Effect of Removing the Lower Leaves on the Physiological Features and Aroma Constituents of Flue-Cured Tobacco

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Abstract: Yunyan 97 was selected as the raw material. The effects of removing different number (0, 2, 3 and 4) of lower leaves on root activity, chlorophyll content, physiological features and contents of neutral aroma constituents in the flue-cured tobacco were analyzed. The results showed that the removal of lower leaves could significantly increase the root activity, chlorophyll content, net photosynthetic rate ($P_n$) and transpiration rate ($T_r$), and delay the photosynthetic functional decline. Such effects were the greatest in lower leaves, followed by middle leaves and upper leaves. Moreover, the degree of the effects increased with higher number of leaves removed. After the lower leaves were removed, the water use efficiency ($WUE$) of leaves in the first 10 d became higher with more leaves removed. In the later periods (24 d, 38 d), $WUE$ decreased with more leaves removed. For the middle and upper leaves, the removal of three leaves (T2) and two leaves (T1) resulted in the highest contents of aroma constituents, respectively. For the tobaccos cultured in soil with moderate fertility under the experimental conditions, the appropriate number of lower leaves removed should be 2-3.

Key words: Physiological features, aroma constituents, flue-cured tobacco.

1. Introduction

The State Tobacco Monopoly Administration launched the program of “optimizing the grade structure of tobacco leaves and improving the capacity of high-quality tobacco leaf supply” in 2011. The purpose of this program is to address the issues of large inventory of tobacco leaves, the elevating grade structure of tobacco leaves and the structural conflict of raw material. This is also in accordance with the strategy of “upgrading the brand of tobacco” and developing “532” and “461” as large brands and large market. The removal of poor-quality tobacco leaves in the field is the critical point of this strategy \cite{1}. So far, some studies have been carried out for optimizing the grade structure of tobacco leaves. The research by Huang et al. \cite{2} showed that the timely removal of bottom leaves and top leaves could optimize the grade structure and economic traits of the tobacco leaves considerably. Zhong et al. \cite{3} believed that the removal of two bottom leaves and two top leaves and the remaining of 18 leaves for the tobacco grown in Wuling County, Fujian Province could make the tobacco leaves easy to be flue-cured. Moreover, the mean price and output value were also the highest.

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According to the report by Zhang and Liu [4], the high cutting of bottom leaves and double topping not only increased the quantity and quality of tobacco leaves and optimized the grade structure, but also enhanced the usability. The effects of the removal of some poor-quality tobacco leaves on the physiological features and aroma constituent contents of the tobacco plants have not been reported yet. An experiment was carried out in Wulong County, Chongqing City in this article. The effects of the number of leaves removed on root activity, chlorophyll content, photosynthetic characteristics and aroma constituent content were studied. The optimal number of tobacco leaves removed under the climate conditions of Chongqing was determined. The results provide a reference for optimizing grade structure of tobacco leaves and increasing the capacity of high-quality tobacco leaf supply.

2. Materials and Methods

2.1 Experiment Design

The experiment was carried out in Xingshun Town, Wulong County, Chongqing in 2013, with Yunyan 97 as the material. The experimental field had an altitude of 1,300 m, with a flat terrain and uniform fertility. The soil was medium loam and the pH value was 6.25. The contents of organic matter, alkali hydrolysable nitrogen, available phosphorus and available potassium were 25.24 g/kg, 89.01 mg/kg, 19.95 mg/kg and 202.31 mg/kg, respectively. The application dosage of nitrogen fertilizer was 113.45 kg/hm², with N:P:K = 1:1.5:2.5 and basal dressing:hole applied fertilizer:after fertilizer = 7:2:1. The floating system was used to cultivate the seedlings. Transplanting was performed on May 4, with a row spacing of 110 cm × 55 cm. The bottom leaves were removed on June 20, and the topping was carried out on July 1. After topping, 18 leaves were retained for each plant. During the topping period, two (T1), three (T2) and four (T3) lower leaves were removed, respectively. No lower leaves were removed in the control check (CK). There were 100 plants in each treatment, which were arranged randomly in six ridges with three replicates.

2.2 Determination Method

Twelve representative tobacco plants in each replicate were selected and marked. Three plants were tested for photosynthetic indicators. Before removal, the 5th, 10th and 16th (from bottom to top) leaves representing the lower, middle and upper leaves were marked. The remaining nine plants were tested for root activity. The root samples were taken and brought back to laboratory to test the root activity.

2.2.1 Photosynthetic Indicators

Starting from 10 d of treatment, the measurement was done once every 14 d, and a total of three measurements were performed. Li-6400 Portable Photosynthesis System (Licor, USA) was used to determine net photosynthetic rate \( \left( P_{n} \right) \) and transpiration rate \( \left( T_{r} \right) \) at 9:00-11:00, and water use efficiency \( WUE = P_{n}/T_{r} \). The experimental conditions were controlled as follows: CO₂ concentration 360 \( \mu\text{mol/mol} \), light intensity 1,200 \( \mu\text{mol/m}^2\text{s} \), and temperature 25 °C.

2.2.2 Chlorophyll Soil and Plant Analyzer Development (SPAD) Value

SPAD-502 Plus Chlorophyll Meters (Konica, Japan) was used to determine the SPAD value. For each point, 30 measurements were done and the mean was taken.

2.2.3 Root Activity

The root activity was determined by the ability to reduce 2, 3, 5-triphenyltetrazolium chloride (TTC) [5].

2.2.4 Statistical Analysis

All data presented are the mean values. The data were subjected to the analysis of variance, appropriate to the design of the experiment. Significant differences among treatment means were determined using the least significant difference \( (P < 0.05) \) at the 1% level of probability, using statistical product and service solutions (SPSS) 13.00.
3. Results

3.1 Effect on Root Activity

The effect of removal of lower leaves on the root activity of tobacco is shown in Table 1. As seen from the Table 1, the root activities at the three measurement time points were enhanced with greater number of leaves removed. Except for the removal of T1, the differences with CK all reached significance level. The differences between the removal of T2 and T3 were not extremely significant at three measurement time points. For all measurement periods, the results were always the highest in T3 treatment. According to the law of root activity attenuation, the sharpest decline of root activity occurred from July 11 to 25 in all treatments. The decline speed was accelerated with the increasing number of leaves removed. When the soil fertility is higher, the excess removal of lower leaves may probably enhance the absorption ability of the roots. As a result, the maturity of the tobacco leaves will be affected due to over-nutrition.

3.2 Effect on SPAD Value of Leaves

The measurements of SPAD value after the removal of lower leaves are given in Table 2. It can be seen that the removal of lower leaves had a great impact on the SPAD value. The SPAD values of leaves in different parts and time periods all rose with more leaves removed. This tendency of SPAD value seemed to enhance with the going of the growth period. The effects were the greatest in the lower leaves, followed by the middle leaves; the effects were the smallest in the upper leaves. This is because the removal of lower leaves improved the ventilation and light penetration, which created a favorable environment for the growth of middle and upper leaves [4]. Such effects tended to be weakened from bottom to top. Therefore, the improper removal of lower leaves may lead to increased content of chlorophyll in the middle and upper leaves and hence late maturing. The economic benefits reaped from tobacco growing will be lowered.

Table 1  Differences in tobacco root vigor under different treatments of fresh leaf.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>July 11</th>
<th>July 25</th>
<th>August 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>160.64 ± 4.81 A</td>
<td>102.88 ± 1.50 A</td>
<td>103.66 ± 0.65 A</td>
</tr>
<tr>
<td>T1</td>
<td>203.42 ± 3.62 A</td>
<td>132.02 ± 4.19 AB</td>
<td>116.21 ± 2.15 AB</td>
</tr>
<tr>
<td>T2</td>
<td>271.51 ± 4.70 B</td>
<td>152.87 ± 2.12 B</td>
<td>134.74 ± 5.83 BC</td>
</tr>
<tr>
<td>T3</td>
<td>311.94 ± 3.30 B</td>
<td>156.16 ± 1.07 B</td>
<td>144.89 ± 1.21 C</td>
</tr>
</tbody>
</table>

Different capital letters stand for significant differences at 0.01 levels.

Table 2  Differences in chlorophyll content under different treatments of fresh leaf.

<table>
<thead>
<tr>
<th>Position</th>
<th>Treatment</th>
<th>July 11</th>
<th>July 25</th>
<th>August 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower leaf</td>
<td>CK</td>
<td>36.25 ± 0.65 A</td>
<td>30.55 ± 1.05 A</td>
<td>26.90 ± 0.60 A</td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>37.40 ± 1.10 A</td>
<td>33.85 ± 0.35 B</td>
<td>30.50 ± 0.70 B</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>39.25 ± 0.45 B</td>
<td>36.05 ± 0.75 C</td>
<td>33.90 ± 0.30 C</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>41.90 ± 0.80 C</td>
<td>38.00 ± 0.80 D</td>
<td>36.20 ± 0.60 D</td>
</tr>
<tr>
<td>Middle leaf</td>
<td>CK</td>
<td>39.90 ± 0.50 A</td>
<td>38.80 ± 0.90 A</td>
<td>30.65 ± 0.95 A</td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>41.15 ± 0.75 AB</td>
<td>40.00 ± 0.70 A</td>
<td>33.70 ± 0.50 B</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>41.90 ± 0.70 B</td>
<td>42.00 ± 0.90 B</td>
<td>35.70 ± 0.70 C</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>44.85 ± 0.65 C</td>
<td>44.35 ± 0.35 C</td>
<td>41.05 ± 0.45 D</td>
</tr>
<tr>
<td>Upper leaf</td>
<td>CK</td>
<td>46.15 ± 0.55 A</td>
<td>45.90 ± 0.70 A</td>
<td>43.70 ± 0.40 A</td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>46.75 ± 0.25 A</td>
<td>47.35 ± 0.50 A</td>
<td>45.23 ± 1.35 B</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>48.10 ± 0.30 B</td>
<td>50.00 ± 0.60 B</td>
<td>46.55 ± 0.85 B</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>49.60 ± 0.70 B</td>
<td>52.50 ± 0.60 C</td>
<td>46.91 ± 0.55 C</td>
</tr>
</tbody>
</table>

Different capital letters stand for significant differences at 0.01 levels.
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3.3 Effect on Photosynthetic Indicators

3.3.1 Effect on Net Photosynthetic Rate

As shown in Fig. 1, after the lower leaves were removed, the $P_n$ of leaves of different parts for different time periods became higher with more leaves removed. This indicates that the removal of lower leaves helped improve the photosynthetic ability and facilitated the accumulation of photosynthetic products. At 10, 24 and 38 d of treatment, the $P_n$ of T1, T2 and T3 in the lower leaves was increased by 15.17%, 39.71%, 45.92%, and 34.54%, 41.27%, 47.46%, and 25.37%, 40.00%, 50.27% compared with the control, respectively. In the middle leaves, the $P_n$ was increased by 16.32%, 36.48%, 43.45%, and 22.00%, 28.57%, 40.55%, and 17.18%, 26.08%, 34.71%, respectively. The more lower leaves removed, the greater the effect on $P_n$ would be. The effect was the greatest in lower leaves, followed by middle leaves and upper leaves in sequence.

As shown by Fig. 2, with the progression of growth period, the photosynthetic functions of leaves declined constantly. The decline became especially sharper in middle and lower leaves with more leaves removed. However, for the upper leaves, the decline speed was gradually slowed down with more leaves removed.

3.3.2 Effect on Transpiration Rate

It is seen from Fig. 3 that the removal of lower leaves had a great effect on $T_i$ of the leaves. As more leaves were removed, the $T_i$ gradually increased, and the differences between the treatments were significant. Except for the upper leaves (10 d), $T_i$ was
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the highest in T3 in all time periods. As shown in Fig. 4, \( T_r \) of lower leaves in T1, T2 and T3 was increased by 41.74%, 23.16% and 30.71% compared with the control, respectively; the increase was 24.42%, 47.21% and 43.39% in the middle leaves, and 21.67%, 32.74% and 54.24% in the upper leaves, respectively. Thus, the removal of lower leaves did improve \( T_r \) of the leaves. Such effects became more apparent with

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Fig. 3 Effect on \( T_r \) of different treatments of fresh leaf.

Fig. 4 Change of \( T_r \) of different treatments of fresh leaf.

Fig. 5 Effect on WUE of different treatments of fresh leaf.
the progression of the growth period.

3.3.3 Effects on WUE

As seen in Fig. 5, the removal of lower leaves had great impact on WUE. The WUE of leaves of different parts all showed similar variation rule. After 10 d, WUE increased as more leaves were removed. At 24 d and 38 d, the opposite variation was found. WUE was the highest in CK or T1. The more leaves removed, the lower the WUE would be. As seen from Fig. 6, WUE gradually decreased over time. The more leaves removed, the faster the WUE declined. In the lower leaves, the WUE of T1, T2 and T3 decreased by 36.40%, 42.00% and 58.31% compared with the control, respectively; in the middle leaves, the decrease was 41.60%, 65.31% and 62.50%, respectively; in the upper leaves, the decrease was 46.57%, 53.00% and 66.75%, respectively.

3.4 Effects on the Contents of Aroma Constituents

Gas chromatograph-mass spectrometer was used to determine the contents of neutral aroma constituents, which were analyzed as phenylalanines, maillard reaction products, cembranenoids, carotenoids, neoplytadiene and other. The results are given in Table 3.

It is seen that the removal of lower leaves had a significant impact on the content of aroma constituents in the middle leaves. In the middle leaves, phenylalanines, cembranenoids, neoplytadiene and total aroma constituents increased as more leaves were removed. The contents were the highest in T2 treatment. The contents of cembranenoids and other showed an increasing trend with more leaves removed. The variation of the content of maillard reaction products was not regular. The content was the highest in T3 treatment and lowest in T2 treatment. The contents of carotenoids, neoplytadiene, other and total aroma constituents in the upper leaves first increased and then decreased as more leaves were removed. The highest contents were found in T1, and after that, the

![Fig. 6  Change of leaf WUE in different treatments of fresh leaf.](image)

<table>
<thead>
<tr>
<th>Volatile aroma components</th>
<th>Middle leaf</th>
<th>Upper leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CK</td>
<td>T1</td>
</tr>
<tr>
<td>Phenylalanines</td>
<td>12.36</td>
<td>10.43</td>
</tr>
<tr>
<td>Maillard reaction products</td>
<td>23.46</td>
<td>18.01</td>
</tr>
<tr>
<td>Cembranenoids</td>
<td>41.13</td>
<td>39.48</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>66.92</td>
<td>58.18</td>
</tr>
<tr>
<td>Neoplytadiene</td>
<td>701.61</td>
<td>871.36</td>
</tr>
<tr>
<td>Other</td>
<td>1.35</td>
<td>1.63</td>
</tr>
<tr>
<td>Total aroma constituents</td>
<td>846.82</td>
<td>999.08</td>
</tr>
</tbody>
</table>
contents gradually decreased. The contents of pheylalanines, maillard reaction products and cembranenoids were the lowest in T2 and T1. Therefore, the content of aroma constituents was the highest in the middle leaves in T2, and in T1, the highest content was found in the upper leaves.

4. Discussion

Root is the major organ of water and mineral uptake in plants. Root activity is the comprehensive indicator of the absorption ability of plants [6]. It is found that leaf-cutting and topping can increase the root activity of tobacco seedlings and tobacco plants [7]. Our results also indicated that the removal of lower leaves facilitated the root activity of tobacco plants. The root activity was higher and attenuated more rapidly as more leaves were removed. However, it was still the highest in T3 in all time periods. The reason is that the removal of lower leaves has the similar effect as leaf-cutting and topping. By improving the field microclimate and nutrient allocation in plants, the root growth could be facilitated. Therefore, improper treatment of lower leaves might enhance the root absorption ability and disrupt the nutrition balance. One of the consequences is late maturing and poor flue-curing quality.

As the site of photosynthesis, chlorophyll plays an important role in photosynthesis [8]. The content of chlorophyll can directly reflect the yield and quality traits. In addition to the nutritional status of plants, the chlorophyll content is also subjected to the influence of field microclimate. Cui et al. [9] reported that shading could considerably lower the chlorophyll content in leaves of summer maize. Bi et al. [10] believed that the chlorophyll content in the flue-cured leaves tended to decrease with the increasing planting density. As shown by the present article, the removal of lower leaves increased the chlorophyll content by improving the ventilation and light penetration. Such effects were weakened from bottom to top. In production practice, the lower leaves should be properly removed depending on soil and the nutritional status of the tobacco plants, so as to ensure the normal maturity of the leaves.

Photosynthesis is associated with the substance accumulation and persistence of metabolism in plants [11]. The detection of photosynthetic level is an important means of analyzing the impacts of environmental factors [12]. Photosynthetic products are the basis for the formation of crop quantity and quality. Over 90% of the dry matter is directly or indirectly produced by photosynthesis [13]. Thus, improving the photosynthetic performance of the tobacco is a basic method of increasing yield and quality [14, 15]. Our experiment showed that the removal of lower leaves had remarkable effect in improving the photosynthetic performance and the accumulation of photosynthetic products. The effect was the greatest in lower leaves, followed by the middle leaves and upper leaves. As more leaves were removed, the effect was enhanced. Moreover, the removal of lower leaves could delay the photosynthetic function decline of the leaves. This is because the removal of lower leaves stimulated the secondary growth of the root system and enhanced the absorption ability. By improving the micro-ecological environment of the population, the chlorophyll content and nutrition level of tobacco plants were increased. As a consequence, the photosynthetic function of the leaves was elevated. The report by Wang et al. [16] also demonstrated the effect of decreased planting density: it not only improved the micro-ecological environment and $P_n$ of tobacco leaves, but also delayed the photosynthetic function decline.

Photosynthesis and transpiration of leaves are the gas exchange processes that occur simultaneously. The leaf stomata regulate the changes of photosynthesis and transpiration. The $P_n/T_r$ ratio determines $WUE$ of the leaves [17]. $WUE$ refers to the level of organic matter produced per unit of water consumed by the plant [6]. $WUE$ is an important indicator of the relationship between plant water
consumption and substance production [18]. The value of WUE reflects the adaptability of plant to environmental stress [19]. According to the results of the present study, the removal of lower leaves had a significant impact on \( T_r \) and WUE of leaves. The more leaves removed, the greater the \( T_r \) was. The reason was that the removal of lower leaves improved the microclimate of population as well as the ventilation and light penetration, thereby increasing the transpiration rate. Zhang et al. [20] reported that similar variation was found in soybeans. The number of leaves removed had a complex effect on WUE. In the early stage, WUE increased as more leaves were removed. In later stage, WUE decreased, the reason for which needs to be further studied. Therefore, the improper removal of lower leaves may result in the increase of transpiration and the decrease of WUE, which further affects the resistance to drought stress.

The aroma of tobacco leaves is an important measure of industrial usability. The aroma quality is determined by the contents of aroma constituents. The formation of aroma constituents is controlled by many factors, including ecology, variety, cultivation and modulation. As shown by our results, the removal of lower leaves had a considerable effect on the content of aroma constituents in the middle and upper tobacco leaves. When three leaves were removed (T2), the contents of phenylalanine, carotenoid degradation products, neoplytadiene and aroma constituents of the middle leaves were the highest. For the upper leaves, the removal of two leaves resulted in the highest contents of carotenoid degradation products, neoplytadiene, other aroma constituents and total aroma constituents.

5. Conclusions

To sum up, the removal of lower leaves enhanced the root activity and the mineral absorption ability of tobacco plants. By improving the microclimate of the population, the chlorophyll content and net photosynthetic rate of the leaves were increased, and the photosynthetic function decline was delayed. Thus, the leaves could accumulate more photosynthetic products. By causing the increase of transpiration rate, the WUE of the leaves was reduced, and the water demand was increased. The contents of aroma constituents in leaves were increased, and the quality of tobacco leaves was improved. In practice, the number of lower leaves removed should be determined by the soil fertility, nutrition of tobacco plants and the climate conditions. This is important to prevent the disruption of nutrition balance of tobacco plants or the late maturing, so as to increase the economic benefits for the tobacco growers. The measurements of root activity, chlorophyll content, photosynthetic performance, water use efficiency and aroma constituent contents showed that the optimal number of lower leaves removed should be 2-3 on the medium fertility soil under the corresponding ecological environment.

References


