Leaf Positions of Potato Suitable for Determination of Nitrogen Content with a SPAD Meter

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Abstract: Hand-held SPAD meter can be used to evaluate the leaf nitrogen status of potato. For practical use, it is necessary to select a proper compound leaf, a proper leaflet within compound leaf and position of leaflet suitable for measurement. Therefore, field experiments were conducted in northern China in 2009 and 2010. The SPAD values, plant growth, N uptake of potato plants at tuber initiation and tuber bulking stages under different N supply levels, and final tuber yields were examined. The criteria for determining the most suitable leaf, leaflet and position within a leaflet are that the SPAD values show less variation at a given N supply level, and show a more sensitive response to different nitrogen levels. Our results showed that the coefficients of variance of SPAD values ranged from 8.7 to 25.9% with a leaf N concentration range of 2.1 to 3.8 gN 100 g⁻¹ at tuber initiation stage, and 7.2 to 21.6% with leaf N concentration range of 0.96 to 1.26 gN 100 g⁻¹ at the tuber bulking stage. The SPAD values of the 4th compound leaf from apex were more stable and more sensitive to the nitrogen level than those of other leaves, suggesting that the 4th compound leaf is suitable for estimating the leaf N status using a SPAD meter. Within a compound leaf, the SPAD value of the top leaflet was more sensitive than the other leaflets to nitrogen supply, whereas it was less stable, making it difficult to chose the leaflet for measurement. However, the top leaflet emerges and expands much earlier than the side leaflets, and should be better for SPAD value measurement. The SPAD measurements at the top point of the top leaflet of the 4th leaf demonstrated both less variation and higher sensitivity to nitrogen supply. Therefore, we conclude that the top point of the top leaflet of the 4th compound leaf is the best position for potato N status diagnosis using a SPAD meter.

Key words: Leaf position, N status, Potato leaf, SPAD value.

Potato can be planted over a wide range of soils and climatic conditions. Optimal nutrient management is critical to obtain a high tuber yield and good quality of potato. Improper nutrient application may lead to lower yield or environmental pollution. It is well known that matching crop N requirements with mineral N supply throughout the growing season can minimize the risk of either environmental pollution or yield loss. This is particularly important for potato that has a shallow root system resulting in lower efficiency of taking up N than other crops such as cereals, sugar beet, and maize. For example, only 20% of mineral N applied at planting is taken up by the potato crop in northern China (Dai et al., 2000). Nitrogen present in soil layers deeper than 50 cm is poorly available to the potato crop. Therefore, the most appropriate time, rate and placement of N fertilization will be very important to optimize N use in potato cultivation.

In order to apply N fertilizer to potato accurately, we must identify the N demand of plants. One of the tools used to diagnose N status of plant is SPAD chlorophyll meter that was first proposed by Inada in Japan in 1963. Inada (1963) found that SPAD values were well correlated not only with chlorophyll concentration, but also with nitrogen concentration of crop leaves. Since then, many research groups had reported the use of an SPAD meter to assess the nitrogen status of crops, e.g., for rice (Turner and Jund, 1991; Johnkutty and Palaniappan, 1995; Peng et al., 1996; Balasubramanian, 2000; Singh et al., 2002), wheat (Follett and Follett, 1992; Reeves, 1993; Singh et al., 2002) and maize (Pickielek and Fox, 1992; Blackmer and Schepers, 1995; Carlos et al., 2001). The first attempt to use the SPAD chlorophyll meter for potato was reported by Vos and Bom (1993). They showed that chlorophyll meter values were well correlated with analytical measurements.
of the chlorophyll content and with the N concentration in the leaves of potato \((r^2=0.95)\). Further investigations have been conducted later by Minotti et al. (1994), Gianquinto et al. (2003, 2004) and Olivier et al. (2006). Previous studies strongly suggest that SPAD hand-held chlorophyll meter is a promising tool to assess the nitrogen status of potato foliage, and offers opportunities to minimize the influences of other factors on N estimation based on SPAD measurements.

Leaf position is critical for determining the N status diagnosis of potato using an SPAD meter. Vos and Bom (1993) measured the distal leaflet of the youngest fully expanded 4th or 5th leaf; Minotti et al. (1994) measured the leaflet adjacent to the terminal leaflet of the 4th or 5th leaf; and Gianquinto et al. (2003) chose the apical leaflet of the 3rd–5th leaf. However, it is unknown whether there is a difference between leaves or leaflets in accuracy of detecting potato N status. As the best leaf position, it has to be sensitive to variations in plant N concentration, precise, and able to detect N deficiency as early as possible. A typical potato leaf is a compound leaf that consists of several leaflets, being more complex than cereal crop leaf. Therefore, it is important to test variation of SPAD values with the position of the leaf and leaflet in potato under different N supply levels.

The objectives of this study were to investigate: (i) variations in the SPAD values with leaf positions in potato plants at the tuber initiation stage to tuber bulking stage, which correspond to the time of N fertilizer supply, (ii) variations in the SPAD values with the position of leaflet in one leaf, (iii) variations in the SPAD values with the measuring points within leaflet, and (iv) the response of the SPAD values to nitrogen levels.

**Materials and Methods**

1. **Experiment 1**

The field experiment was conducted at Salaqi (40°55′N, 110°52′E), an important potato production region in northern China in 2009. The soil properties are shown in Table 1. The prior crop was spring wheat. Potato (cv. Kexin-1) was planted on 5 May, and was harvested on 27 September. The plot size was 30 m², and plant density was 45000 plants ha⁻¹ with 90 cm of row distance and 25 cm of plant distance. The treatments included six nitrogen rates of 0, 75, 150, 225, 300, and 375 kg N ha⁻¹ as urea (denoted by N0, N1, N2, N3, N4 and N5) with two times of applications, 50% at planting, 50% at 15 days after emergence. All plots were treated with 150 kg P₂O₅ ha⁻¹ and 450 kg K₂O ha⁻¹ as K₂SO₄.

**Table 1.** Soil properties of the two different experimental sites.

| Exp. No | Sites     | Soil texture | pH | Organic matter (g 100 g⁻¹) | Nmin (mg kg⁻¹) | P (Sodium bicarbonate) mg kg⁻¹ | K (Sodium bicarbonate) mg kg⁻¹ |
|---------|-----------|--------------|----|--------------------------|---------------|-------------------------------|-------------------------------|
| 1       | Salaqi    | Sandy loam   | 7.2| 1.1                      | 40.7          | 16.1                          | 92.0                          |
| 2       | Damaoqi   | Sandy loam   | 7.5| 1.0                      | 34.2          | 14.0                          | 102.0                         |

SPAD values in the same way as in Experiment 1.

2. **Experiment 2**

The field experiment was conducted at Damaoqi (41°72′N, 110°25′E) in 2010, another important potato production region in northern China. The soil characters are shown in Table 1. Potato (cv. Kexin-1) was planted on 9 May after spring wheat, and was harvested on 26 September. The plot size was 30 m², plant density was 45000 plants ha⁻¹ with 90 cm of row spacing and 25 cm plant distance. The treatments included six nitrogen rates of 0, 75, 150, 225, 300, and 375 kg N ha⁻¹ as urea (denoted by N0, N1, N2, N3, N4 and N5) with two times of applications, 50% at planting, 50% at 15 days after emergence. A randomized block design was used with four replications. All plots were treated with 150 kg P₂O₅ ha⁻¹ and 450 kg K₂O ha⁻¹ as K₂SO₄.

Biomass: Potato plants were harvested at 30 and 50 days after emergence. Five plants per plot were harvested each time. Plants of each treatment were divided into leaves,
stems, stolons and roots. Samples were dried in an oven at 60ºC for 48 hr before weighing. Leaf N: Dried leaf samples were ground to pass through a 1-mm sieve, and mineralized in Kjeldahl flasks using the wet method with H₂SO₄ and catalysts H₂O₂ and CuSO₄. After digestion, solutions were cooled and diluted with deionized water to the specified volume. Total nitrogen was determined using the standard Kjeldahl method based on distillation of ammonia with steam from the alkalized solution, and determining the ammonia content in the distillate by titration.

3. **Statistical analysis**

Results were subjected to analysis of variance (ANOVA) to determine F values of different variation factors, and Duncan’s multiple range test was used to determine the differences between the treatments at 0.05 significant level, coefficient of variation was also used for the analysis (SPSS Ver.16.0 for Windows, SPSS Inc., Chicago, IL, USA).

**Results and Discussion**

1. **Variation of the SPAD values with the measuring point in a leaflet**

The results of analysis of variance in experiment 1 shows that SPAD values of potato leaves varied with the position of the measuring point in a leaflet (Table 2). The SPAD values at the top point of a leaflet (Table 3) were higher than those at the middle and basal points at 30 and 50 days after emergence, i.e., at the stages of the initiation and the bulking of tuber. The variation of SPAD values at different measuring points within a leaflet might be mostly due to the different developmental stage or thickness of the tissue of the leaflet.

If the SPAD readings at the measuring point vary too much with the plant under same N level, it must interfere the accuracy of N diagnosis. Therefore, the readings at a certain point in a leaflet should be stable among different plants under the same N level. The coefficients of variation (CV) of SPAD values with the measuring point of leaflet are shown in Table 4. At both harvests, the CV of the values obtained at the top measuring point was least (Table 4), suggesting that the top position of a leaflet meets the requirement for the N diagnosis.

2. **Variation of SPAD values with the position of measured leaflets**

The SPAD values significantly varied with the position of the measured leaflets in a compound leaf at both stages of
tuber initiation and tuber bulking. The SPAD values of the top leaflet were higher than those of the 1st side and 2nd side leaflets (Table 3).

The coefficients of variation (CV) of the SPAD values of the leaflet at different positions in a compound leaf as shown in Table 4. The results showed that the CVs of the SPAD values decreased with the age of the leaflets. At both sampling times, the CV of top leaflet (oldest) SPAD value was higher than those of 1st side and 2nd side leaflets. This suggests that top leaflet may not be suitable for plant N diagnosis, and side leaflets might be more suitable with respect to variation.

3. Variation of the SPAD values with the position of measured compound leaf

The SPAD values of the 3rd, 4th and 5th compound leaves from the apex were examined in this study. There was a significant difference among SPAD values obtained on different potato compound leaves at the stages of tuber initiation and tuber bulking. The SPAD value of the 3rd compound leaf was higher than that of the 4th and 5th leaves at the tuber initiation stage. At the tuber bulking stage, the SPAD values of both 3rd and 4th compound leaf were higher than that of the 5th (Table 3), but the SPAD values of all 3 leaves decreased.

At both tuber initiation and tuber bulking stages, the CV of SPAD value of the 3rd compound leaf was higher than those of 4th and 5th leaves, and the CV in the 4th compound leaf was least (Table 4). The CVs in all compound leaves decreased with the growth stage. This study indicates that the 4th compound leaf may be the suitable leaf, because it had least CV of SPAD values at both tuber initiation and tuber bulking stages.

4. Relationships between the SPAD values and the leaf nitrogen concentration

Results in Table 5 show that the biomass and leaf N concentration of potato increased with N levels. The leaf N concentrations at tuber bulking stage were lower than those at tuber initiation stage. Correlation analysis shows that SPAD values were highly correlated with leaf nitrogen concentrations regardless of measuring point, leaflet or compound leaf (Table 6). The SPAD value at the top point of the leaflet more closely correlated with leaf nitrogen concentration at both growth stages. The coefficient of correlation of SPAD value of the top leaflet with the leaf N concentration was higher than those of other leaflets at tuber initiation stage. At the tuber bulking stage, however the coefficients of correlation of the SPAD value of the top leaflet or 1st side leaflet with the foliar nitrogen concentration was greater than those of the 2nd leaflets, and the coefficients of correlation of the SPAD value of the 3rd or 4th compound leaf with the leaf N concentration was higher than those of the 5th compound leaf at the tuber initiation stage, whereas at tuber bulking stage, the correlation coefficient in the 4th compound leaf was

| Measuring points | CV (%) | Leaflets | CV (%) | Compound leave | CV (%) |
|------------------|--------|----------|--------|----------------|--------|
| Tuber initiation stage |        |          |        |                |        |
| Top               | 9.0    | Top      | 10.2   | 3rd            | 25.9   |
| Middle            | 9.1    | 1st side | 9.4    | 4th            | 15.2   |
| Basal             | 9.2    | 2nd side | 8.7    | 5th            | 19.7   |
| Tuber bulking stage |       |          |        |                |        |
| Top               | 7.2    | Top      | 9.1    | 3rd            | 21.6   |
| Middle            | 7.5    | 1st side | 7.1    | 4th            | 12.9   |
| Basal             | 7.5    | 2nd side | 7.1    | 5th            | 13.6   |

| N levels (kg ha\(^{-1}\)) | DW (g plant\(^{-1}\)) | Leaf N Content (gN 100 g\(^{-1}\)) | Tuber yield (t ha\(^{-1}\)) |
|---------------------------|----------------------|-----------------------------------|--------------------------|
|                           | 35 DAE               | 55 DAE                           | 35 DAE                   | 55 DAE                     |                           |
| 0                         | 20.00 b*             | 34.00 d                          | 2.10 c                   | 0.96 c                     | 21.0 d                   |
| 75                        | 22.35 b              | 40.14 c                          | 2.70 d                   | 0.97 c                     | 24.3 c                   |
| 150                       | 29.40 a              | 47.40 b                          | 3.10 c                   | 1.11 bc                    | 29.0 b                   |
| 225                       | 30.00 a              | 54.00 a                          | 3.50 b                   | 1.16 b                     | 35.0 a                   |
| 300                       | 33.10 a              | 56.40 a                          | 3.60 ab                  | 1.20 ab                    | 33.0 a                   |
| 375                       | 28.80 a              | 58.92 a                          | 3.80 a                   | 1.26 a                     | 34.5 a                   |

*The same letter in the same column denotes that there is no significant differences (P=0.05) by Duncan’s multiple range test.
higher than those in the others.

The sensitivity of the response of leaf SPAD values to nitrogen levels is important to determine the best leaf position. This study showed that the SPAD value of the 4th compound leaf was more closely correlated with leaf N concentration than those of the 3rd and 5th (Table 6), which indicates that the SPAD values of 4th compound leaf respond to nitrogen levels more sensitively and confirmed that 4th compound leaf from apex is the best leaf for N status diagnosis of potato by using a SPAD meter.

The closer correlation of the SPAD value in the top leaflet with the foliar nitrogen concentration suggests that the top leaflet responds to nitrogen supply rates sensitively; therefore, the top leaflet might be better for N status diagnosis. However, this conflicts with conclusion from the variation analysis of the SPAD values among leaflets, which suggested that side leaflets are more suitable, because side leaflets had more stable SPAD values than the top leaflet (Table 4). However, the top leaflet emerges and expands much earlier than side leaflets, therefore, the top leaflet may be better for SPAD value in practice.

Among the three measuring points in a leaflet, the SPAD value at the top point was most closely correlated with leaf N concentration at both growth stages suggesting that the SPAD value at the top point is most sensitive to nitrogen supply, confirming that top point in a leaflet can be ideal position for taking SPAD values (Table 4).

It is well known that tuber initiation and tuber bulking stages are two key periods for potato N nutrition. Therefore, N fertilizer is usually applied at the early stages of these two periods. Insufficient N at these key periods may lead to tuber yield loss, and excess N may not only cause environmental problems but also tuber quality problem. Therefore, the optimum time for diagnosis of N status in potato should include these two stages, although this research was not designed to evaluate the effects of N diagnosis throughout the potato growth period.

In conclusion, this study suggests that the top point of the top leaflet of the 4th compound leaf (Fig. 1) could be the best position for taking SPAD values to evaluate the potato plant N status with a SPAD meter.

Table 6: Correlation coefficient ($r$) of leaf N contents with SPAD values at different measuring points, and on different leaflets or compound leaves.

| Measuring point | Tuber initiation stage | Tuber bulking stage |
|-----------------|------------------------|---------------------|
| Top             | 0.98                   | 0.97                |
| Middle          | 0.90                   | 0.92                |
| Basal           | 0.92                   | 0.93                |
| Leaflet         |                        |                     |
| Top             | 0.98                   | 0.97                |
| 1st side        | 0.94                   | 0.97                |
| 2nd side        | 0.86                   | 0.82                |
| Compound leaf   |                        |                     |
| 3rd             | 0.97                   | 0.92                |
| 4th             | 0.97                   | 0.97                |
| 5th             | 0.91                   | 0.83                |

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