BRIEF COMMUNICATION

Estimation of total nitrogen content in sugar beet leaves based on chlorophyll fluorescence parameters

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Abstract

Two sugar beet cultivars, Beta 356 and KWS9147, were grown in field trials at four different nitrogen contents (0, 75, 150, and 225 kg ha^{-1}), and each fertilizer treatment was divided into four applications (100% prior to seeding; 70% prior to seeding and 30% at canopy development; 50% prior to seeding and 50% at canopy development; 30% prior to seeding and 70% at canopy development) in two consecutive growing seasons. Leaf chlorophyll fluorescence (ChlF) parameters and leaf nitrogen content were measured simultaneously at different growth stages, establishing an evaluation model of leaf nitrogen nutrition. The results showed that the correlation between ChlF parameters and leaf nitrogen content reached 0.7099[**] (canopy development), 0.8266[**] (storage root development) and 0.8607[**] (sugar accumulation stage). We conclude that the ChlF parameters can provide a decision-making method for N diagnosis and regulation in field production.

Additional key words: electron transport rate; maximum electron transfer efficiency; monitoring; nitrogen nutrition index.

Nitrogen (N) is involved in the synthesis of amino acids, proteins, chlorophyll (Chl), and other substances in plants; it is one of the essential nutrients for growth, yield, and quality in crop (Choi *et al.* 2016, Jay *et al.* 2017). Yield and quality all increased with increasing N (within limits) application levels in cotton, maize, and so on. Excessive nitrogen fertilization may cause plant lodging, undesirable delayed senescence at later stages of growth, an increase in the incidence of pests and diseases, deterioration of crop yield and quality, and damage to the environment (Yang *et al.* 2012, Cordero *et al.* 2019). Timely diagnosis and timely quantitative fertilization are of great significance for crop growth. The traditional nitrogen management needs destructive sampling, which not only takes time and effort, but also delays the fertilization period. Fast, nondestructive, accurate monitoring and diagnosis of crop nitrogen nutritional status become more and more important and will help to determine the best management strategy and dynamic regulation of nitrogen use in crop.

Recently, the use of nondestructive plant phenotype technology has attracted much attention in crop nitrogen nutrition diagnosis and recommendation of nitrogen application. Recently, a *Greenseeker* (*N Tech*, USA) meter has been applied to chrysanthemum, cotton, grass, and other crops to evaluate crop nitrogen status (Jia *et al.* 2014, Bu *et al.* 2016, Colaco *et al.* 2018, Bracke *et al.* 2019). SPAD instrument (*Konica Minolta*, Japan) has been successfully applied to corn, *Macadamia*, potato, and other crops by measuring the Chl content of plant leaves to reflect the nitrogen nutrition of crops (Edalat *et al.* 2019, Galanti *et al.* 2019, Li *et al.* 2019). The digital imaging technology reflects the nitrogen nutrition status of crops, which is also related to wheat, coffee, and other crops (Elsayed *et al.* 2018, Putra and Soni 2018). Hyperspectral remote sensing technology has been shown to be a promising tool to rapidly monitor crop growth status, it has been used for nitrogen nutrition monitoring of soybean, rice, and other crops (Bi *et al.* 2018, Chlingaryan *et al.* 2018, Zhou *et al.* 2018). SPAD monitoring of plant nitrogen nutrition is easily affected by crop varieties and growth period. Digital imaging technology, *GreenSeeker* technology, and hyperspectral remote sensing technology are three technologies that have the drawback of providing a mixed measurement of signal originating from both the plants and the soil (Feng *et al.* 2015).

As an active remote sensing technology, chlorophyll fluorescence (ChlF) has a major advantage that fluorescence signals originate only from the plants. ChlF detection has been successfully used to monitor the health and growth of plants. Presently, there have been few reports on ChlF

and the quantitative relationship with plant N status. The primary aim of this work was to evaluate the suitability of ChlF to accurately determine the plant N status in crops (sugar beet) using a hand-held device under field conditions. The anticipated results can provide technical support and a theoretical basis for diagnosing N nutrition and for recommending fertilization of the crop using the ChlF technique.

This experiment was conducted at the experiment station of Agricultural College, Shihezi University, China (44°20'N, 88°30'E) in 2017–2018 growing seasons. Sugar beet seeds (Beta356, *Beta Seed Company*, USA) were sown on 18 April, 2017 and 21 April, 2018 with a row spacing of 20 cm and a plant spacing of 50 cm. Sugar beet seeds (KWS9147, *KWS Seed Company*, Germany) were sown on 21 April, 2018 with a row spacing of 20 cm and a plant spacing of 50 cm. It was used to verify the reliability of the model. There were four N $[CO(NH_2)_2]$ (46% N) treatments (0, 75, 150, and 225 kg(N) ha^{-1}, as N0, N75, N150, and N225), and each fertilizer treatment was divided into four applications (100% prior to seeding as R1; 70% prior to seeding and 30% at canopy development as R2; 50% prior to seeding and 50% at canopy development as R3; 30% prior to seeding and 70% at canopy development as R4). Irrigation (7,500 m^3 ha^{-1}) was applied during the entire growth, with one drip irrigation tape laid between two rows of sugar beets. P and K fertilizers (P$_2$O$_5$: 345 kg ha^{-1}; K$_2$O: 210 kg ha^{-1}) were applied once as base fertilizers.

After all N fertilizer was applied, functional leaves [at the canopy development stage (the 10th leaf measured 50 d after emergence), at the storage root development stage (the 15th leaf measured 75 d after emergence), and at the sugar accumulation stage (the 35th leaf measured 91 d after emergence)] were selected to measure the fast light response curve by using the *PAM-2500* chlorophyll fluorometer (*Walz*, Germany). First, leaves were dark-adapted for 30 min using *PAM-2500s* (*Walz*, Germany) leaf clamp. The light intensity was set at 0, 9, 34, 67, 104, 144, 201, 366, 622, 984; 1,163; and 1,666 μmol(photon) m^{-2} s^{-1}; and data were obtained after stabilization. The maximum electron transport rate (ETR$_{max}$) was obtained by the instrument according to equation:

$$\text{ETR} = \text{ETR}_{max}\left(1 - e^{-\alpha \text{PAR}/\text{ETRmax}}\right)e^{-\beta \text{PAR}/\text{ETRmax}} \qquad (1)$$

where ETR is the electron transfer rate [μmol(e$^-$) m^{-2} s^{-1}]; ETR$_{max}$ is maximum electron transfer efficiency [μmol(e$^-$) m^{-2} s^{-1}]; PAR is the photosynthetic active radiation [μmol(photon) m^{-2} s^{-1}]; α is the initial slope of the curve (dimensionless); β is photoinhibition parameter (dimensionless). Leaf nitrogen concentration (LNC [%]) is the ratio between the total nitrogen accumulation in the leaves and the corresponding total dry mass at each period. All sample components were placed in a forced-air oven, killed for 30 min at 105°C, and dried to a constant mass at 80°C. The samples were ground, and the Kjeldahl method was used to determine the leaf nitrogen content (Bao 2000):

$$N = (V \times 0.05 \times 14 \times 100)/(1{,}000 \times M) \qquad (2)$$

where N is the leaf nitrogen content [%]; V is the volume of HCl consumed [ml]; M is the mass of the sample [g].

The plant nitrogen nutrition index (NNI) was calculated according to Ata-Ul-Karim *et al.* (2014):

$$NNI = N_a/N_{opt} \qquad (3)$$

where N_a is the measured value of the nitrogen concentration [%] in the leaf; N_{opt} is the optimum nitrogen concentration obtained by the sugar yield nitrogen response model [%] across the no-N treatment and the excessive-N treatment.

Statistical analyses were conducted with experimental data of three years using *SPSS 12.0* software (*SPSS Inc.*, Chicago, USA). Correlation analyses were conducted between ChlF parameters and leaf and plant N concentrations to determine the relationship between ChlF parameters and nitrogen concentration in leaves and plants. Plotting was completed with software *Origin 8.5* (*OriginLab*, USA).

Regression analysis showed that at the canopy development, storage root development, and sugar accumulation stage, the relationships between ETR$_{max}$ and leaf N concentration (LNC) had a unified regression equation. The relationship of LNC and ETR$_{max}$ exhibited a greater difference between canopy development, storage root development, and sugar accumulation stage, so the regression equations were established separately based on growth stages (Fig. 1*A*–*C*). At the canopy development, storage root development, and sugar accumulation stage, the differences in ETR$_{max}$ can reflect changes in LNC. There was a significant positive correlation between ETR$_{max}$ and LNC in three growth periods, and the coefficient of determination (R^2) was 0.7099 ($P<0.001$), 0.8266 ($P<0.001$), and 0.8607 ($P<0.001$), respectively. There was a significant positive correlation between measured value and analog value in three growth periods, and the correlation coefficient (r) was 0.906** ($P<0.001$), 0.946** ($P<0.001$), and 0.930** ($P<0.001$), respectively (Fig. 1*D*–*F*). The best fit equation for LNC at different growth stages *vs.* sugar yield was parabolic, and the determination coefficient of $R^2>0.8086$ (Fig. 2). It can be seen from the curve that when the LNC exceeds a certain value in different growth periods, the sugar production decreases. According to the equation, we obtained the optimal LNC of three growth periods, which were 4.44, 3.32, and 4.01%.

Nitrogen (N) is a critical element for plant growth and productivity that influences photosynthesis and Chl fluorescence and LNC is a major biochemical parameter for estimating photosynthetic efficiency and crop yields (Yang *et al.* 2019). Previous studies have found that the yield and quality of crops decrease when the amount of nitrogen fertilizer exceeds a certain amount (Draycott and Christenson 2003, Bagherzadeh *et al.* 2014, Fei *et al.* 2019). So nondestructive and timely monitoring of crop structural and biochemical traits is of major importance to assess the physiological and phenological status of the
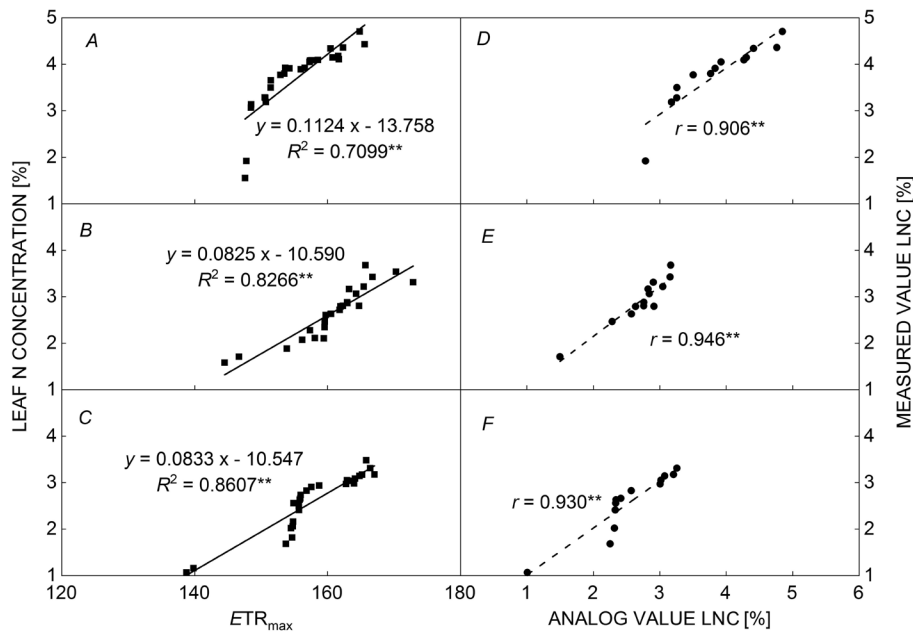
Fig. 1. Linear relationship between leaf nitrogen content (LNC) and maximum electron transport rate (ETR$_{max}$) in sugar beet and 1:1 linear regression between simulated value and measured value. (*A,D*) Canopy development; (*B,E*) storage root development; (*C,F*) sugar accumulation stage.
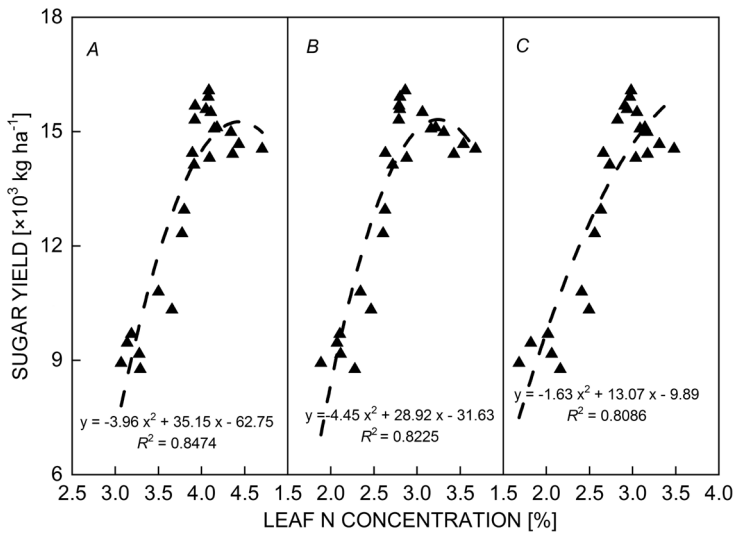

Fig. 2. Regression curves between sugar yield and leaf N concentration at different developmental stages of sugar beet in 2017 and 2018. (*A*) Canopy development; (*B*) storage root development; (*C*) sugar accumulation stage.

plants and to further understand their functioning over time (Jay *et al.* 2019). The traditional methods of crop nitrogen diagnosis mainly include soil mineral nitrogen, plant total nitrogen, nitrate, and diphenylamine. Due to the difference of heredity and stage and the time needed, the application of these detection methods is restricted by some factors, such as poor adaptability, long processing time (Feng *et al.* 2015). Wang *et al.* (2020) pointed out that although hyperspectral and digital cameras were successfully applied to some crops, the results were very vulnerable to environmental factors. Some other studies have found that blue light-induced chloroplast movement in the leaves affects the accuracy of SPAD value (Nauš *et al.* 2010). Meanwhile, SPAD instrument may also cause irreversible damage to leaves. Chl fluorescence technology is an important noninvasive technology, which is used to evaluate and quantify the damage of photosynthetic

apparatus, especially the PSII activity under environmental stress (Baker and Rosenqvist 2004). This method can avoid the influence of chloroplast movement by dark adaptation of the measurement area. Low-N stress significantly decreased Chl content and rapid light-response curves of F_m', F_s, q_N, F_v/F_m, F_v/F_0, and actual photochemical efficiency of PSII of leaves in maize (Wu *et al.* 2019). N fertilizer application significantly increased electron donor and acceptor performance of the PSII reaction center in winter wheat (Yang *et al.* 2018). Our results showed that LNC in sugar beet and the corresponding ChlF parameters varied significantly at different N levels, providing a rich source of information and a theoretical basis for estimating plant nitrogen status using the ChlF technique. There was a significant correlation between LNC and ETR$_{max}$. The overall accuracy of the simulation equation at leaf was high ($R^2>0.7$), indicating that estimating a diagnosis standard

for plant N status by ETR$_{max}$ measurement of a single leaf was reliable (Fig. 1*A*–*C*). Some studies have proposed that if the NNI = 1, the nitrogen nutrition is appropriate. If the NNI > 1, the nitrogen nutrition is excessive; if the NNI < 1, the nitrogen nutrition is insufficient (Ma *et al.* 2018). Calculating the NNI according to Eq. 3, we can make corresponding nitrogen management measures. Our study confirmed that chlorophyll fluorescence parameters can be used to monitor nitrogen nutrition of crops (sugar beet). This model can significantly facilitate the estimation of in-season crop (sugar beet) N requirement and provide strong technical support for the precision management of N fertilization.

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