination. We determined the nitrate reductase (NR), glutamine synthetase (GS), glutamate synthase (GOGAT), glutamate dehydrogenase (GDH) and polyphenol oxidase (PPO) using the kit provided by Beijing Solarbio Science & Technology Co., Ltd.

2.2.7 Determination of other physiological indicators. We determined the free amino acids using inhydrin coloration method, measured the carotenoids using 96% ethanol extraction method, measured the nicotine using the acetic acid extraction method, and determined the total phenols using Folin-Ciocalteu method, and measured the moisture content water using the method specified in *Evaluation for Curing Characteristics of Flue-cured Tobacco* (YC/T 311-2009).

2.3 Data analysis We carried out the statistical analysis, curve fitting, analysis of variance and independent sample T test on the experimental data with the aid of Excel 2010 and SPSS 20.0 statistical software.

3 Results and analysis

3.1 Main agronomic traits As indicated in Table 2, the plant height of NC71 was significantly lower than that of Yunyan 87, which was reduced by 4.53 cm, and the leaf area, leaf length, and leaf width (extremely) were significantly greater than that of Yunyan 87, which increased by 153.89 cm^2, 3.4 cm, and 1.59 cm, respectively. There were no significant differences in leaf number, stem girth, and internodes space, showing that NC71 has a lower plant height but the yield may be higher than that of Yunyan 87.

Table 2 Survey results of main agronomic traits (mean of 3 places in 2 years)

Variety	Plant height//cm	Leaf number//piece	Stem girth//cm	Internodes space//cm	Leaf length//cm	Leaf width//cm	Leaf area//cm^2
Yunyan 87	121.64	18.18	11.01	4.82	74.30	35.00	1 650.02
NC71	117.11**	19.92	11.29	4.23	77.70*	36.59*	1 803.91**

Note: * and ** denote significant differences and extremely significant differences from Yunyan 87 through independent sample T test ($P<0.05$, $P<0.01$). The same in Table 3 - 5.

3.2 Disease resistance As indicated in Table 3, the natural incidence of NC71 climatic scab, wildfire, TMV, and PVY was significantly lower than that of Yunyan 87; while the natural incidence of black shank, brown spot, angular leaf spot, root knot nematode and CMV was extremely significantly higher than Yunyan 87, indicating that NC71 and Yunyan 87 have different disease resistance.

Table 3 Survey results of natural incidence of diseases (mean of 3 places in 2 years) %

Disease	Yunyan 87	NC71	Disease	Yunyan 87	NC71
Black shank	3.03	3.55**	Angular leaf spot	0.00	2.00**
Brown spot	2.00	2.50**	Root knot nematode	0.00	1.50**
Bacterial wilt	0.00	0.00	TMV	1.33	0.00**
Climate scab	16.35	15.67**	CMV	0.00	1.00**
Tobacco wildfire	15.74	9.03**	PVY	3.86	0.95**

3.3 Appearance and intrinsic quality of raw tobacco leaves

3.3.1 Appearance quality of tobacco leaves. As shown in Table 4, except for oil content, chroma, and luster, other indicator scores of NC71were significantly higher than Yunyan 87, and the total score was increased by 1.96, indicating that NC71 has better appearance quality than Yunyan 87.

Table 4 Appearance quality evaluation (mean of 3 places in 2 years)

Indicator	Yunyan 87	NC71	Indicator	Yunyan 87	NC71
Color	7.50	7.88**	Chroma	6.17	5.88**
Maturity	7.83	7.88**	Leaf tissue	5.17	6.13**
Leaf structure	8.00	8.38**	Suppleness	5.17	6.13**
Identity	7.67	8.38**	Luster	6.00	6.00
Oil content	6.67	5.50**	Total	60.17	62.13**

3.3.2 Chemical components of raw tobacco leaves. As indicated in Table 5, the indicators of the two varieties had extremely significant differences. Among them, the nicotine, total sugar, and chlorine content of NC71 was lower than that of Yunyan 87, and the potassium content and potassium chloride ratio of NC71 were higher than that of Yunyan 87.

Table 5 Chemical component analysis (mean of 3 places in 2 years) %

Variety	Nic	TS	Cl	K	K/Cl
Yunyan 87	3.12	21.42	1.04	1.49	1.42
NC71	2.90**	21.34**	0.98**	1.96**	2.01**

3.3.3 Sensory evaluation results. As shown in Fig. 1, the differences in the scores were small between the two varieties. Among them, the aroma volume score of Yunyan 87 was slightly higher than that of NC71.

3.4 Nitrogen metabolism characteristics

3.4.1 Enzyme activity. From Table 6, it can be seen that the overall performance of NR and GOGAT enzyme activities was Yunyan 87 > NC71, and the GDH and GS enzyme activities were NC71 > Yunyan 87, showing that the enzyme activities of NR and

GOGAT in Yunyan 87 tobacco leaves were higher at the maturity stage, while GDH and GS enzyme activities were lower, while NC71 was the opposite.

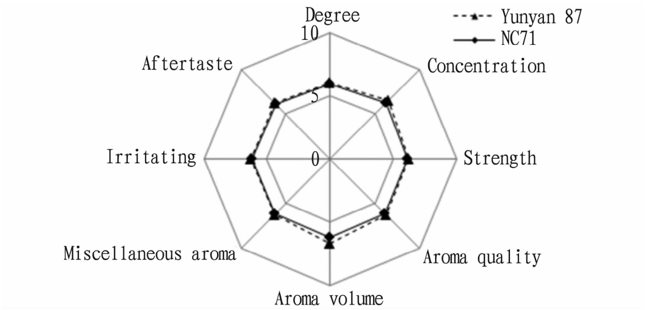

Fig. 1 Comparison of sensory evaluation results (mean of 3 places in 2 years)

There were also certain differences in the activities of key enzymes in nitrogen metabolism among different nitrogen application amount. The enzyme activities of NR, GS and GOGAT increased with the increase of nitrogen application amount; the enzyme activities of NR, GS and GOGAT under low nitrogen treatment were significantly lower than those under medium and high nitrogen treatment. For the GDH activity, NC71 variety increased with the increase of nitrogen application amount, and had the maximum value under high nitrogen treatment; by comparison, GDH activity of Yunyan 87 increased first and then decreased with the increase of nitrogen application amount, and had a maximum value at medium nitrogen level, indicating that there is an interaction between variety and nitrogen usage in GDH activity. The NR enzyme activity under NC71 high nitrogen level was not significantly different from Yunyan 87 medium nitrogen treatment; there was no significant difference between the GOGAT enzyme activity under NC71 high nitrogen level and Yunyan 87 medium nitrogen treatment, showing that the enzyme activity of NC71 at high nitrogen level was similar to that of Yunyan 87 at medium nitrogen level, which suggests that NC71 has weaker nitrogen assimilation and absorption capacity than Yunyan 87.

Table 6 Comparison of the activities of key enzymes in nitrogen metabolism under different nitrogen application amount (mean of 2 years)

Variety	Nitrogen application amount	NR//U/g	GS//nmol/mg/min	GOGAT//nmol/mg/min	GDH//U/g
Yunyan 87	N1	1.42^{c}	7.88^{d}	149.63^{d}	35.11^{c}
	N2	1.60^{b}	14.57^{c}	200.05^{b}	36.64^{b}
	N3	1.73^{a}	19.02^{b}	226.91^{a}	35.93^{c}
NC71	N1	1.15^{d}	6.64^{d}	128.08^{e}	33.11^{d}
	N2	1.47^{c}	20.76^{b}	190.38^{c}	37.12^{b}
	N3	1.59^{b}	22.75^{a}	205.45^{b}	39.17^{a}

Note: Different lowercase letters in the same column denote significant differences between treatments ($P \leqslant 0.05$), the same in Table 7.

3.4.2 Secondary metabolism. As indicated in Table 7, the free amino acid content, nicotine content and carotenoid content in different varieties showed the overall performance of Yunyan 87 was better than NC71. The total phenol content was also different between the two varieties, the overall performance of NC71 was better than Yunyan87, showing that the content of free amino acids, carotenoids and nicotine in Yunyan 87 tobacco leaves at maturity stage was higher than that of NC71.

Besides, there were also certain differences in nitrogen metabolism and secondary metabolite content among different nitrogen application amount. The content of free amino acid and nicotine increased with the increase of nitrogen application amount. The carotenoid content of Yunyan 87 increased with the increase of nitrogen application amount, and had a maximum value at the high nitrogen level; the carotenoid content of NC71 first increased and then decreased with the increase of nitrogen application amount, and had a maximum value at the medium nitrogen level. The total phenol content NC71 increased with the increase of nitrogen application amount, and had a maximum value at high nitrogen level; the total phenol content of Yunyan 87 increased first and then decreased with the increase of nitrogen application amount, and had a maximum value at medium nitrogen level, suggesting that the content of carotenoids and total phenols has an interaction between variety and nitrogen usage.

The content of free amino acids of NC71 at the high nitrogen level was not significantly different from Yunyan 87 at the medium nitrogen level; the nicotine content and carotenoid content of NC71 at the high nitrogen level were significantly lower than the nicotine content and carotenoid content of Yunyan 87 at the medium nitrogen level, suggesting that NC71 needs to be treated with high nitrogen to maintain better secondary metabolism, while Yunyan 87 can maintain better secondary metabolism at medium nitrogen levels.

Table 7 Comparison of the content of nitrogen metabolites and secondary metabolites at different nitrogen application amount (mean of 2 years)

Variety	Nitrogen application amount	Free amino acid//mg/100 g	Nicotine//%	Carotenoid//mg/g	Total phenols//%
Yunyan 87	N1	155.46^{d}	0.97^{c}	0.26^{ab}	5.16^{d}
	N2	165.11^{b}	1.37^{b}	0.29^{a}	6.20^{c}
	N3	167.39^{a}	1.74^{a}	0.31^{a}	6.15^{c}
NC71	N1	158.67^{c}	0.90^{c}	0.15^{c}	6.14^{c}
	N2	159.70^{c}	0.94^{c}	0.24^{b}	6.36^{b}
	N3	162.98^{b}	1.40^{b}	0.22^{b}	6.65^{a}

3.5 Curing characteristics

3.5.1 Moisture content. During the tobacco curing process, the moisture content of tobacco leaves decreased in a downward slope curve (Fig. 2A – C). The changing trends of different varieties and harvesting maturity were consistent, and they all showed a downward trend with the increase of the curing time. Before 48 h (color fixation stage), the moisture content of Yunyan 87 decreased more smoothly than NC71, and after 48 h, the moisture content decreased significantly faster. As the maturity of field harvesting increased, the moisture content of tobacco leaves of various varieties decreased, and the moisture content of tobacco leaves of all varieties was unripe > ripe > overmature.

According to the change rules of tobacco leaf moisture content during curing process of different varieties and harvest maturity (Fig. 2A – C), we used the equation $y = ax^3 + bx^2 + cx + d$ to fit the curve, where y is the moisture content (%) of tobacco leaves with different harvest maturities, x is the curing time (h), and a, b, c, d are parameters to be determined (Table 8). The P values of the regression equation obtained by the F test were all less than 0.000 1, reaching an extremely significant level, suggesting that the fitting equation is statistically significant. Through further calculating the second derivative of time, we obtained the change rules of the water loss rate of each treated tobacco leaf. From Fig. 1D, 1E, 1F, it can be seen that the changing trends of different varieties and harvest maturity were consistent. The water loss rate of NC71 was faster than that of Yunyan 87 in unripe and mature, but the overmature was opposite. The water loss rate of NC71 was unripe > ripe > overmature, and the water loss rate of Yunyan 87 was overmature > unripe > ripe, indicating that different varieties had different rates of water loss at different harvest maturity levels.

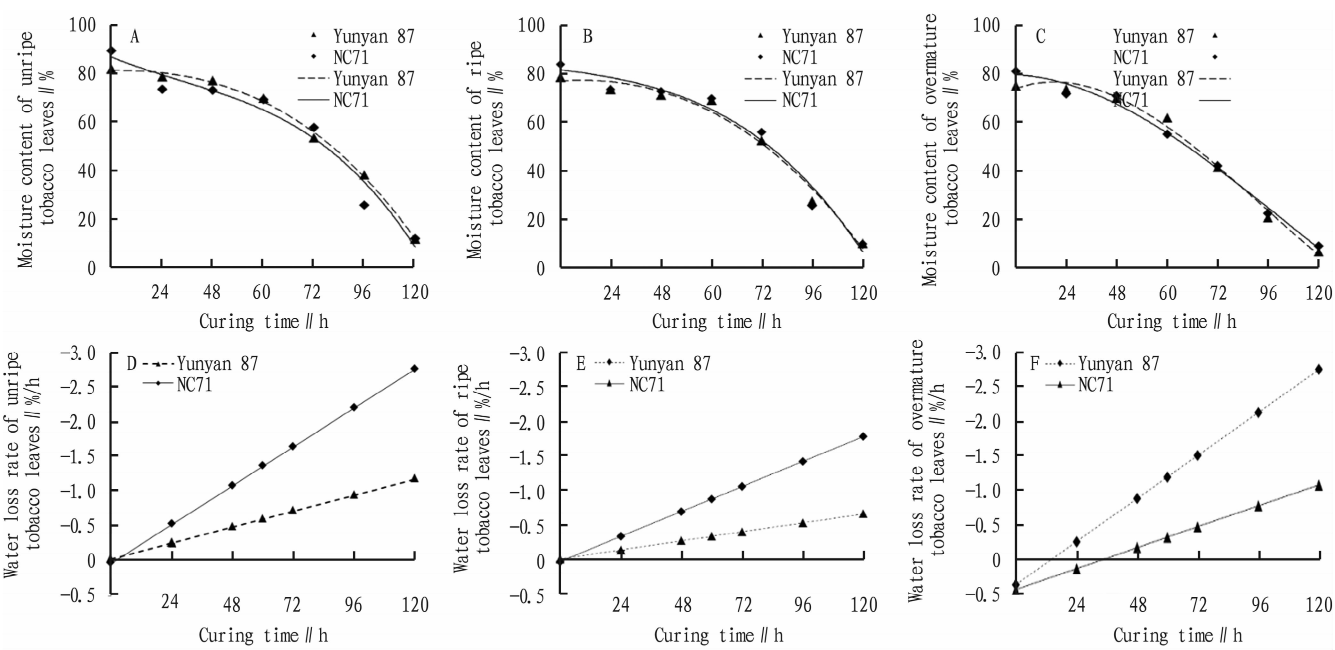

Fig. 2 Change rules of moisture content and water loss rate of tobacco leaves with different maturity levels (mean of 2 years)

Table 8 Fitting characteristic parameters of moisture content in different treatments

Treatment	Variety	*a*	*b*	*c*	*d*	R^2	*P*
Unripe	Yunyan 87	–0.001 6	–0.004 6	0.014 4	0.801 8	0.997 0	<0.000 1
	NC71	–0.003 9	0.028 1	–0.129 9	0.970 1	0.961 3	<0.000 1
Ripe	Yunyan 87	–0.000 9	–0.013 1	0.042 8	0.741 4	0.983 4	<0.000 1
	NC71	–0.002 5	0.006 1	–0.033 4	0.846 3	0.971 5	<0.000 1
Overmature	Yunyan 87	0.003 7	–0.064 5	0.193 1	0.608 3	0.992 8	<0.000 1
	NC71	0.001 8	–0.034 8	0.055 6	0.774 5	0.991 3	<0.000 1

3.5.2 PPO activity. As shown in Fig. 3A – C, the PPO activity of tobacco leaves during the curing process of tobacco plants took on a "W" curve change, and the change trend was the same among different varieties and harvest maturity, and they all showed low PPO content at 48 h and 96 – 120 h, and high PPO content at 0 – 24 h and 72 h. At the unripe and ripe stages of tobacco leaves, the PPO content of Yunyan 87 changed more than that of NC71. The PPO activity of tobacco leaves with different harvest maturity levels showed a slow decline first, then a rapid rise, and finally a rapid decline and maintained a low level trend. The PPO activity was always unripe > ripe > overmature.

According to the change rules of tobacco leaf PPO content during curing process of different varieties and harvest maturity (Fig. 3A – C), we used the equation $y = ax^5 + bx^4 + cx^3 + dx^2 + ex + f$ to fit the curve, where y is the is the PPO content of tobacco leaves with different harvest maturity (U/(g · min), x is the curing time (h), and a, b, c, d, e, and f are parameters to be determined (Table 9). The P values of the regression equation obtained by the F test were all less than 0.000 1, reaching an extremely significant level, suggesting that the fitting equation is statistically significant. Through further calculating the second derivative of time, we obtained the change rules of the PPO content of each treated tobacco leaf. From Fig. 3D – F, it can be seen that except for NC71 at the overmature stage, the changing trends of different varieties and harvest maturity were consistent. The accumulation rate of PPO of Yunyan 87 at different harvest maturity was faster than that of NC71. The PPO accumulation rates of Yunyan 87 and NC71 were unripe > ripe > overmature, and the PPO accumulation rate of NC71 tobacco leaves at the overmature stage did not change much with the increase of curing time, indicating that different varieties had different PPO accumulation rates at different maturity levels of tobacco leaves.

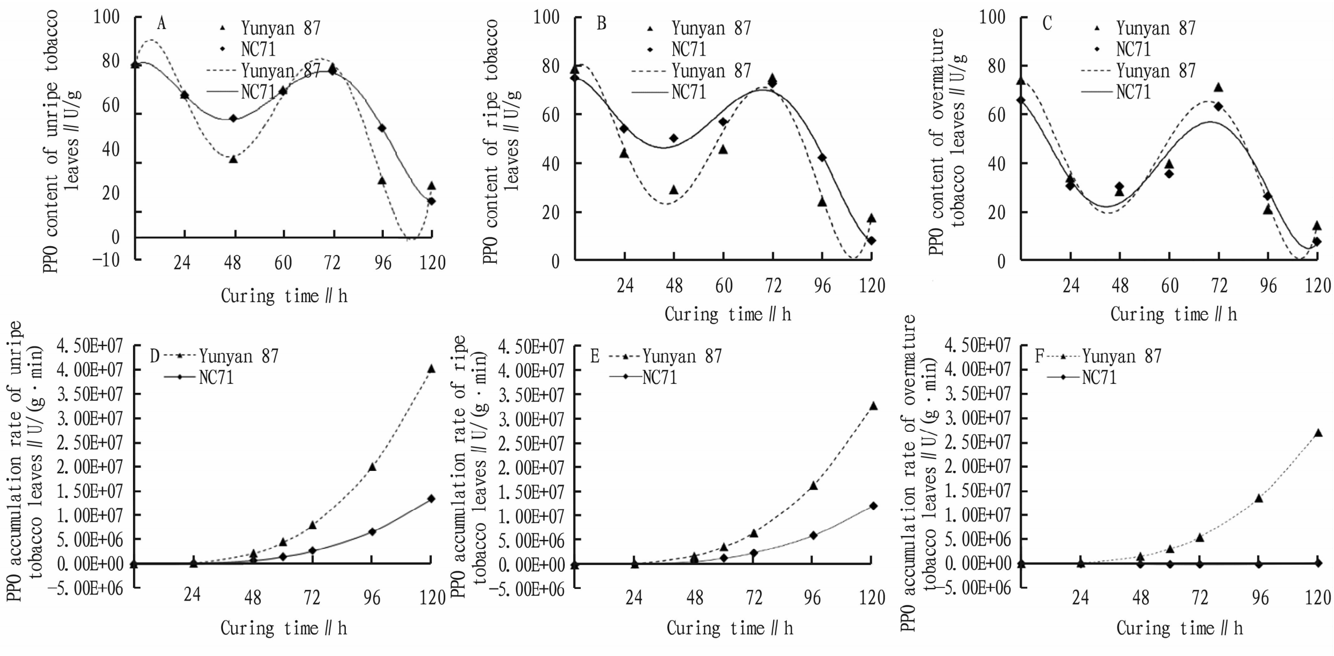

Fig. 3 Change rules of PPO content and accumulation rate of tobacco leaves with different maturity levels (mean of 2 years)

Table 9 Fitting characteristic parameters of PPO in different treatments

Treatment	Variety	a	b	c	d	e	f	R^2	P
Unripe	Yunyan 87	1.283 7	–24.976 0	177.630 0	–563.270 0	766.760 0	–278.530 0	0.997 5	<0.000 1
	NC71	0.428 3	–8.519 2	60.905 0	189.690 0	243.760 0	–28.559 0	0.999 7	<0.000 1
Ripe	Yunyan 87	1.038 7	–20.055 0	140.160 0	–428.500 0	541.240 0	–155.530 0	0.965 4	<0.000 1
	NC71	0.385 4	–7.564 7	52.728 0	–156.720 0	183.510 0	2.454 3	0.984 7	<0.000 1
Overmature	Yunyan 87	0.862 9	–16.426 0	112.200 0	–329.930 0	387.490 0	–80.749 0	0.932 7	<0.000 1
	NC71	0.514 3	–9.799 1	66.083 0	–186.760 0	196.710 0	–1.352 9	0.925 3	<0.000 1

3.5.3 Total phenol content. As shown in Fig. 4A – C, the total phenol content of tobacco leaves during the curing process of tobacco plants changed in an "M" curve, and the changing trends were consistent among different varieties and harvest maturity. With the increase of the curing time, the total phenol content increased in 0 – 48 h, then slowly decreased, and rose again after 72 h and reached the highest value. Proper maturity can effectively increase the total phenol content of tobacco leaves. The total phenol content of the two varieties of ripe tobacco leaves was significantly higher than that of the unripe and overmature tobacco leaves, and the polyphenol content of the unripe tobacco leaves was the lowest.

According to the change rules of total phenol content of tobacco leaves during curing process of different varieties and harvest maturity (Fig. 4A – C), we used the equation $y = ax^5 + bx^4 + cx^3 + dx^2 + ex + f$ to fit the curve, where y is the is the total phenol content of tobacco leaves with different harvest maturity (mg/g), x is the curing time (h), and a, b, c, d, e, and f are parameters to be determined (Table 10). The P values of the regression equation obtained by the F test were all less than 0.000 1, reaching an extremely significant level, suggesting that the fitting equation is statistically significant. Through further calculating the second derivative of time, we obtained the change rules of the total phenol accumulation rate of each treated tobacco leaf. From Fig. 4D – F, it can be seen that the changing trends of different varieties and harvest maturity were consistent. The total phenol accumulation rate of NC71 at different harvest maturity was faster than that of Yunyan 87. The total phenol accumulation rates of Yunyan 87 and NC71 were ripe > overmature > unripe, indicating that different varieties had different total phenol accumulation rates at different maturity levels of tobacco leaves.

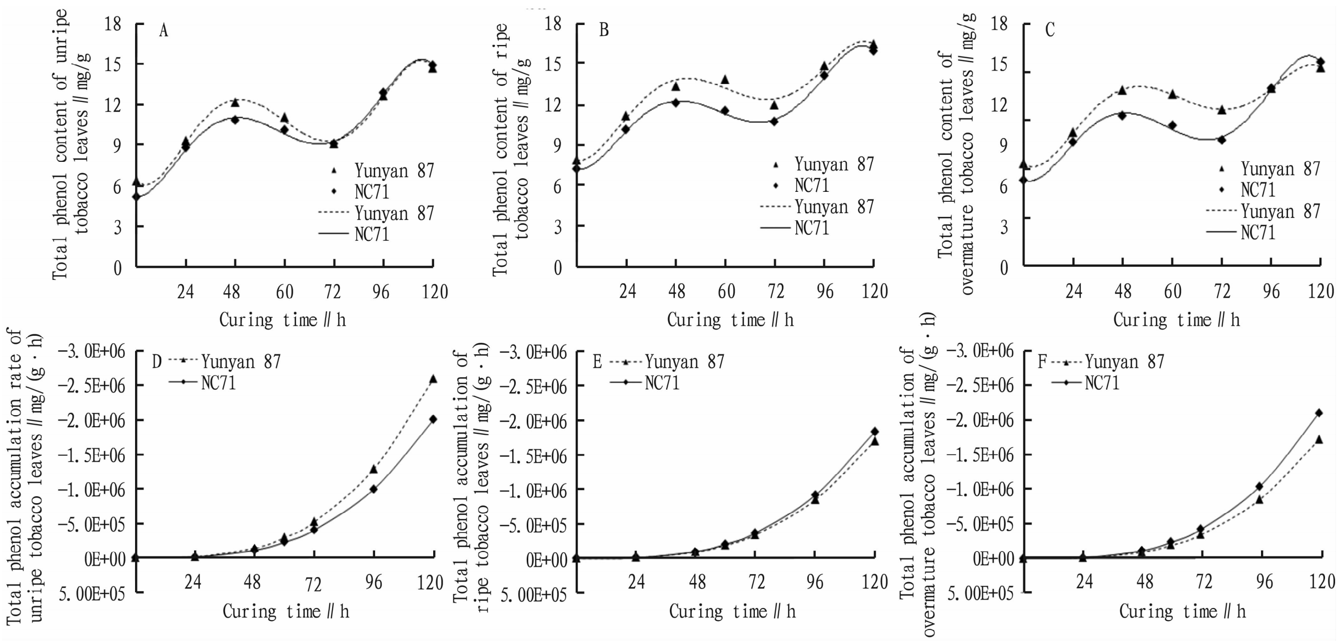

Fig. 4 Change rules of total phenol content and accumulation rate of tobacco leaves with different maturity levels (mean of 2 years)

Table 10 Fitting characteristic parameters of total phenol in different treatments

Treatment	Variety	*a*	*b*	*c*	*d*	*e*	*f*	R^2	*P*
Unripe	Yunyan 87	-0.083 1	1.649 6	-11.898 0	37.719 0	-49.128 0	28.149 0	0.996 0	<0.000 1
	NC71	-0.063 9	1.236 3	-8.617 6	25.980 0	-30.718 0	17.481 0	0.996 1	<0.000 1
Ripe	Yunyan 87	-0.054 7	1.089 7	-7.868 8	24.756 0	-30.738 0	20.733 0	0.980 7	<0.000 1
	NC71	-0.058 8	1.144 8	-8.062 9	24.762 0	-30.407 0	19.885 0	0.997 4	<0.000 1
Overmature	Yunyan 87	-0.055 5	1.133 6	-8.466 5	27.884 0	-37.369 0	24.519 0	0.999 9	<0.000 1
	NC71	-0.067 3	1.312 7	-9.272 7	28.682 0	-36.073 0	21.846 0	0.995 5	<0.000 1

4 Discussion and conclusions

The new strongly aromatic tobacco variety NC71 has a lower plant height, but a larger leaf area, and has better disease resistance to climatic splot, wildfire, TMV, and PVY. The appearance quality of NC71 raw tobacco leaves is better than that of Yunyan 87, and the chemical components of NC71 nicotine, total sugar and chlorine content of NC71 are lower than that of Yunyan 87. The nitrogen absorbed by flue-cured tobacco is mainly nitrate nitrogen. The experimental results show that with the increase of nitrogen application amount, the NR, GS and GOGAT activities are increased, which are consistent with findings of Yue Hongbin[16] and Gao Qin *et al*. [17], showing that the ability of plants to absorb, assimilate and re-assimilate nitrogen is positively correlated with the nitrogen application level.

In this experiment, we collected samples at the maturity stage of tobacco leaves, and GDH showed deamination effect, which is consistent with the results of previous studies[18-19]. Different varieties have largely different nitrogen metabolism characteristics[20], and the nitrogen metabolites in tobacco leaves also show differences in nitrogen absorption and assimilation of different varieties to a certain extent. Yunyan 87 has higher activity of NR and GOGAT at the maturity stage, but lower activity of GDH, indicating that its ability to absorb and assimilate nitrogen is still strong. At the maturity stage of NC71, the activity of NR and GOGAT is weak, and the activity of GDH is strong, indicating that the ability to absorb and re-assimilate nitrogen is weak, and the degradation of nitrogen nutrients is larger. Our research results are consistent with findings of Zhou Jianfei *et al*. [21]. According to results of this study, the content of nitrogen metabolites such as free amino acids and nicotine also increases with the increase of nitrogen application amount[21-22]. Different varieties are different in total phenol content. NC71 increases with the increase of nitrogen application amount, and the total phenol content of Yunyan 87 first increases and then decreases with the increase of nitrogen application amount. The overall performance is NC71 > Yunyan 87, the total phenol content is basically consistent with the changes in nitrogen metabolism-related enzyme activities. The overall performance of carotenoid content is Yunyan 87 > NC71. NC71 has a weak ability to absorb and re-assimilate nitrogen at the maturity stage. High nitrogen (nitrogen application 60 kg/ha) treatment contributes to the accumulation of nitrogen metabolism and its secondary metabolism, which is favorable for the formation of some aroma precursors of flue-cured tobacco and the yellowing of tobacco leaves, thereby improving the quality of tobacco aroma and increasing the yield and quality of tobacco leaves.

Nowadays, the curing characteristics of tobacco are mostly evaluated in terms of easy to cure and curing resistance. The curing characteristics of flue-cured tobacco varieties are closely related to the curing quality and curing process. Thus, establishment of suitable curing methods for varieties is a prerequisite for improving the quality of tobacco leaves[23]. Different varieties have different curing characteristics[24]. In actual production, tobacco farmers do not have adequate knowledge about the curing characteristics of new varieties, so that the tobacco leaves are easy to burn. Using water loss, chlorophyll reduction rate and polyphenol oxidase activity, Wang Chuanyi[25] made a comprehensive evaluation of the curing characteristics of tobacco varieties. This experiment indicates that the curing characteristics of Yunyan 87 and NC71 are good, which is consistent with the research results of Zhu Feng *et al.*[26]. During the curing process, the moisture loss and yellowing characteristics of tobacco leaves directly reflect the easy curing characteristics. The tolerance of tobacco leaves to environmental temperature and humidity during the yellowing and color fixation period reflects the resistance to curing[27]. This study shows that Yunyan 87 and NC71 are more coordinated with yellowing and dehydration, and show better easy curing characteristics. Varieties with high polyphenol oxidase activity will act on polyphenols to produce enzymatic browning reactions during color fixation, leading to a larger proportion of variegated tobacco after curing[28]. In this experiment, the PPO activity of each variety had an obvious peak during the fixation period, which is consistent with the findings of Lan Junrong *et al.*[29]. This is possibly due to the continuous moisture loss in tobacco leaves, and the rapid lysis of tobacco leaf cells during the curing process leads to the destruction of cell intactness[30]. In terms of the fixation period (72 h), the polyphenol oxidase activity of Yunyan 87 and NC71 is similar, and the polyphenol oxidase content declines rapidly after 72 h, the tobacco quality after curing and the resistance to curing are better. In summary, the yellowing of Yunyan 87 and NC71 is more coordinated with moisture loss, faster yellowing, and easier color fixation, better appearance quality after curing. It is recommended to coordinate the yellowing and moisture loss, and conduct curing process by three steps during the color fixation.

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