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Optimized Nitrogen Application Rate Significantly Increases Total Economic Value and Quality of Flue-Cured Tobacco due to the Improvement of Superior Tobacco Yield *

by

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SUMMARY

With the improvement of irrigation and transportation infrastructures, single tobacco cultivation has been converted into tobacco–vegetable double cultivation in Yunnan Province. High residual nitrogen (N) levels in soil before tobacco transplanting induced by the excessive N input during the vegetable cultivation season resulted in a reduction of economic income and the quality of flue-cured tobacco. Therefore, the objective of this paper is to describe the optimization of N management and to provide a better understanding of the mechanism of optimal N application rate on the economic benefit and quality of tobacco. A field experiment with six N application rates (0, 45, 60, 75, 90, and 105 kg N ha^{-1}) was carried out with a randomized block design in 2021 and 2022 in Yunnan Province. The economic value and yield, intrinsic chemical and processing quality, leaf growth rate and agronomic characters were determined. Compared with the currently recommended 105 kg N ha^{-1} rate, 75 kg N ha^{-1} significantly increased the total economic value and superior tobacco yields, improved the integrated grade of chemical compounds and resulted in leaf midrib proportions in an appropriate range. The total economic value positively correlated with the superior tobacco leaf yields ($R^2 = 0.91$, $p < 0.001$), while not with medium and inferior leaf yield. The daily leaf growth rate in prosperous growth stage significantly correlated with the yield of superior tobacco and reached its maximum at a N rate of 75 kg N ha^{-1}. The sum of N application rate and soil residual N before transplanting correlated with the total economic value ($R^2 = 0.66$, $p < 0.05$) and superior leaf yield ($R^2 = 0.64$, $p < 0.05$), respectively. Based on the amount of soil residual N before transplanting, the optimal N application rate was 66 kg N ha^{-1} which was 39 kg N ha^{-1} lower than the currently recommended N rate (105 kg N ha^{-1}). Our results highlighted that the technical consultants and farmers should adjust the

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N application rate appropriately according to the residual N amount before transplanting and optimize the water and fertilizer management especially in the prosperous growth stage. An optimized N rate is not only of economic benefit and the improvement of quality of tobacco cultivation, but also environment friendly. [Contrib. Tob. Nicotine Res. 33 (2024) 136–147]

KEYWORDS

Nitrogen application rate; leaf growth rate; prosperous growth stage; soil residual N

ZUSAMMENFASSUNG

Mit der Verbesserung der Bewässerungs- und Transportinfrastrukturen wurde in der Provinz Yunnan der einfache Tabakanbau in einen kombinierten Tabak-Gemüseanbau umgewandelt. Der hohe Reststickstoffgehalt des Bodens vor dem Auspflanzen des Tabaks, der durch die übermäßige Stickstoffzufuhr während der Gemüseanbausaison verursacht wurde, führte zu einer Verringerung des wirtschaftlichen Einkommens und der Qualität des Flue-Cured-Tabaks. Ziel dieser Arbeit ist es daher, das Stickstoffmanagement zu optimieren und den Mechanismus der optimalen Stickstoffausbringungsrate auf den wirtschaftlichen Nutzen und die Qualität des Tabaks zu verstehen. In den Jahren 2021 und 2022 wurde ein Feldversuch mit sechs N-Dosier-mengen (0, 45, 60, 75, 90 und 105 kg N ha^{-1}) in einem randomisierten Blockversuch durchgeführt. Ermittelt wurden der wirtschaftliche Wert und der Ertrag, die chemische und verarbeitungstechnische Qualität, das Blattwachstum und agronomische Merkmale. Im Vergleich zu den bisher empfohlenen 105 kg N ha^{-1} steigerte 75 kg N ha^{-1} den wirtschaftlichen Gesamtwert und den überdurchschnittlichen Tabakertrag deutlich, verbesserte die integrierte Qualität der chemischen Verbindungen und führte zu einem Blattmittelrippenanteil in einem angemessenen Bereich. Der wirtschaftliche Gesamtwert korrelierte positiv mit dem Ertrag der oberen Tabakblätter ($R^2 = 0,91$, $p < 0,001$), nicht jedoch mit dem mittleren und unteren Blattertrag. Die tägliche Blattwachstumsrate im Wachstumsstadium korrelierte signifikant mit dem Ertrag von Superior-Tabak und erreichte das Maximum bei einer N-Rate von 75 kg N ha^{-1}. Die Summe der N-Ausbring-ungsmenge und des Rest-N im Boden vor dem Umpflanzen korrelierte mit dem wirtschaftlichen Gesamtwert ($R^2 = 0,66$, $p < 0,05$) bzw. mit dem Ertrag der besseren Blätter ($R^2 = 0,64$, $p < 0,05$). Ausgehend von der Reststickstoffmenge im Boden vor dem Umpflanzen lag die optimale Stickstoffdosis bei 66 kg N ha^{-1} und damit 39 kg N ha^{-1} niedriger als die zuvor empfohlene Stickstoffdosis (105 kg N ha^{-1}). Unsere Ergebnisse verdeutlichen, dass der technische Berater und die Landwirte die N-Ausbringungsrate entsprechend der Reststickstoffmenge vor dem Umpflanzen anpassen und das Wasser- und Düngemanagement insbesondere in der Wachstumsphase optimieren sollten. Die optimierte N-Rate ist nicht nur für einen hohen wirtschaftlichen Nutzen und eine hohe Qualität des Tabakanbaus, sondern auch für die Umwelt von Vorteil. [Contrib. Tob. Nicotine Res. 33 (2024) 136–147]

RESUME

Avec l'amélioration des infrastructures d'irrigation et de transport, la monoculture du tabac a été convertie en une double culture tabac-légumes dans la province du Yunnan. L'azote résiduel élevé dans le sol avant la transplantation du tabac, induit par l'apport excessif d'azote pendant la saison de culture des légumes, a entraîné une réduction du revenu économique et de la qualité du tabac flue-cured.
L'objectif de cet article est donc d'optimiser la gestion de l'azote et de comprendre le mécanisme du taux d'application optimal de l'azote sur le bénéfice économique et la qualité du tabac. Des expériences de terrain avec six taux d'application d'azote (0, 45, 60, 75, 90 et 105 kg N ha^{-1}) ont été menées avec un plan en blocs aléatoires en 2021 et 2022. La valeur économique et le rendement, la qualité chimique intrinsèque et la qualité de transformation, le taux de croissance des feuilles et les caractères agronomiques ont été déterminés. Par rapport à la dose de 105 kg N ha^{-1} recommandée précédemment, 75 kg N ha^{-1} a augmenté de manière significative la valeur économique totale et le rendement supérieur du tabac, a amélioré la qualité intégrée des composés chimiques et a permis d'obtenir une proportion de nervure médiane des feuilles dans une fourchette appropriée. La valeur économique totale est positivement corrélée avec le rendement supérieur des feuilles de tabac ($R^2 = 0,91$, $p < 0,001$), alors qu'elle ne l'est pas avec le rendement des feuilles moyennes et inférieures. Le taux de croissance journalier des feuilles au stade de croissance prospère est en corrélation significative avec le rendement du tabac supérieur et a atteint son maximum à un taux d'azote de 75 kg N ha^{-1}. La somme du taux d'application de l'azote et de l'azote résiduel dans le sol avant la transplantation est en corrélation avec la valeur économique totale ($R^2 = 0,66$, $p < 0,05$) et le rendement foliaire supérieur ($R^2 = 0,64$, $p < 0,05$), respectivement. Sur la base de la quantité d'azote résiduel dans le sol avant la transplantation, le taux d'application optimal de l'azote était de 66 kg N ha^{-1}, soit 39 kg N ha^{-1} de moins que le taux d'azote recommandé précédemment (105 kg N ha^{-1}). Nos résultats ont mis en évidence que le conseiller technique et les agriculteurs devraient ajuster le taux d'application de l'azote de manière appropriée en fonction de la quantité résiduelle d'azote avant la transplantation et optimiser la gestion de l'eau et de l'engrais, en particulier au stade de croissance prospère. L'optimisation du taux d'azote permet non seulement d'accroître les avantages économiques et la qualité de la culture du tabac, mais aussi de respecter l'environnement. [Contrib. Tob. Nicotine Res. 33 (2024) 136–147]

1. INTRODUCTION

The tobacco industry is the largest tax contributor in China, accounting for 8.7% of the Chinese total tax revenue in 2022 (1). The annual cultivation area of flue-cured tobacco in China is 1.01 million hectares with an annual tobacco yield of 2.13 million tons. Yunnan Province is the most important tobacco-producing area, well known for its high-

quality tobacco. The cultivation area accounts for about 40% of the total production in China (2). With the increasing demand for land use for vegetable production in winter and spring, and the improvement of irrigation and transportation infrastructure, annual single tobacco mono-cropping has gradually been converted into vegetable–tobacco double cultivation in Yunnan Province. The growth period of vegetables is from October to February, while tobacco cultivation is from April to August. Excessive use of fertilizer and water for vegetable production has become a common phenomenon, aiming to achieve high yields of vegetables. As a result, the soil residual nitrogen (N) amount increases before the tobacco transplanting in the late spring. Thus the recommended optimal N rate might not be suitable for the production of tobacco of high quality (3). Based on the amount of soil residual N before tobacco transplanting, optimizing N management and understanding the mechanism of the effect of the optimal N application rate on plant growth are an important issue, not only for the economic benefit and quality of tobacco, but also for a better protection of the environment.

Yunnan Province has unique climatic conditions, and it is of high economic benefits to cultivate open field vegetables in winter and spring after tobacco-harvesting. However, in order to achieve high yields as well as an economic income and due to N leaching induced by flooding irrigation, the average N application rate is as high as 450 kg ha^{-1} $season^{-1}$ (4), which exeeds plant uptake by 3–4 times (5). For this reason the amount of residual N in soil increases year by year (6). This practice not only wastes fertilizer resources, but also increases the risk of water and air pollution (7–8). In addition, the soil quality, such as the deterioration of soil structure and cultivability (9), decreases the soil pH and beneficial microorganisms (10–12). Furthermore, it leads to a decrease in plant resistance and imbalance of soil nutrients (8, 13) and especially to an increase of soil residual N, which affects the production of high-quality tobacco (14).

Flue-cured tobacco is very sensitive to nitrogen (15–16). Insufficient N supply leads to slow growth rates, leaf yellowing and premature aging, poor tobacco quality and low yield while oversupply of N results in late ripening, abnormal yellowing of tobacco leaves, and substantial reduction of tobacco quality and economic value (17–18). Soil N is the main source of N absorbed by flue-cured tobacco, accounting for over 50% of total N uptake (19). Excessive soil residual N will inevitably result in the optimal N rate recommended by the demonstration experiments of tobacco industry over the years, which might not meet the requirements for the producing high-quality tobacco. The results of a large soil sample survey showed that the residual inorganic N amount has increased by 93% in the tobacco–vegetable double cultivation system in the past 10 years (3), which is the highest classification standard of soil nutrients in tobacco field (20). In order to meet the tobacco N requirement at different growth stages, it is necessary to re-optimize N application rate and to establish the nitrogen-effect-equation based on the current soil residual N amount and plant demand at different growth stages.

The tobacco growth stages are roughly divided into four periods, namely resetting, prosperous, mature and harvesting stage (19). Among them, in the prosperous stage, the tobacco middle and upper leaves grow rapidly, which is the key period to determine the economic value and quality of flue-cured tobacco (19). Compared with lower tobacco leaves, middle and upper leaves have a higher economic value with better chemical and processing qualities (18), such as higher contents of nicotine and total nitrogen as well as a lower leaf midrib proportion. Ensuring timely and appropriate N supply in the prosperous growth stage plays a decisive role in obtaining high quality and good yields of flue-cured tobacco. The evaluation indexes of high-quality flue-cured tobacco include commodity quality, intrinsic chemical and processing quality (21). Commodity quality determines the sales price of flue-cured tobacco, ranging from 5 to 50 RMB kg^{-1} (0.8 to 6.6 U.S. Dollars kg^{-1}), which is the most important concern for farmers. The intrinsic chemical quality determines the flavor of flue-cured tobacco, which mainly includes six indexes and is important to flue-cured tobacco purchasing enterprises. Leaf midrib proportion as an important parameter of processing quality, is a major concern for flue-cured tobacco processing enterprises (22). Excepting the varieties and ecological conditions of cultivation areas, the above three categories of indicators are mainly affected by fertilization management (23), especially by the amount and time of N application.

A field experiment was conducted from 2021 to 2022 in Yuxi City, which is a major area producing high-quality flue-cured tobacco in Yunnan Province. Parameters as yield and economic value, intrinsic chemical and processing quality, daily leaf growth rate, agronomic characters and soil N amount were determined. Objective of this study was to optimize N management and understand the mechanism of the effect of the optimal N application rate on plant growth for the better economic benefit and high quality of flue-cured tobacco.

Our hypotheses were:

1. Yield decrease of superior leaf tobacco results in the reduction of total economic value and quality, caused by excessive or deficient N application rate.
2. The optimal N application rate including soil residual N might be significantly lower than the currently recommended rate.
3. The nitrogen effect equation including soil residual N can be used to guide the cultivation of high-quality flue-cured tobacco.

2. MATERIALS AND METHODS

2.1. Experimental site information and design

The experimental site is located in Guojia Village (N24°38'25", E102°52'26"), Yuxi City, Yunnan Province, with an altitude of 1730 m. The average precipitation, temperature and sunshine duration during the growth period is 126 mm, 20.5 °C, and 165 h (see supplementary tables at the end of publication, Table S1).

Prior to the experiment beginning in 2021, soil tests of the 0–20 cm topsoil showed:

- bulk density: 1.35 g cm^{-3},
- pH: 6.28,

- organic matter: 35.0 g kg^{-1},
- alkali-hydrolyzed N: 188 mg N kg^{-1},
- available phosphorus: 67.4 mg P kg^{-1}, and
- available potassium: 375 mg K kg^{-1}.

Before our experiment began, the previous vegetable crop was fresh faba beans with an N application of 180 kg N ha^{-1}. The field experiments were conducted from April 22nd to August 30th, 2021, and from April 25th to August 30th, 2022. Six N application rates (0, 45, 60, 75, 90 and 105 kg N ha^{-1}) were set in a completely randomized block with three replications and a total of 18 plots. Each plot was 9.6 m long and 7.8 m wide.

2.2. Fertilization and field management

On April 5th, 2021 and 2022, the raised beds were set up after deep ploughing of 20 cm and rotating ploughing twice, with a height of 30 cm and a width of 60 cm. On April 23rd, base fertilizer was applied in small holes with a diameter of 10 cm and a depth of 5 cm, where the seedling would be transplanted. On the next day, 45 days old seedlings of local popular tobacco variety "Cinnabar Yan No. 2" were transplanted with plant space of 0.6 m and row space of 1.2 m. Immediately after transplanting the seedling, 2 L water were irrigated for each planting hole and insecticide beta-cyfluthrin (Bayer, Leverkusen, Germany) for controlling tobacco aphids was sprayed. Hereafter black plastic film (15 μm in thickness) was covered immediately to avoid evaporation and weeds as well as to increase soil temperature. On May 21st, the plastic film was removed and soil was added around the seedlings. On June 1st, the topdressing fertilizer was applied. On June 25th, the tobacco was topped. 20 to 30 mm water was given every 5–7 days by drip irrigation system according to the precipitation and soil moisture. On the 10th, 25th and 40th day after transplanting, dimethomorph (BASF, Ludwigshafen, Germany) and fluopicolide and propamocab hydrochloride (Bayer, Munich, Germany) were sprayed to control black root rot and black shank, respectively.

According to N application rates, special compound fertilizer "Hongye Brand" for flue-cured tobacco was used with a ratio 1:0.5:2 of $N:P_2O_5:K_2O$ (Honghe Henglin Chemical Co., LTD, Yunnan, China). For control treatment (0 kg N ha^{-1}), only phosphorus and potassium fertilizer were applied. The total application amount of phosphorus (45 kg P_2O_5 ha^{-1}) and potassium fertilizer (270 kg K_2O ha^{-1}) were the same for all treatments. The insufficient phosphate and potassium fertilizer in each treatment were supplemented with calcium superphosphate (16% P_2O_5) and potassium sulfate (51% K_2O). The ratio of base to topdressing fertilizer was 1:1. The base fertilizer was applied in the planting hole (25 cm in diameter, 15 cm in depth) and mixed with approximately 0.5 kg soil. Topdressing fertilizer was dissolved in water and then poured into the planting hole.

2.3. Agronomic characters investigation and soil sample collection

Plant samples were collected at 35th, 70th and 105th days after transplanting. Three tobacco plants with even growth were selected from the same position in each plot each time and numbered with the label. The tobacco plants were cut close to the soil surface, put into nylon mesh bags and brought back to the laboratory, put in the oven for 30 min at 105 °C and dried to constant weight at 75 °C. The dry weight of the leaves was measured.

Soil samples of 0–20 cm was collected by a soil auger with a diameter of 38 mm in the middle of the two plants. Air-dried soil was sieved through a 2-mm diameter sieve. 10 g soil was extracted with 100 mL 0.1 mol L^{-1} $CaCl_2$ solution. The $NO3^-$-N and $NH4^+$-N concentrations were determined by an auto-analyzer (AA3, Hamburg, Germany).

2.4 Tobacco harvest, yield calculation and quality parameter determination

During the harvest period July 25th to August 25th, the matured tobacco leaves were collected on four separate dates within a 10 days interval. All the leaves of each plot were tied and flue-cured according to the national standard (GB) for flue-cured tobacco (GB 2630-1992) and for curing tobacco (GB/T 23219-2008) independently. After flue-curing, the tobacco leaves were graded according to GB 2635-92 (Table S2). The dry weight of flue-cured tobacco of each grade was recorded respectively for the subsequent calculation of the tobacco economic value and yield. The total economic value of flue-cured tobacco was calculated as Σ yield of different grades per hectare × responding price (Table S4). The nitrogen economic benefit (NEB) of flue-cured tobacco = (the economic value of each N rate – the economic value of 0 kg N ha^{-1}) / the N rate of each treatment.

Twenty-five tobacco leaves of X2F, C3F and B2F grades (Table S2) were selected from the harvested and flue-cured tobacco on July 25th, August 15th and August 25th, respectively, for the subsequent determination of leaf midrib proportion and chemical quality. From each grade of the tobacco samples in each plot, 10 flue-cured leaves were randomly selected, using the near infrared spectrum analyzer (IAS-8120, Xunjie Guangyuan Technology Co., LTD., Wuxi, China). It measured the water content of the 10 pieces of tobacco leaves paralleled to the middle line of the main vein 1/4, 2/3, 3/4, respectively, taking the average value recorded as water content. Then, the 10 pieces of tobacco leaves were manually separated into laminas and midribs (side veins and main veins with a diameter < 1.5 mm at leaf tip are regarded as laminas), and the laminas and midribs were separately weighed for calculating the leaf midrib proportion. In addition, 5 flue-cured tobacco leaves were randomly selected from each plot and grade. After removing the leaf midrib, they were cut into 0.8 ± 0.1 mm slices, which were baked to constant weight at 60 °C. Then a pulverizer (Cyclone Mill-Twister type, Retsch, Germany) was used and mixed through a 0.25 mm mesh screen. The quality parameters (total sugar, reducing sugar, total nitrogen, nicotine, potassium oxide, chlorine) were determined by Fourier Transform near infrared spectrometer (Antaris II, Thermo Fisher Scientific, Waltham, MA, USA). Integrated grade of tobacco intrinsic chemical quality = scores of various quality parameters × weights of various quality parameters, specific scoring standards and calculation formulas are shown in Table S4.

3. RESULTS

With the increase of N rate, the economic value of flue-cured tobacco and N economic benefit first increased and then decreased (Figure 1). At the N rate of 75 kg N ha^{-1}, the economic value was 122 × 103 RMB ha^{-1} with N economic benefit (NEB) of 1836 RMB kg^{-1} N which was significantly higher than those of other N rates. Compared with the currently recommended N rate of 105 kg N ha^{-1}, economic value and NEB were significantly increased by 37.0% and 32.8% at the N rate of 75 kg N ha^{-1}, respectively.

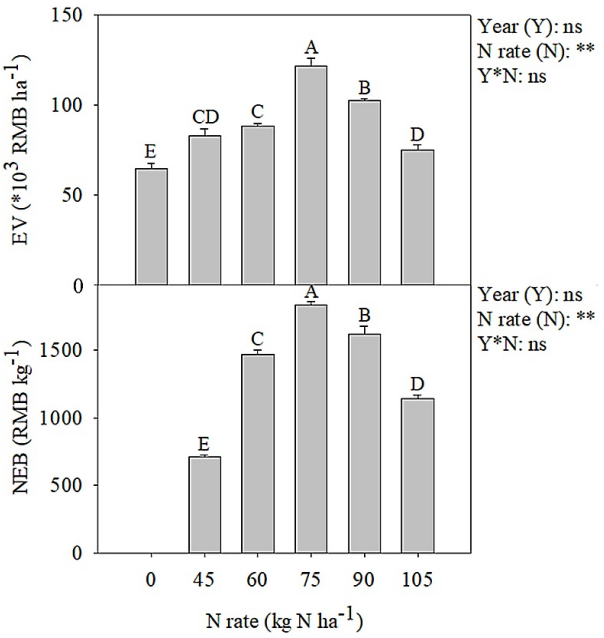


Figure 1. Effects of the N application rate on the economic value (EV) and N economic benefit (NEB) of flue-cured tobacco average over years. Bars labeled with different capital letters indicate a significant difference ($p < 0.05$) among N treatments. Error bars represent the standard error of means.

With the increasing N rate, the integrated grade of chemical quality showed a parabolic trend, increasing up to its highest level at the N rate of 75 kg N ha^{-1} and then decreasing (Figure 2). With the increase of N rate, the contents of nicotine, total nitrogen and potassium K increased first and then decreased (Table S3), but the total sugar and reducing sugar showed a trend of decreasing first and then increasing, while the chlorine content had no significant change. Except for K, the other five parameters of chemical quality mentioned above were in the suitable range at the N rate of 75 kg N ha^{-1}.

The leaf midrib proportion significantly decreased with the increase of N rates (Figure 3). Compared with the currently recommend rate of 105 kg N ha^{-1}, the leaf midrib proportion increased by 3.6–4.4% at 75 kg N ha^{-1} for different grades, but it was lower than that at low N rates. Regardless of N rates, the leaf midrib proportion of B2F tobacco leaves was significantly lower than that of C3F and X2F tobacco leaves (Figure 3).

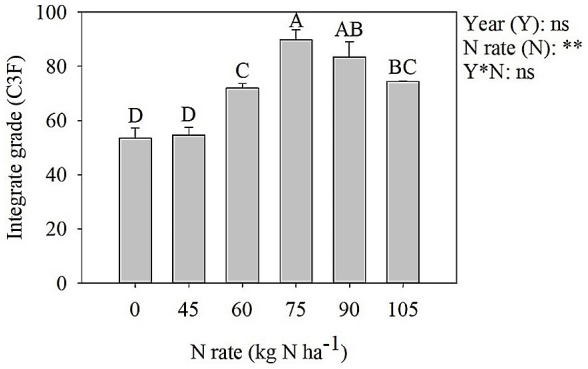


Figure 2. Effect of the N rate on the integrate grade of chemical quality of average over years in C3F grade. Integrate grade was calculated based on Table S2 and Table S4. Different capital letters indicate significant difference among the N rates ($p < 0.05$). Error bars represent the standard error of means.

Similar to the economic value, the total yield and yield of superior flue-cured tobacco first showed a trend of increase and then decreased with a higher N rate (Figure 4). The total yield and yield of superior flue-cured tobacco peaked to 3690 kg ha^{-1} at the N rate of 75 kg N ha^{-1} (Figure 4), which significantly increased by 27.3% and 37.0% compared with the currently recommended N rate of 105 kg N ha^{-1}, respectively.

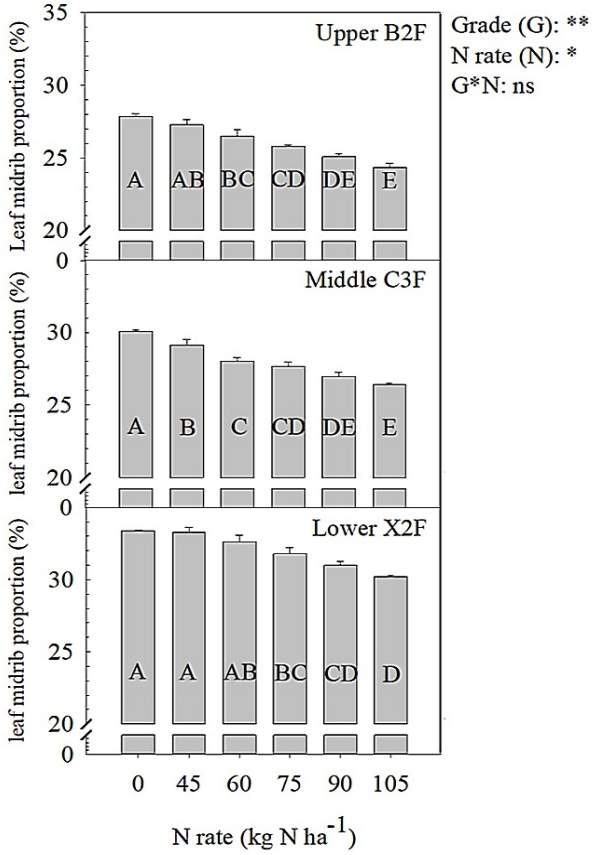


Figure 3. Effect of the N rate on leaf midrib proportions of upper B2F, middle C3F and lower X2F flue-cured tobacco leaves. Different capital letters indicate significant differences among the N rates in the same grade ($p < 0.05$).

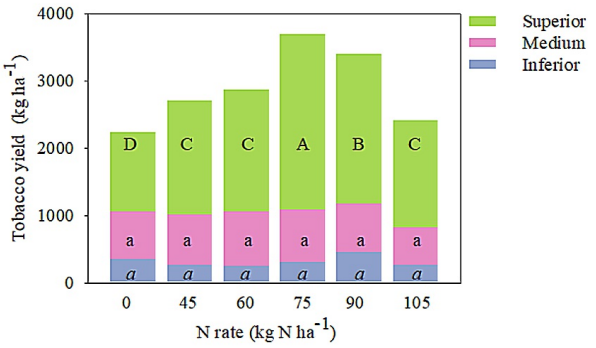


Figure 4. Effect of the N rate on the average yield of superior, medium and inferior flue-cured tobacco. Different capital, lowercase and italics lowercase letters indicate the significant differences in the yields of superior tobacco, medium and inferior tobacco, respectively ($p < 0.05$). The classification criterion and responding sales prices of superior, medium and inferior are shown in Table S2.

However, the yield of medium and inferior flue-cured tobacco was not significantly affected by the N rate. There was a significant positive correlation between the total economic value and the yield of superior flue-cured tobacco (Figure 5; $R^2 = 0.91$, $p < 0.05$), while there was no significant correlation with the yield of medium and inferior flue-cured tobacco.

The leaf growth rate at the prosperous growth stage (resetting - topping) was significantly higher than that at the resetting (transplanting - resetting) and maturing stage (topping - harvesting) (Figure 6). The maximum leaf growth rate appeared at the N rate of 75 kg N ha^{-1} regardless of growth stages. Compared with the current recommendation of 105 kg N ha^{-1}, the leaf growth rate at 75 kg N ha^{-1} was significantly increased by 17.8%. There was a significant positive correlation between the yield of superior tobacco and the leaf growth rate at the prosperous growth stage (Figure 7; $R^2 = 0.66$, $p < 0.05$).

Excluding two data points outside the 95% confidence zone, the sum of applied N and the residual soil inorganic N before transplanting showed a significant positive correlation with the total economic value of flue-cured tobacco and the yield of superior tobacco, respectively (Figure 8). Based on the total economic value and the yield of superior tobacco, the sum of N rate and residual soil N determined by the nitrogen effect equation was 130 kg N ha^{-1}, in which the optimal N rate was 66 kg N ha^{-1} and soil inorganic nitrogen content before transplantation was 64 kg N ha^{-1} (Figure 8).

4. DISCUSSION

Compared with the N rate of 105 kg N ha^{-1} recommended by the tobacco industry after years of field experiments, the optimal N rate of 75 kg N ha^{-1} determined in this paper significantly improved the leaf growth rate during the prosperous growth stage, and increased the total economic value of flue-cured tobacco, the yield of superior tobacco and the integrate grade of intrinsic chemical quality as well as the processing quality. The equation of N effect for the recommendation of optimal N application rate should include the amount of residual soil N before transplanting.

4.1. The leaf growth rate at the prosperous stage determines the economic value and quality of flue-cured tobacco

The economic value and quality of flue-cured tobacco are very sensitive to nitrogen supply. Suitable N application rates significantly increase the yields of superior flue-cured tobacco (Figure 4) (14). At the N rate of 75 kg N ha^{-1}, the economic value, the N economic benefit and the yield of superior flue-cured tobacco are significantly higher than those of all other N rates including the currently recommended 105 kg N ha^{-1} (Figure 1 and Figure 4). This is closely related to the effect of N supply at different growth stages on the commodity grade of flue-cured tobacco (24) which determines the sale price. The average sale price of superior tobacco is about 8 times higher than that of inferior tobacco (Table S2). This verified well by the significant positive correlation between the yield of superior tobacco and the total economic value of flue-cured tobacco (Figure 5). Besides the commodity quality of flue-cured tobacco, the chemical and processing qualities are the most affeceted indexes of tobacco purchasing and processing enterprises. The integrate grade of chemical

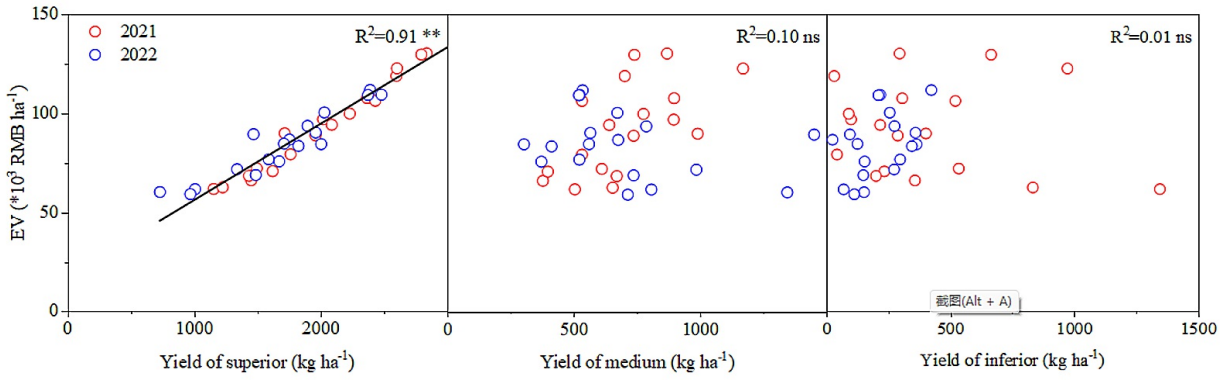


Figure 5. Correlation between the total economic value (EV) and yield of superior, medium and inferior flue-cured tobacco average over two years.

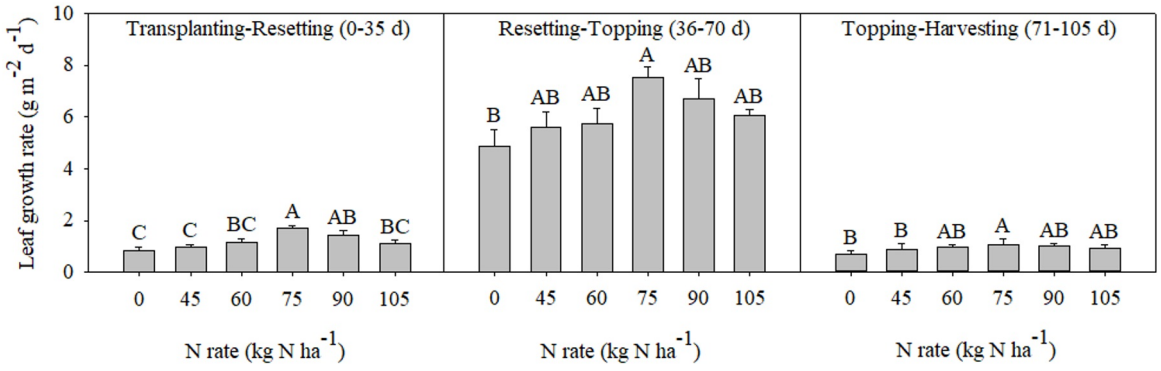


Figure 6. Effect of the N rate on the average leaf growth rate at resetting, topping and harvesting stage average over years. Different capital letters indicate significant difference among the N rates ($p < 0.05$).

quality is an important index to evaluate the coordination of chemical components of flue-cured tobacco (23). The integrate grade of chemical quality of C3F tobacco leaf, the most representative of superior flue-cured tobacco, was significantly higher at the N rate of 75 kg N ha^{-1} than that of all other N rates including the currently recommended N rate of 105 kg N ha^{-1} (Figure 2; Table S3). Leaf midrib proportion is an important index to evaluate the processing quality of flue-cured tobacco. If the leaf midrib proportion is too high, it will reduce the percentage of leaf strips yield. If it is too low, it will reduce the tensile strength and machinability (25). The leaf midrib proportion of flue-cured tobacco decreased significantly with increase of the N rate (Figure 3). The leaf midrib proportion of 75 kg N ha^{-1} was between high and low N rates. Therefore, optimizing the N management is the key to improve the yield and quality of superior flue-cured tobacco (Figure 4 and 5).

Superior tobacco mainly includes five grades, including upper (B1-2F) and middle (C1-3F) leaves mainly formed during the prosperous stage (resetting - topping) (Table S2).

The prosperous stage is the key period for the formation of flue-cured tobacco yield and quality (19). The leaf growth rate of flue-cured tobacco in the prosperous stage is significantly higher than that in both stages of resetting and maturing (Figure 6), and the peak of N uptake also occurs in this stage (26). The leaf growth rate of prosperous growth stage was significantly higher at 75 kg N ha^{-1} than that of other N rates (Figure 6), and showed a significant positive correlation with the yield of superior tobacco (Figure 7). Therefore, we can conclude that the leaf growth

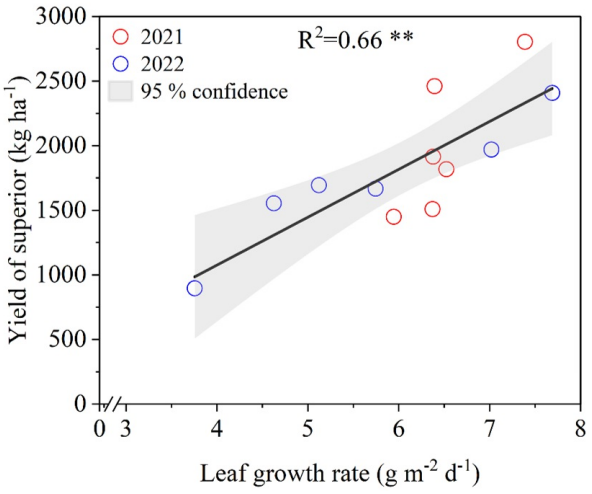


Figure 7. Correlation between the yield of superior tobacco and the leaf growth rate in the prosperous stage over six N rates and two years.

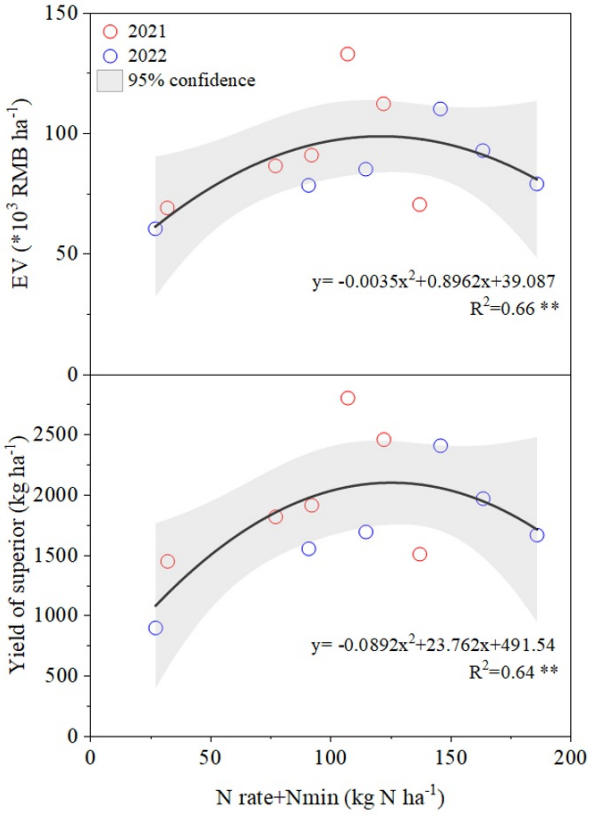


Figure 8. Nitrogen effect equation between the sum of applied N rate and the residual soil inorganic N before transplanting and economic value (EV) of flue-cured tobacco and superior tobacco yield. Two data points outside the 95% confidence were excluded for establish of the effect equation. The residual soil inorganic N content before transplanting is shown in Table S5.

rate of the prosperous growth stage is the key to determine the yield of superior tobacco and the total economic value (Figure 5). Optimizing the N supply rate with attention of soil residual N can not only promote leaf growth and improve the yield and quality of superior tobacco (Figure 4), but also help to avoid late ripening and the abnormal yellowing of tobacco leaves, which leads to a reduction of tobacco quality and sale prices (27) (Figure 2; Table S2).

4.2. Nitrogen effect equation including soil residual N is the optimal scheme to determine the optimal N application rate

The high quality and yield of flue-cured tobacco are the results of the combined action of N from soil and fertilizer, and at least 50% of the N absorbed by tobacco plants comes from the soil (28). Therefore, to recommend reasonable N application rates based on the residual soil N before transplanting, it should be the basic principle to optimize N management of flue-cured tobacco. This was well verified by the significantly positive correlation between the sum of soil N and N rate and the economic value and yield of superior flue-cured tobacco respectively (Figure 8). When the sum of soil N and N rate was 130 kg N ha^{-1}, the economic value and the yield of superior flue-cured tobacco reached the maximum (Figure 8). However, the currently reported nitrogen effect equation often ignored the effect of soil N (29). In particular, with the improvement of irrigation and transportation infrastructure, single tobacco cultivation has been converted into tobacco and vegetable double cropping.

The excessive N input in vegetable production results in a large amount of soil residual N before tobacco transplanting, which will not only affect the quality and economic value of flue-cured tobacco, but also leads to a high nicotine content and poor smoking qualities (30).

Too high or too low N application rates will affect the economic value and quality of flue-cured tobacco. Except the N rate, the percentages of base- and topdressing fertilizer also have a crucial impact (23). The prosperous stage has the highest leaf growth rate (Figure 6) and is the key growth stage for the formation of superior C3F and B2F tobacco leaves, which have reasonable chemical (Figure 2, Table S3) and processing qualities (Figure 3). Therefore, ensuring timely and sufficient N supply in the prosperous stage is the key to improving the chemical quality and sensory quality of flue-cured tobacco (31–32). If residual soil N was high before transplanting (Table S5), one should not only reduce the total N application rates, but also appropriately reduce base fertilizer rates, while increasing topdressing fertilizer rates to improve leaf growth rate in the prosperous stage (Table S6). On the contrary, the total N rate and proportion of base fertilizer should be appropriately increased when the residual soil N is low. In conclusion, optimizing the total N rate and the proportion in different growth stages according to soil residual N content before transplanting and N effect equation is the key to improve the total economic value and quality of flue-cured tobacco.

5. CONCLUSIONS

Conversion of single-tobacco-cultivation into tobacco–vegetable double production leads to high residual soil N before tobacco transplanting due to the excessive N fertilizer application rate in vegetable production. The currently recommended N rates of 105 kg N ha^{-1} without consideration of soil residual N is no more suitable for the production of flue-cured tobacco with high quality and economic value. According to the current soil residual N, our results demonstrate that the optimal N rate should be reduced to 66 kg N ha^{-1}, which significantly increases the economic value, yield and quality of flue-cured tobacco due to the improvement of superior tobacco yield and the daily leaf growth rate especially in the prosperous stage. Therefore, it is suggested that consultants and tobacco farmers should measure their soil residual N content before transplanting in order to determine the total N application amount and to adjust the N application ratio at different growth stages according to the nitrogen effect equation obtained in this paper.

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Supplementary Information.

Table S1. The annual average (1981–2020) and the experimental year (2021–2022) of precipitation, mean temperature and sunshine hours during the flue-cured tobacco growing season (April–August).

	Precipitation (mm)			Temperature (°C)			Sunshine duration (h)		
Month	1981–2020	2021	2022	1981–2020	2021	2022	1981–2020	2021	2022
April	28.0	64.5	35.6	18.2	19.2	16.7	223	231	227
May	80.1	22.5	69.8	20.3	21.8	18.3	208	229	219
June	152	166	43.8	21.2	21.9	21.1	131	129	130
July	210	107	27.6	21.2	21.5	22.6	116	96	106
August	162	199	95.4	21.4	21.5	22.4	149	124	137
Average	126	112	54.4	20.5	21.2	20.2	165	162	164
Max	268	199	95.4	31.8	33.1	31.1	241	231	227
Min	16.0	22.5	27.6	9.4	8.8	3.8	89	96	106

Table S2. The standard of grading superior, medium and inferior flue-cured tobacco and their corresponding purchase price.

Classification	Grade	Maturity	Structure	Thickness	Oil content	Chroma	Length (cm)	Mutilation (%)	Price (RMB kg^{-1})
Superior	B1F	Maturity	Slight loose	Slight thick	Many	Very strong	45	15	41.4
	B2F	Maturity	Slight loose	Slight thick	Medium	Strong	40	20	34.0
	C1F	Maturity	Loose	Medium	Many	Very strong	45	10	48.5
	C2F	Maturity	Loose	Medium	Medium	Strong	40	15	43.8
	C3F	Maturity	Loose	Medium	Medium	Medium	35	25	38.8
Medium	B3F	Maturity	Slight dense	Slight thick	Medium	Medium	35	30	22.0
	X2F	Maturity	Loose	Slight thin	Less	Medium	35	25	22.9
	X3F	Maturity	Loose	Slight thin	Less	Weak	30	30	11.9
	X2V	Slight maturity	Loose	Slight thin	Less	Medium	35	15	8.6
Inferior	CX1K	Slight maturity	Loose	Slight thin	Medium	—	35	20	5.8

Table S3. Effects of nitrogen N application rate on the average content of the chemical compound as intrinsic quality indexes of superior C3F tobacco leaf average over years (n = 6).

N rate (kg N ha^{-1})	Nicotine (%)	Total N (%)	Reducing sugar (%)	Total sugar (%)	K (%)	Cl (%)	Total sugar / nicotine	Total N / nicotine	K / Cl
0	1.40 ± 0.04	1.44 ± 0.01	21.5 ± 0.06	36.0 ± 0.79	1.43 ± 0.02	0.44 ± 0.04	30.1 ± 2.12	1.31 ± 0.09	3.43 ± 0.40
45	1.42 ± 0.03	1.51 ± 0.03	22.2 ± 0.47	36.7 ± 1.34	1.40 ± 0.02	0.47 ± 0.08	26.2 ± 0.72	1.22 ± 0.07	4.43 ± 0.38
60	1.56 ± 0.05	1.56 ± 0.02	19.8 ± 0.59	34.0 ± 0.91	1.66 ± 0.13	0.39 ± 0.02	22.2 ± 2.11	1.08 ± 0.06	4.72 ± 1.37
75	2.49 ± 0.07	2.20 ± 0.07	18.6 ± 0.94	28.1 ± 1.17	1.48 ± 0.07	0.42 ± 0.06	16.4 ± 0.33	0.79 ± 0.02	3.04 ± 0.06
90	2.38 ± 0.05	1.91 ± 0.08	20.6 ± 0.70	31.8 ± 0.96	1.40 ± 0.03	0.35 ± 0.04	12.2 ± 0.11	0.82 ± 0.03	4.42 ± 0.18
105	2.08 ± 0.04	1.72 ± 0.02	19.2 ± 0.08	31.2 ± 0.42	1.46 ± 0.02	0.37 ± 0.03	15.1 ± 0.77	0.82 ± 0.02	3.57 ± 0.34
$LSD_{0.05}$	0.28	0.23	2.84	5.59	0.29	0.27	6.16	0.31	2.73
Best range	2.20–2.80	2.00–2.50	18.0–22.0	26.0–30.0	≥ 2.50	≤ 0.60	8.50–9.50	0.95–1.05	≥ 8.00

Table S4. Range of chemical compound content and the corresponding grade of flue-cured tobacco style characteristics evaluation of the first most influential tobacco areas in Yunnan province – chemical composition evaluation table (C3F).

Compound	Grading rules				
	100	100–90	90–80	80–70	70–60
Nicotine (%) (B1)	2.20–2.80	2.20–2.00 2.80–2.90	2.00–1.80 2.90–3.00	1.80–1.70 3.00–3.10	1.70–1.60 3.10–3.20
Total N (%) (B2)	2.00–2.50	2.00–1.90 2.50–2.60	1.90–1.80 2.60–2.70	1.80–1.70 2.70–2.80	1.70–1.60 2.80–2.90
Reducing sugar (%) (B3)	18.0–22.0	18.0–16.0 22.0–24.0	16.0–14.0 24.0–26.0	14.0–13.0 26.0–27.0	13.0–12.0 27.0–28.0
K (%) (B4)	≥ 2.50	2.50–2.00	2.00–1.50	1.50–1.20	1.20–1.00
Total sugar / Nicotine (B5)	8.50–9.50	8.50–7.00 9.50–12.0	7.00–6.00 12.0–13.0	6.00–5.50 13.0–14.0	5.50–5.00 14.0–15.0
Total N / Nicotine (B6)	0.95–1.05	0.95–0.80 1.05–1.20	0.80–0.70 1.20–1.30	0.70–0.65 1.30–1.35	0.65–0.60 1.35–1.40
K/Cl (B7)	≥ 8.00	8.00–6.00	6.00–5.00	5.00–4.50	4.50–4.00
Integrated grade (IG)	IG = B1 × 0.18 + B2 × 0.10 + B3 × 0.15 + B4 × 0.09 + B5 × 0.26 + B6 × 0.12 + B7 × 0.10				

Table S5. Inorganic nitrogen content in 0–20 cm soil before tobacco transplanting (BefTrp) and after harvesting (AftHarv) in the experimental site (kg N ha^{-1}).

Time	N rate	Inorganic N	NH_4^+–N	NO_3^-–N
BefTrp (April 22, 2021)		32.0 ± 2.20	1.45 ± 0.65	30.5 ± 2.69
AftHarv (August 31, 2021)	0	23.3 ± 1.37	0.98 ± 0.12	22.4 ± 1.49
	45	25.9 ± 0.07	1.00 ± 0.05	24.9 ± 0.12
	60	28.1 ± 0.85	1.01 ± 0.11	27.0 ± 0.82
	75	31.7 ± 1.27	1.03 ± 0.04	30.7 ± 1.24
	90	34.8 ± 0.83	1.33 ± 0.26	33.5 ± 0.60
	105	47.4 ± 4.22	1.13 ± 0.16	46.3 ± 4.36
BefTrp (April 25, 2022)	0	27.0 ± 3.13	9.81 ± 0.45	17.2 ± 2.93
	45	45.7 ± 7.79	8.12 ± 0.24	37.6 ± 7.72
	60	54.5 ± 5.41	8.19 ± 0.21	46.3 ± 5.21
	75	70.6 ± 8.21	9.71 ± 0.33	60.8 ± 7.90
	90	73.3 ± 10.4	9.21 ± 1.54	64.1 ± 10.0
	105	80.7 ± 1.14	9.79 ± 1.09	70.9 ± 2.23
AftHarv (September 05, 2022)	0	21.0 ± 6.65	2.58 ± 0.40	18.5 ± 6.57
	45	56.2 ± 7.92	7.75 ± 0.42	48.4 ± 7.82
	60	62.9 ± 8.98	12.1 ± 2.71	50.8 ± 7.35
	75	76.3 ± 9.50	6.80 ± 0.29	69.5 ± 9.23
	90	90.4 ± 9.17	7.04 ± 1.54	83.4 ± 7.92
	105	97.7 ± 7.66	7.29 ± 2.40	90.4 ± 7.12

Table S6. Recommendation of the optimal total N application rate, and the N rate as base fertilizer at resetting and as topdressing at prosperous growth stage according to the soil inorganic N content before transplanting and nitrogen effect equation, and nitrogen application amount.

	Recommend N rate		
Soil N min (kg N ha^{-1})	Total (kg N ha^{-1})	Base fertilizer (kg N ha^{-1})	Top application (kg N ha^{-1})
20	110	44	66
30	100	40	60
40	90	36	54
50	80	32	48
60	70	28	42
70	60	24	36
80	50	15	35
90	40	5	35
100	30	0	30