 Spatial distribution of SPAD value and determination of the suitable leaf for N diagnosis in cucumber

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Abstract. To determine the best leaf position for nitrogen diagnosis in cucumber with SPAD meter, greenhouse experiments were carried out to study spatial distribution of SPAD value of different position of the 3rd fully expanded cucumber leaf in the effect of different nitrogen levels, and the correlations between SPAD values and nitrogen concentration of chlorophyll. The results show that there is remarkable different SPAD value in different positions of the 3rd fully expanded leaf in the flowering and fruiting stage. Comparing the coefficients of SPAD value variation, we find that the coefficient of variation of leaf edge was significantly higher than the edge of the main vein, and the coefficient of variation of triangular area of leaf tip is significantly higher than any other leaf area. There is a significant correlation between SPAD values and leaf nitrogen content. Preliminary study shows that triangular area of leaf tip from the 20% leaf tip to leaf edge is the best position for nitrogen diagnosis.

1. Introduction

Nitrogen is the main component of chlorophyll, which is also a part of the enzyme associated with chlorophyll synthesis. 75% nitrogen in the leaf is located in chloroplast [1-2]. Chlorophyll meter (SPAD) has advantages of easy and fast operation and nondestructive monitoring of plant nitrogen nutrition level, etc. [3]. In recent years, it is widely applied in soybean, rice, lettuce, peanut and other plants [4-12], it is obvious that the chlorophyll meter values and plant nitrogen content have strong correlation. Leaf is the primary organ for photosynthesis of plants. The research result of Zhang Yanli etc. [13] show that the leaf SPAD value in each cucumber leaf position is increased with the increase of nitrogen application rate. However, the increase of leaf SPAD value is prominently different in different leaf positions. It is concluded that the third leaf of the cucumber seedling stage and flowering stage, and the 7th leaf of the fruiting stage have the most sensitive reaction to nitrogen application level. They can be regarded as the best positions for diagnosing deficiency of cucumber nitrogen. It is obvious that there is prominent spatial difference among different leaf positions. Li Lantao [14] believes the follows after comprehensive: the middle of the 4th fully expanded leaf at the top of stem is the best test leaf position and site for monitoring rape nitrogen nutrition by SPAD meter, the correlation among SPAD value, chlorophyll content, leaf nitrogen content and plant total nitrogen content reaches significant or extremely significant level at the site, which meets the requirements of nitrogen nutrition rapid diagnosis.
The above analysis shows that the most sensitive blade of cucumber on nitrogen in different growth stages has been studied. However, the research on sensitivity of different sites of the same cucumber leaf to nitrogen is not reported till present. In the paper, the correlation between leaf SPAD value and leaf nitrogen content is analyzed by exploring the change rule of SPAD value on different sites of the cucumber, thereby determining the leaf sensitive site for cucumber leaf nitrogen nutrition diagnosis, thereby providing the best measurement site for cucumber nitrogen nutrition diagnosis.

2. Materials and methods

2.1. Test design
Cucumber variety for testing was Jinyou No. 1. After cucumber seeds were sprouted on a 25/20 °C wet gauze four days later, the seedlings with the same size were transplanted into a basin. The basin diameter was 22 cm, its height was 20cm, and the planting matrix was perlite. Cucumber seedlings grew in the greenhouse of agricultural equipment engineering college of Jiangsu University. The humidity was kept at 70 ±10%, and cucumber was planted with Yamazaki formula. When the cucumber grew to four leaves and one core (seedling stage) nitrogen treatment was started, and data were measured at seven leaves and one core (flowering stage). Cucumber was divided into four groups for nitrogen treatment experiment: N0- severe nitrogen deficiency treatment; N20-moderate nitrogen deficiency treatment; N100- normal nitrogen treatment; N200-nitrogen overtreatment. Normal nitrogen treatment (N100) belonged to complete Yamazaki formula. Ca(NO$_3$)$_2$.4H$_2$O and KNO$_3$ were eliminated from the nutrient solution of nitrogen deficiency group, and CaCl$_2$ and KC1 were respectively used for supplementing calcium and potassium. NaNO$_3$ was used for adding nitrogen in the over-nitrogen group.

2.2. Data collection and analysis

2.2.1. Sampling method and determination of chlorophyll meter (SPAD) value, SPAD-502 was used for respectively measuring the SPAD value of three positive leaves in the flowering stage in different sites of the leave (the first positive leaf, the second positive leaf and so on from the bottom up). Each leaf was divided into 78 position points shown in fig.1, which are symmetrical on both sides of the leaf main vein, and the SPAD value of each point was determined. 8 leaves were measured in each treatment, and the average value was obtained.

![Figure 1. Sampling methods of leaf](image)
2.2.2 Leaf sampling method and determination of nitrogen content, the third positive leaf of the flowering stage was 23.2±0.8 long and 27.8±0.6 wide. A homemade thin iron plate with circular hole was used for cutting blade, wherein the blade was 28 cm long and 30 cm wide. The 7th positive leaf of fruiting stage was 24.4±0.9 long and 29.6±0.9 wide, and a homemade thin iron plate with circular hole was used for cutting blade, wherein the blade was 30 cm long and 30 cm wide. The homemade thin iron plate with circular hole was divided into ten parts respectively and equally along the length and width, a total of 100 holes were formed, which were placed on the fully spread positive 3rd leaf blade. The blade was cut according to the holes on the thin iron plate with square hole, the branches on the sampling sheet were eliminated after sampling. The leaves symmetrical on both sides of the main vein were placed together during classification. 8 leaves were measured in each treatment, the average value was obtained, and they were numbered and placed in an envelope according to figure 1. The leaves were killed out at 105 °C for 30 min, and dried at 80 °C to constant weight. The dry weight was weighed. The leaves were ground, a suitable amount of leaves was weighed, the Kjeldahl method is used for measuring leaf nitrogen content.

2.2.3 Data analysis, Origin 8.0 software was used for data analysis, wherein variation coefficient CV = (standard deviation/average value) *100%. In the paper, the variation coefficient referred to the SPAD value change degree in different sites of the cucumber leaf under different nitrogen nutrition levels. The variation coefficient was larger, the site was more sensitive to the change of nitrogen.

3. Results and analysis

3.1 Blade distribution rules of cucumber SPAD value during flowering stage

![Figure 2. The SPAD values of different distance from main vein to leaf edge](image-url)
Figure 3. The SPAD values of different distance from leaf base to leaf tip

There are significant distribution differences in SPAD value of different sites on the third fully expanded leaf of cucumber during flowering stage (FIG. 2 and FIG. 3). Figure 2 shows that SPAD value shows gradually decreasing change trend from the main leaf vein to the leaf margin. The SPAD value close to the main vein part is significantly higher than that on the leaf edge with more serious nitrogen deficiency, and the change trend is more obvious. Figure 3 showed that the SPAD value had the change trend of decreasing after increasing from the leaf base to the leaf tip. The SPAD value was higher, and the change was the minimum in 40%–80% area from the leaf base. The SPAD value was smaller in the leaf base and the leaf tip. The results were similar to the measurement result on corn and wheat. It was obvious that the SPAD value under different nitrogen levels had prominent difference. The SPAD value at different sites of the leaf were also greatly different. The SPAD value difference at different sites was also greater and greater with aggravation of nitrogen stress.

3.2. Variation coefficient analysis on chlorophyll meter value at different sites of cucumber leaf during flowering stage

The variation coefficients of SPAD value at different sites of the third fully expanded leaf of cucumber under different nitrogen nutrition levels during flowering stage were compared. It was obvious that the variation coefficient of SPAD values at different sites were prominently different. It was not consistent with uniform yellowing of the leaf during cucumber nitrogen deficiency, which is consistent with prominently different SPAD values in different sites. The variation coefficient average value of SPAD value in the whole leaf is 17.8%. In the relative distance 0–60% scope at both sides close to the main vein (yellow and green areas in figure 4), the variation coefficient average value of the SPAD value in 4A, 4B, 5B, 5C, 6A, 6B, 6C, etc. is 15.6%. It was obvious that the area was not sensitive to the change of nitrogen. The variation coefficient of SPAD value in the area between the leaf edge and relative distance 40% away from the leaf edge (orange area in figure 4) was 18.1% on average. The variation coefficient was higher in the area. It was obvious that the area from the leaf edge to the 6cm in the middle of the blade or so had high sensitivity to the change of different nitrogen levels. The variation coefficient with the highest SPAD value in the whole blade was mainly located in the triangle part at the tip of the blade (red area in figure 4). The result showed that the surrounding area at the tip of the blade was the most sensitive to the change of different nitrogen levels. Therefore, the whole blade showed that the leaf tip triangle area between the relative distance 20% at the tip of the leaf and the leaf edge was the most sensitive to nitrogen (red area in figure 4), which can reflect the change of nitrogen nutrition most easily.
Figure 4. The map of distribution of coefficients of SPAD value variation in different position (CV%) in the flowering stage

4. Conclusion
The cucumber leaf SPAD value is highly correlated with leaf nitrogen content. The distribution condition of SPAD value at different sites of the blade is analyzed. It is discovered that the SPAD value among different sites are greatly different. The nitrogen stress is more severe, the difference is more prominent. It is obvious that SPAD value can be used for diagnosing the sensitivity of different site nitrogen changes. The SPAD value variation coefficient at different sites are prominently different. It is inconsistent with the overall yellowing features visible by eyes during blade nitrogen deficiency. In general, the SPAD value variation coefficient in the area at both sides close to the main vein outside the tip of the leaf is the minimum. It is obvious that the sites in the area are not sensitive to the nitrogen changes. The SPAD value variation coefficient in the area 6cm in the middle of the blade edge is commonly high, and the value has relatively small difference, which is stable. It is obvious that the sites in the area are more sensitive to the changes of nitrogen. However, the SPAD value variation coefficient in the triangle area at the tip of the cucumber leaf between the relative distance 20% to the leaf edge is the highest in the whole leaf. It is obvious that the triangle area has faster and more sensitive response to the nitrogen supply change than other area of the blade. It can most represent the nitrogen nutrition status of the whole blade. Therefore, it is the most ideal area for nitrogen nutrition diagnosis of cucumber blade. In the paper, the method of determining the best nitrogen diagnosis measurement site in the cucumber leaf by studying the change law of SPAD value in different sites of the cucumber blade is also applicable to other crops. However, the blade shape of different crops and blade nutrition distribution rules are greatly different, the sites of different crops with the highest sensitivity to the nitrogen changes should be further tested and studied.

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