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Precision harvesting technology of flue-cured tobacco based on SPAD value

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Abstract. The correct control of the maturity of flue-cured tobacco leaves is an important guarantee for improving the intrinsic quality of tobacco leaves, and it is also the premise for ensuring and improving the quality and external quality of cured tobacco leaves. In order to quantify the maturity of flue-cured tobacco, the chlorophyll meter was used to measure the leaves with different harvesting maturity levels of the middle and upper leaves, and the correlation between the measured value of chlorophyll meter (SPAD) and chlorophyll, yield and output value was established, and the precise harvesting technology of fixed leaf position was verified. The results showed that the SPAD values of the 3rd, 6th, 9th and 12th leaves had a linear relationship with the chlorophyll content, reaching a significant level, and the SPAD values of the four leaf positions showed a quadratic curve relationship with the yield quality of the cured tobacco leaves, and the SPAD threshold when the yield quality was optimal was obtained through the regression equation, combined with the measured SPAD values of each leaf position, the prediction interval of the SPAD values of the four leaf positions was as follows: 3rd leaves (10.9, 15.0), 6th leaves (13.7, 17.3), 9th leaves (16.9, 20.6), 12th leaves (20.7, 24.0), the tobacco leaves harvested at this time are 8~10 mature, the maturity is good, the yield quality of tobacco leaves is high, and the maturity grade of the middle and upper leaves is higher.

Keywords: precise harvesting, SPAD value, flue-cured tobacco, maturity.

1. Introduction

The maturity of flue-cured tobacco stands as a pivotal determinant impacting both its yield and quality, thus serving as a crucial aspect in ensuring the industrial viability and quality assurance standards of this tobacco variety [1].

Extensive research efforts, both domestically and internationally, have been dedicated to understanding the nuanced facets of flue-cured tobacco maturity. A prevailing consensus suggests that the optimal maturity, termed technological maturity, of flue-cured tobacco manifests when a harmonious balance of chemical components is achieved in the harvested leaves, resulting in desirable aroma, ample quantity, and superior yield and quality, all of which are conducive to the advancement of the cigarette industry [2-4].

SPAD values have emerged as valuable indicators of chlorophyll content, indirectly aiding in the assessment of tobacco leaf maturity. Recent years have witnessed a paradigm shift, with chlorophyll content becoming the primary quantitative index for evaluating tobacco leaf maturity, reflecting changes in plant physiological activity. However, it is pertinent to note that SPAD values are subject to variations influenced by factors such as tobacco varieties, growth stages, and measured leaf positions. Notably, previous studies have highlighted the middle part of fully expanded leaves as the optimal location for SPAD value determination in flue-cured tobacco.

Despite the significance of SPAD values, scant attention has been paid to their utilization in quantifying flue-cured tobacco maturity. Therefore, this study endeavors to fill this gap by investigating the changes in SPAD values and chlorophyll content across different leaf positions at varying harvest maturities, and their correlation with yield and quality. By analyzing the optimal SPAD values corresponding to desirable yield and quality outcomes across different maturity levels, this research aims to lay a theoretical foundation for accurately quantifying flue-cured tobacco maturity.

2. Materials and methods

2.1 Experimental site

Experiments were conducted in Gaoshui Village, Mashi Town, Shixing County, Shaoguan City, Guangdong Province from 2018 to 2019. A flue-cured tobacco variety K326 was used. The previous crop was rice, and the soil type was purple soil. The chemical properties of the 0-20cm soil profile were as follows: pH 7.38, organic matter 26.5 g kg-1, total nitrogen 1.77 g kg-1, total potassium 0.76 g kg-1, total potassium 15.7 g kg-1, available nitrogen 139.69 mg kg-1, available phosphorus 25.78 mg kg-1, available potassium 110.85 mg kg-1.

2.2 Experimental design

At the maturity stage, five maturity levels were set both in the middle leaf and the upper leaf, and each treatment was repeated three times. The treatment of upper and middle leaves in the same maturity treatment was carried out in the same plot, and a total of 15 plots were randomly arranged. About 65 plants were planted in each plot, and protected rows were set around the plot. The leaf number per plant was between 21 and 23, and planting density was 15000 plants ha-1. The different maturity treatments were controlled by advancing and delaying harvesting times (Table 1). Other management measures are carried out according to the standardized production requirements of local high-quality flue-cured tobacco.The leaf sample from each treatment was sampled according to the setting harvest time (Table 1). The samples in each plot were collected using a three-point sampling method between 9:00-11:00 am, and fixed as a replicate. Then the soil and water were cleaned from the tobacco leaves and cut them into two parts along the main veins. One part of the samples was determined by physiological indexes, and the other part of the samples was killed at 105 ℃ for 10 min and dried at 80 ℃ to constant weight, followed by ground them into powder and sealed for chemical analysis.

Based on the results from 2018, a verification experiment with flue-cured tobacco precision harvesting technology was further carried out in 2019. Two harvesting methods, i.e. normal harvesting and precision harvesting based on SPAD value, were set up in the middle and upper leaves of flue-cured tobacco. At the maturity stage, the sample time according to the testing SPAD value of the specified leaf, once which has reached the threshold SPAD value of middle or upper leaf, which can be harvested, respectively. Each treatment had three replicates, and the treatment of upper and middle leaves in the same maturity treatment was carried out in the same plot, a total of six plots were random block arrangements.

Table 1 The treatment code and harvest time used in the present study.

Leaf position	Grade of maturity	Treatment code	Harvest time
Upper leaves	Unripe	UM0	8 days earlier than UM2
	Mature	UM1	4 days earlier than UM2
	Ripe	UM2	Normal harvest time
	Mellow	UM3	4 days later than UM2
	Overmature	UM4	8 days later than UM2
Middle leaves	Unripe	MM0	8 days earlier than MM2
	Mature	MM1	4 days earlier than MM2
	Ripe	MM2	Normal harvest time
	Mellow	MM3	4 days later than MM2
	Overmature	MM4	8 days later than MM2

2.3 Experimental sampling and measurements

2.3.1 Leaf appearance description

In accordance with the tobacco industry standard of the "Tobacco agronomic traits investigation methods" in China, the leaf color, vein color (including main vein and branch vein), leaf surface fuzz, stem-leaf angle, leaf tip, leaf edge and auricle color were described.

2.3.2 Leaf age

After the tobacco seedlings were transplanted, 10 plants were randomly selected from each plot. The birth and harvest time of each middle and upper leaf were recorded during 10:00-11:00 am. The leaf birth time was recorded when the length of the leaf was above 5 cm.

2.3.3 Chlorophyll content

Chlorophyll content (SPAD) was measured using an SPAD-502 (Konica Minolta Inc., Tokyo, Japan) portable chlorophyll detector. At the maturity stage, the middle position between the leaf margin and the leaf vein from 3rd, 6th, 9th, 12th, 15th leaves in different treatments were detected six times, respectively （Fig.1; Fig.2）. The average value was the SPAD value of specific leaf.

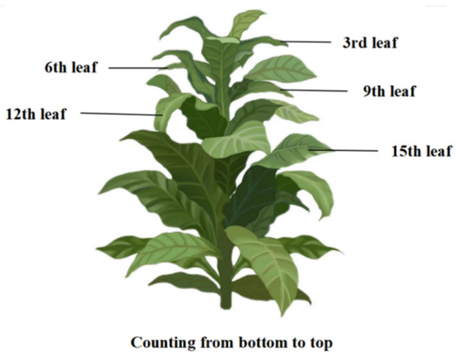

Fig 1 Tobacco K326 simulation model, the number of leaves are all counting from bottom to top.

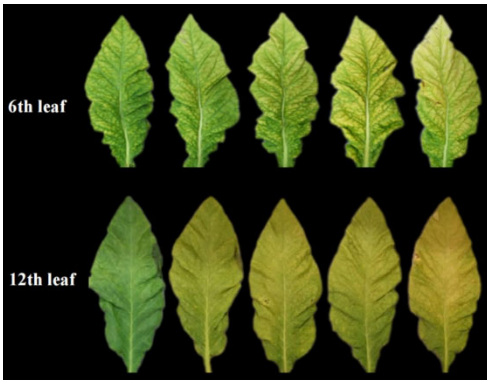

Fig 2 Photo of the 6th leaf and 12 th leaf of tobacco K326 at different growth stages.

Accordingly, the contents of chlorophyll a and chlorophyll b from 3rd, 6th, 9th, 12th, 15th leaves were also determined in different treatments. The fresh leaf was extracted with 95% alcohol and measured with a spectrophotometer at 649nm and 665nm, respectively.

2.3.4 Chemical compositions

C3F and B2F samples of flue-cured tobacco leaves were collected from each plot. The chemical compositions of flue-cured tobacco leaves of C3F and B2F grades were determined to Wang Ruixin [5].

2.3.5 Economic trait

The plots were listed to harvest and bake mature tobacco leaves.The flue-cured tobacco leaves were graded according to GB 2635-1992 [6].The price of each grade of tobacco leaf should refer to the local purchasing price of tobacco leaf, and calculate the yield, output value, average price and the proportion of upper and middle tobacco.

2.3.6 Data analysis

Charts were produced by Microsoft Excel 2007. Statistical analyses of data were carried out with SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1 Appearance and morphological characteristics

With the increase of maturity, the color of the leaves, main veins and branches, tip of leaf, auricles, the angle between stems and leaves, the hair on the leaf surface and the area of mature spots on the leaf surface have all changed significantly. The color of the leaves changed from green to varying degrees of yellow, and the color of the upper leaves varied greatly (Table 2). The leaf age and field growth period of flue-cured tobacco were prolonged with the delay of harvest.

3.2 Chloroplast pigment content and SPAD value

Chlorophyll is one of the main components affecting the quality and availability of tobacco leaves. It not only determines the color of tobacco leaves after modulation, but also its related degradation products are closely related to the aroma and aroma quantity of tobacco leaves. The chlorophyll content and the SPAD value of various parts of flue-cured tobacco gradually decreased with the increase of maturity (Fig. 3A; Fig. 3B). It can be seen that the improvement of maturity is conducive to the increase of chlorophyll degradation products in the upper leaves, that is, the increase of aroma preconditions (Fig. 3A). Compared with M 1, the SPAD values of each mature grade significantly decreased with the increase of leaf position, and the decrease in SPAD values of each part decreased with the decrease of leaf position when reaching M 4 grade (Fig. 3B). It can be seen that increasing the maturity of tobacco leaves has a significant impact on the SPAD values of the upper leaves compared to the middle leaves, and the SPAD values respond most intuitively to the changes in chlorophyll content of the upper leaves.

3.3 Correlation analysis between SPAD value and chlorophyll content

As maturity increases, there is a significant or extremely significant linear positive correlation between SPAD values and chlorophyll content in the middle and upper leaves. However, the correlation varies among different leaf positions, with the 3 rd leaf having the best correlation. As leaf positions decrease, the correlation coefficient decreases (Fig. 4). This indicates that the correlation between SPAD values and chlorophyll content in the upper leaves is relatively high in the later stage of maturity, which can accurately estimate the amount of chlorophyll content in the upper leaves. This further reflects the maturity level of tobacco leaves. Therefore, the SPAD values of the 3rd, 6th, 9th, 12th can be used as quantitative indicators to determine the maturity of flue-cured tobacco.

Table 2 The appearance maturities characteristic of the fresh tobacco leaves in five maturity levels.

Leaf position	Treatment	Leaf age (d)	Growth periods (d)	Appearance characteristic							
				Leaf color	Main vein color	Branch vein color	Leaf tip and leaf edge condition	Leaf ear	Angle between stem and leaf	Pubescence	Mature macula
Upper leaves	UM0	65.68	129	Strong green	Green-white	Green	Slightly undulating leaf margin	Strong green	Larger(60~90°)	Much	No
	UM1	72.34	135	Green	1/2 whitened	About 1/2 turn white	Wrinkled leaf edge	Dark green	Larger(60~90°)	More	No
	UM2	76.22	136	Yellow-green	4/5 whitened to full white	1/2~2/3 turn white	Starting to scorch	Light yellow	Large(80~90°)	Partly shedding	Begin to appear
	UM3	80.56	141	Light yellow	All white hair bright	2/3 turn white	More withered tips and withered edges	Light yellow	Very large(>90°)	Mostly shedding	Clearly mature macula
	UM4	86.28	145	Yellow bubble through white	All white hair bright	All white hair bright	Withered tips with burnt edges	All yellow	Very large	2/3 shedding	Much
Middle leaves	MM0	66.23	108	Strong green	Green-white	Light green	Flat leaf margin	Dark green	Smaller(40~50°)	Much	No
	MM1	61.68	113	Dark green	1/2 whitened	About 1/3 turn white	Slightly undulating leaf margin	Green	Small(50~60°)	More	No
	MM2	66.89	117	1/2 yellow	1/2~2/3 turn white	1/3~1/2 turn white	Leaf tip is scorched and hooked down	Light green	Larger(60~90°)	Partly shedding	Begin to appear
	MM3	70.00	121	2/3 yellow	4/5 all white hair bright	2/3 turn white and light yellow	1~2cm scorched	Light yellow	Large(80~90°)	More shedding	Less
	MM4	76.28	127	Yellow bubble through white	All white hair bright	All white hair bright	More withered tips and withered edges	Light yellow	Very large(>90°)	2/3 shedding	More

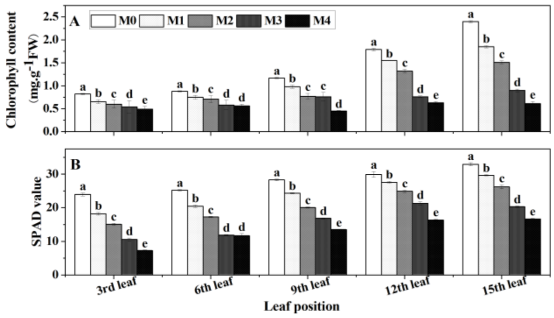

Fig 3 Effects of different parts of the 3rd leaf, 6th leaf, 9th leaf, 12th leaf and 15th leaf of tobacco K326 treated with M 0, M 1, M 2, M 3 and M 4 on chlorophyll content and SPAD value. (A) Chlorophyll content. (B) SPAD value. Values with the same letter are not significantly different at P<0.05, comparisons within the same parameter only. Bars denote the standard error of the means.

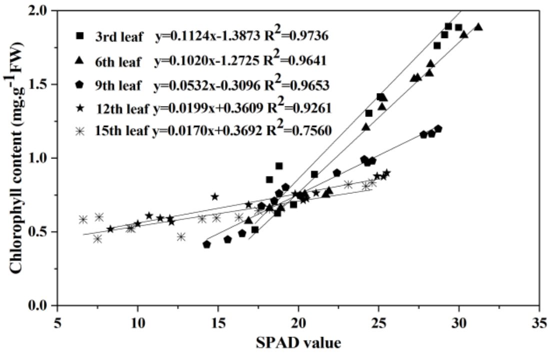

Fig 4 Correlation analysis of interaction between different SPAD values and chlorophyll content in the 3rd leaf, 6th leaf, 9th leaf, 12th leaf and 15th leaf. These straight lines are all fitted.

3.4 Research on precision harvesting technology of flue-cured tobacco based on SPAD instrument

3.4.1 Correlation analysis between SPAD value and maturity

According to the maturity discrimination method in production, tobacco leaves with different maturity levels are assigned values, with a score of 10.0 representing ten mature, i.e. fully mature. Therefore, the scores of each maturity level from low to high are 4.0 points, 6.0 points, 8.0 points, 10.0 points, and 12.0 points. The relationship between SPAD and maturity was linear, and the correlation reaches a significant or extremely significant level (Fig. 5). It can be seen that after grading maturity, the SPAD values can be used to more intuitively represent the maturity level of tobacco leaves. By using a linear regression equation, the SPAD harvest values corresponding to different maturity levels of each leaf position can be derived,and the SPAD value intervals corresponding to each maturity level can be determined. This result provides a theoretical basis for further research on the SPAD threshold for precise harvesting of flue-cured tobacco.

3.4.2 Economic characters

Yield, output value, proportion of top grade tobacco, and average price are the main indicators for measuring the economic characteristics of flue-cured tobacco. The yield of tobacco leaves harvested too early and too late is significantly reduced, but delayed harvesting significantly improves the maturity of tobacco leaves (FIG 5). Output value, proportion of top grade tobacco, and average price have little impact on the proportion of medium grade tobacco in flue-cured tobacco. The economic characteristics of M 0 and M 1 treatments are relatively poor, so delaying harvesting for 4 to 8 days is appropriate. The output value and proportion of high-quality tobacco in flue-cured tobacco are higher, and the economic characteristics are better.

3.4.3 SPAD harvesting threshold

The SPAD values of the four leaf positions show a clear quadratic relationship with the yield (Fig. 6A) and output value (Fig. 6B) of cured tobacco leaves. As the maturity increases and the SPAD value decreases, the yield of cured tobacco leaves first increases and then decreases. Therefore, combining the regression equations of the eight curves, the SPAD values corresponding to the highest yield and output value of each leaf position are calculated. Therefore, the SPAD value range for mature harvesting of the 3rd leaf is 10.9~15.0; 13.7~17.3 for the 6th leaf; The number of the 9th leaf ranges from 16.9 to 20.6, and the 12th leaf is 20.7~24.0, and the interval value is substituted into the regression equation between SPAD value and maturity score. It is found that the maturity of flue-cured tobacco at each leaf position was M 2 - M 3 when the highest yield and output value are achieved.

Table 3 Effect of different maturity on economic characters of cured tobacco leaves. The analysis of variance for the data in the table was conducted using Duncan's new complex range method. The difference between two data with the same letter in the same column and different treatments did not reach a significant level of 5%, while the difference between two data with different letters reached a significant level of 5%.

Treatment	Yield/ (Kg.hm-2)	Converted output value/ (yuan.hm-2)	Proportion of superior tobacco/(%)	Proportion of medium tobacco/(%)	Average price/ (yuan/kg)
M0	2790.36±32.34b	34358.15±113.34d	18.32±0.62c	29.56±0.22b	12.31±0.24c
M1	3062.79±40.82a	39944.37±135.91c	19.32±0.33c	34.32±0.78b	13.04±0.21b
M2	3186.9±45.88a	42035.95±123.96b	21.29±0.40c	52.25±0.46a	13.57±0.17b
M3	3097.20±39.73a	42978.45±213.71a	46.04±0.91a	45.96±0.62a	13.88±0.16b
M4	2766.70±46.75b	42413.28±202.77ab	31.55±0.62b	46.48±0.83a	15.33±0.17a

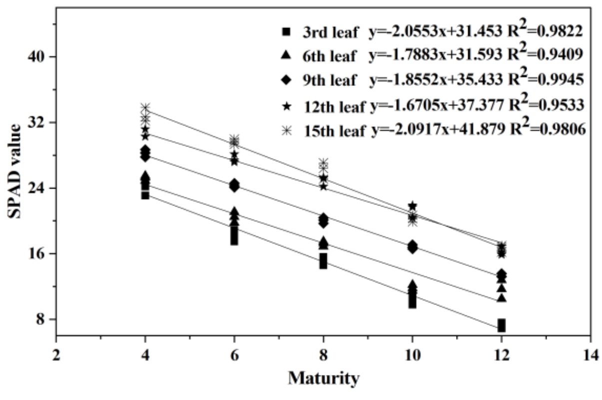

Fig 5 Correlation analysis of the interaction between different maturity and SPAD values in the 3 rd leaf, 6 th leaf, 9 th leaf, 12 th leaf and 15 th leaf. These lines are all fitted.

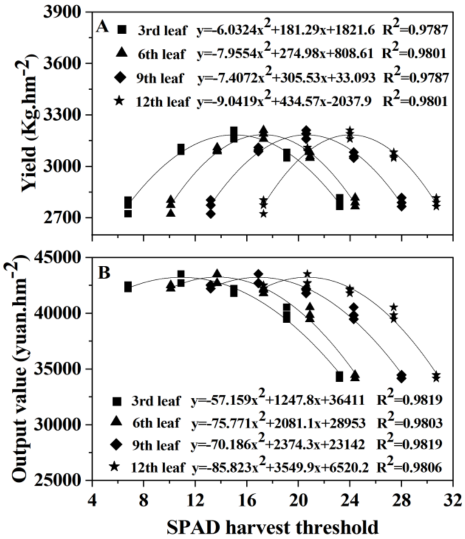

Fig 6 Correlation analysis of the 3 rd leaf, 6 th leaf, 9 th leaf and 12 th leaf harvest threshold with the output value and yield. (A) Yield. (B) Output value. The curves are the fit lines.

3.5 Precision harvesting and productive validation of flue-cured tobacco based on SPAD instrument

The precise harvesting method of flue-cured tobacco based on SPAD instrument has little impact on the yield of cured tobacco leaves (Table 4). The precise harvesting method based on SPAD values reduces the subjective bias in empirical judgment of maturity, significantly improves the maturity of tobacco harvesting, improves the quality of tobacco, and thus increases the output value.

The soluble sugar content in the upper and middle leaves of precision harvested flue-cured tobacco increased (Table 5). The variation trend of reducing sugar content varies among different parts, with a significant increase in the upper leaves and a significant decrease in the middle leaves. The high potassium content of tobacco leaves in the Shixing tobacco region is one of its characteristics, and the potassium content in various parts of accurately harvested tobacco leaves significantly increases compared to that harvested by farmers. The quality of flue-cured tobacco is not determined by the content of a single component, but by the coordination between the various components. The main factor affecting the taste of flue-cured tobacco is the balance and coordination of acidic and alkaline substances formed during the pyrolysis of tobacco leaves during combustion. Accurately harvested roasted tobacco leaves result in more coordinated carbon and nitrogen metabolism, and good leaf balance.

Table 4 Effect of different harvesting methods on economic characters of cured tobacco leaves.The analysis of variance for the data in the table was conducted using Duncan's new complex range method. The difference between two data with the same letter and different treatments in the same column did not reach a 5% significant level, while the difference between two data with different letters reached a 5% significant level.

Treatment	Yield/ (Kg.hm-2)	Converted output value/ (yuan.hm-2)	Proportion of superior tobacco/(%)	Proportion of medium tobacco/(%)	Average price/ (yuan/kg)
Farmer harvesting(CK)	3065.34±35.34 a	39233.54±179.59 b	39.34±0.23 b	44.32±0.78 a	12.80±0.26 b
Precision harvesting(M3)	2997.20±39.73 a	44158.33±254.79 a	49.58±0.41 a	45.87±0.66 a	14.73±0.16 a

Table 5 Effect of different harvesting methods on chemical composition of cured tobacco leaves. The analysis of variance for the data in the table was conducted using Duncan's new complex range method. The difference between two data with the same letter and different treatments in the same column did not reach a 5% significant level, while the difference between two data with different letters reached a 5% significant level.

Grade	Treatment	Soluble sugar/(%)	Starch/(%)	Reducing sugar/(%)	Nicotine/(%)	Total nitrogen/(%)	Potassium/(%)	The ratio between sugar and alkali	The ratio between nitrogen and nicotine	Shi Muke value
B2F	Farmer harvesting(CK)	18.68±0.32b	4.68±0.14a	14.56±0.09b	3.63±0.05a	2.38±0.04a	1.78±0.23b	4.01±0.31b	0.65±0.04a	1.57±0.08b
	Precision harvesting(M3)	20.32±0.10a	3.57±0.11b	18.45±0.16a	3.01±0.01b	1.98±0.01b	2.51±0.15a	6.13±0.15a	0.66±0.03a	2.13±0.21a
C3F	Farmer harvesting(CK)	21.60±1.26b	4.19±0.26a	24.39±0.63a	2.82±0.01a	1.69±0.01b	2.53±0.34b	9.66±0.23a	0.60±0.02a	2.24±0.32a
	Precision harvesting(M3)	23.21±0.65a	3.83±0.15b	21.96±0.18b	2.62±0.10b	1.81±0.10a	2.89±0.25a	8.38±0.26b	0.69±0.02a	2.79±0.17b

4. Discussion

The investigation into the relationship between SPAD values and chlorophyll content, yield, and quality of middle and upper leaves using the SPAD instrument yielded significant insights. Notably, a significant or extremely significant positive correlation was observed between SPAD values at different maturity levels and leaf positions and the total chlorophyll content of flue-cured tobacco. Among these, the 3rd, 6th, 9th, and 12th leaf positions exhibited the strongest correlation, making them ideal indicators for assessing flue-cured tobacco maturity. Additionally, a clear quadratic curve was evident in the relationship between SPAD value and both yield and output value of flue-cured tobacco. This underscores the importance of establishing SPAD threshold ranges for each leaf position.

Optimal yield and output value of flue-cured tobacco were achieved when the SPAD threshold ranges were as follows: 10.9-15.0 for the 3rd leaf, 13.7-17.3 for the 6th leaf, 16.9-20.6 for the 9th leaf, and 20.7-24.0 for the 12th leaf. These findings align with practical production scenarios, indicating that the maturity of flue-cured tobacco falls within the range of 8 to 10. Importantly, experiments conducted over two consecutive years corroborated the efficacy of utilizing the SPAD instrument for assessing flue-cured tobacco maturity, suggesting its potential for widespread adoption in production settings.

Overall, the establishment of SPAD threshold ranges at different leaf positions provides valuable guidance for assessing flue-cured tobacco maturity. The findings of this study support the feasibility and utility of employing the SPAD instrument in practical production contexts, offering a promising approach to enhance productivity and quality in flue-cured tobacco cultivation.

5. Conclusions

In conclusion, this study provides a valuable reference for the precise harvesting method of fixed leaf position. However, more research is needed to further deepen this approach and better apply it in practice.

Acknowledgments

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