# Hand-held chlorophyll meter: a promising tool to assess the nitrogen status of potato foliage

### J. VOS and M. BOM

Department of Agronomy, Wageningen Agricultural University, Haarweg 333, 6709 RZ Wageningen, the Netherlands

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#### Summary

A field experiment with potato (Solanum tuberosum L., cv. Vebeca) was conducted on a sandy soil near Wageningen (52° North) in 1992. The treatments included a zero-nitrogen control and combinations of three amounts of nitrogen, viz. 110, 180 and 250 kg N ha<sup>-1</sup>, and splitting of the N dose in one (early May), two (early May and June) or three (early May, June, July) applications. The chlorophyll content of the uppermost fully grown leaves was assessed with a SPAD-502 chlorophyll meter (Minolta, Osaka, Japan) throughout the season. The pattern of change with time in SPAD-values differed between treatments. SPAD-502 readings correlated well with laboratory measurements of the chlorophyll content and with the nitrogen concentration in leaves ( $r^2 > 0.95$ ). Data on the nitrate concentration in petiole sap (included as a reference) showed that this variable responded much more to split nitrogen applications than the SPAD-value. Future research will need to consider other factors which may affect the chlorophyll content of the foliage.

### Introduction

Correct nitrogen nutrition of potato crops is important from the view point of yield and quality and the prevention of environmental pollution. Nitrogen is usually applied in spring. However, it can be argued that careful management of nutrients in crop production requires that nitrogen is supplied in at least two split applications (Vos & Struik, 1992). For each application, the amount of additional nitrogen needs to be determined using quantitative data on the current nitrogen status of the crop or the soil or of both. In addition, reasonable estimates should be made of the nitrogen requirement of the crop and the rate of nitrogen supply from the soil in order to facilitate a decision as to when the next application might be needed. Vos & Struik (1992) called this 'dynamic optimization of nitrogen supply'.

Dynamic optimization of nitrogen nutrition requires tools to assess its status in the crop or the soil, or both, during the growing season. One tool that aroused interest is the analysis of the concentration of nitrate in petioles or stem ends, using test strips that change colour in proportion to the nitrate concentration in combination with a refractometer to measure the colour density (Nitsch & Varis, 1991). A calibration curve (e.g. Van Loon et al., 1987) is needed to interpret the result.

A second method that has attracted attention recently is based on measuring the chlorophyll content, an idea which was first explored in Japan (Inada, 1963; Matsuzaki et al., 1980; Takebe et al., 1990). Following the introduction of a hand-held

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chlorophyll meter, several workers have recently described its application to assess the nitrogen status of crops, including rice (Turner & Jund, 1991), cotton (Wood et al., 1992) and maize (Piekielek & Fox, 1992).

This paper deals with the first use of the SPAD-502 chlorophyll meter in potato crops. Our objectives were to investigate (i) changes with time in cholorophyll content of the youngest fully grown leaf throughout the growing season for treatments differing in the timing and amount of nitrogen applied, and (ii) correlations between the instrument readings and the results of analytical analyses of both the chlorophyll content and the concentration of nitrogen in the leaves. For reference we included data on changes with time in the nitrate concentration in petiole sap.

## Material and methods

Field experiment. A field experiment with cv. Vebeca was conducted on lighttextured sandy soil near Wageningen (52° N) in 1992. The treatments included a zero-nitrogen control (N0) and all combinations of three amounts of nitrogen (viz. 110, 180 and 250 kg N ha<sup>-1</sup>, denoted N1, N2 and N3) with splitting the nitrogen dose. Applications were made on May 11, shortly after planting (T1); in equal amounts on May 11 and on June 17, which was 30 days after emergence (T2); in equal amounts on May 11, June 17 and July 15, which was 58 days after emergence (T3). A randomized block design was used with four replications. The net plot size was 22.5 m<sup>2</sup> (two rows wide, 5 m long). The date of emergence was May 18. Irrigation was applied whenever necessary, at rates equivalent to 15 mm on each occasion. The seasonal sum of rainfall and irrigation amounted to 490 mm; the estimated seasonal evapotranspiration amounted to 400 mm. Rain in August caused a precipitation surplus.

The observations included readings of the chlorophyll content of the distal leaflet of the youngest fully expanded compound leaf (i.e. the fourth or fifth leaf from the apex). These observations were made on eight dates, at intervals of 2 weeks. Samples of leaves, encompassing a wide range of SPAD-readings across treatments, were removed from the plants and stored in a deep-freeze. Upon thawing, the nitrogen concentration and the chlorophyll content were measured on a dry weight basis. The petioles of the youngest fully grown leaves were sampled in parallel to SPADreadings, except for the first and the last dates of chlorophyll measurement. The treatments included in systematic SPAD-observations and petiole testing were N0, N2T1, N2T2, N2T3 and N3T1; at the two last dates N3T3 was also included. (The nitrogen utilization and crop production in relation to the timing and the total amount of nitrogen supply were studied in all treatments, but those aspects are beyond the scope of this paper).

In situ measurement of chlorophyll content. The instrument used was a SPAD-502 model manufactured by Minolta Camera Co., Ltd, Osaka, Japan. It determines the amount of chlorophyll by measuring the transmittance of a leaf at two wavelengths, namely approximately 430 nm and 750 nm. The instrument can easily be held in the hand, and up to 30 individual readings can be stored, selectively deleted, replaced and averaged. The measuring head consists of two hinged parts and is clamped onto a leaf. The two sources in the emitting part of the head emit a beam of light; the transmittance across the leaf is measured by the receptors in the opposite part of the

head. A reading takes only a few seconds to make, and the speed of sampling a field is determined by the speed with which one can move and the time needed to select a leaf.

Analytical measurements of chlorophyll, nitrogen and nitrate. The analysis of chlorophyll was conducted according to the method described by Bruinsma (1963). Total nitrate (including nitrate-N) in the dry matter of the leaves was determined as described by Biemond & Vos (1992). In brief: a destruction method was used which prevents loss of nitrate-N; the nitrogen content of the aqueous solution was measured using the equipment and procedures as supplied by Technicon Autoanalyzers (Tarrytown, New York, USA). The nitrate in sap recovered from petioles was also analyzed by Technicon procedures.

# Results

Variation between readings. The coefficient of variation (CV) of individual readings within a plot typically ranged between 5 and 9% during the first 2 months after emergence (n = 20); late in the season the CV increased to values ranging from 10 to 13%. Most of this was random plant-to-plant variation. However, the analysis of treatment effects and seasonal changes was not based on readings of individual leaves, but means of 20 observations per plot were entered into the analysis as the basic units of measurement. With four replications (blocks) this led to an average seasonal Least Significant Difference (LSD) (P = 0.05) between treatment means of 1.6 SPAD-units, with LSD values even lower than 1 SPAD-unit early in the season and LSD values close to 2 units at the last date of observation.

Seasonal trends in SPAD-readings. At the first date of observation, (14 days after emergence), there were no differences between any of the treatments (Fig. 1). SPADunits rose temporarily for the treatments supplied with the largest amounts of nitrogen at planting (N2T1, N3T1). SPAD-units remained initially stable for the treatments N2T2 and N2T3 until about 40 days after emergence (DAE). SPADvalues then declined gradually for all N2 and N3 treatments. SPAD-values for the zero-N treatment declined continuously from the first date of observation. The LSD-values in Fig. 1 illustrate that differences between treatments which were numerically fairly small were statistically highly significant. It is interesting to note that treatments N2T1, N2T2 and N2T3 reversed their relative positions as the season progressed: split nitrogen dressings led to lower initial values, which were compensated for by relatively higher values later in the season. Treatment N3T3 was not included in the treatments which were monitored throughout the season. However, measurements at 98 DAE and 112 DAE showed SPAD-values of 38 and 33, respectively; these were much higher than the SPAD-values on those dates for any of the treatments shown in Fig. 1. Therefore, the occasional observations on N3T3 reinforce the proposition that splitting the nitrogen dressings resulted in flattening the seasonal pattern of change of the chlorophyll content (peak lower; decline slower).

Correlation between SPAD-readings and measurements of cholorophyll and nitrogen. SPAD-readings were highly correlated (r = 0.97) with the analytical measurements of the chlorophyll content (Fig. 2a). These results show that the

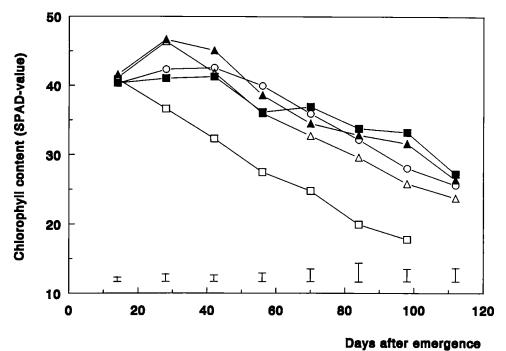


Fig. 1. Chlorophyll content of the uppermost fully grown potato leaves, measured with the SPAD-502 chlorophyll meter (SPAD-value) as a function of time (days after emergence) for five treatments differing in the total amount of nitrogen and the number of split dressings. The vertical bars represent LSD values (P = 0.05). Nitrogen applications:  $\Box$  none;  $\triangle$  180 kg ha<sup>-1</sup> at planting (N2T1);  $\circ$  180 kg ha<sup>-1</sup>, half upon planting, half on June 17 (N2T2);  $\blacksquare$  180 kg ha<sup>-1</sup>, split in three applications upon planting, June 17 and July 15;  $\triangle$  250 kg ha<sup>-1</sup> upon planting (N3T1).

SPAD-readings provided a good estimate of the chloroplyll content of the leaf laminae. The correlation between SPAD-readings and the concentration of nitrogen in the dry matter of leaf laminae was equally high (r = 0.97; Fig. 2b). The SPAD-readings therefore also provided a good estimate of the nitrogen concentration.

Seasonal trends in petiole nitrate concentrations. The features of the change with time in petiole nitrate concentrations were (Fig. 3):

(i) initially high values early in the season, declining continuously with time, at least in treatments given only one nitrogen dressing at planting;

(ii) higher values were found for higher rates of nitrogen supply, with very low nitrate concentrations at any date for the zero-N treatment (see LSD's in Fig. 2b);

(iii) a temporary and large, significant reversion of the declining trend in response to split N-applications (treatments N2T2 and N2T3); petiole nitrate concentrations responded much more to split nitrogen applications than did the SPAD-value (Fig. 1).

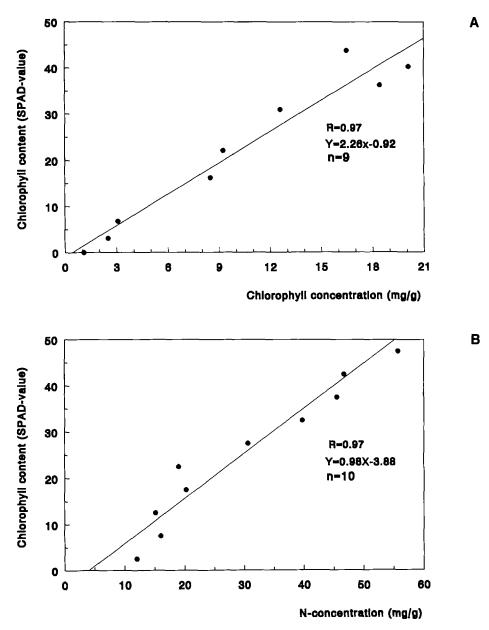


Fig. 2. Correlations between SPAD-readings and laboratory analyses. (a) Correlation between the chlorophyll content measured with the SPAD-502 chlorophyll meter (SPAD-values) and the chlorophyll measured with an analytical method (Bruinsma, 1963; dry weight basis); (b) The correlation between the concentration of nitrogen in the dry matter of leaf laminae and the readings with the SPAD-502 chlorophyll meter (SPAD-value).

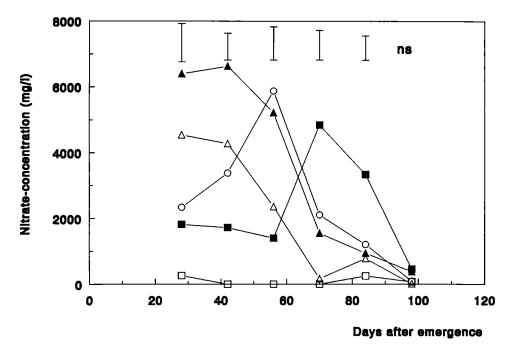
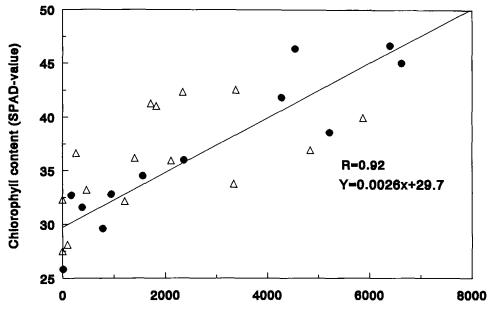


Fig. 3. Concentration of nitrate in the sap recovered from the petioles of the uppermost fully grown potato leaves as a function of time (days after emergence) for five treatments differing in total amount of nitrogenand the number of split dressings. The vertical bars represent LSD values (P = 0.05). For explanation of symbols see Fig. 1.

Correlation between SPAD-readings and the concentration of nitrate in petioles. There was no close correlation between the nitrate concentration in petiole sap and SPAD-readings when all the data were bulked (Fig. 4) (both measured on comparable leaves). Data points from treatments with one application of nitrogen in spring (N2T1 and N3T1) are identifiable by closed dots in Fig. 4. For that sub-set of data, there was a high correlation between the two methods (r = 0.92). The correlation is much weaker (r = 0.76) when data from all treatments are entered into the regression. Clearly this was due to the much stronger response in petiole nitrate than in chlorophyll to split dressings.

### Discussion

An important result of this study is that the chlorophyll content of potato leaves can be assessed by the SPAD-502 chlorophyll meter. In this respect the current study confirms similar reports for other crop species (see also Dwyer et al., 1991). The assessment of chlorophyll also provides an estimate of the nitrogen concentration of leaf laminae (Fig. 2b). This is an important requirement for a technique which is being considered for its suitability as a tool for assessing the nitrogen status of a crop.



Nitrate-concentration (mg/l)

Fig. 4. Correlation between the concentration of nitrate in sap recovered from petioles of the uppermost fully grown leaves and the chlorophyll content as measured with the SPAD-502 chlorophyll meter (SPAD-values). • data points from treatments with one single nitrogen application in spring;  $\triangle$  data points from treatments with split applications and the control; the regression equation pertains to treatments with a single nitrogen application.

Taking SPAD-readings of chlorophyll has many advantages that make the method attractive as a tool for decision-making in nitrogen nutrition. Firstly, the instrument performs the same function as the grower when judging a crop: i.e. assessing its colour. However, the instrument operates quantitatively rather than subjectively. The second advantage is the ease of operation: to apply the method one does not need to master any analytical skills; the test result is obtained instantaneously, without further steps. The chlorophyll meter is much easier to use than any nitrate test. The tremendous response of the nitrate concentration in petioles to split applications of nitrogen is a phenomenon that renders a 'standard calibration curve' useless when one wants to adopt dynamic optimization of nitrogen nutrition, which requires split applications.

Consideration needs to be given to how to interpret a test result in terms of fertilizer need: (i) what are the critical SPAD-levels calling for action, if one does not want to take the risk of a reduction in yield, and (ii) how do such critical levels change with the stage of development of the crop. The current experiment was not designed to answer such questions, and more data need to be collected before it will be possible to develop guidelines for using the method in determining nitrogen recommendations. Continued research will also have to consider other factors that affect the

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colour of the crop, e.g. cultivar, nutritional limitations other than nitrogen, drought and diseases. However, the upper points in Fig. 1 could be used as a provisional reference for the relation between the desired SPAD-value and the age of well managed crops, at least for cultivars in the same maturity class as cv. Vebeca.

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