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Effects of nitrogen application rates on the spatio-temporal variation of leaf SPAD readings on the maize canopy

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Abstract

The spatio-temporal variation of leaf chlorophyll content is an important crop phenotypic trait that is of great significance for evaluating crop productivity. This study used a soil-plant analysis development (SPAD) chlorophyll meter for non-destructive monitoring of leaf chlorophyll dynamics to characterize the patterns of spatio-temporal variation in the nutritional status of maize (*Zea mays* L.) leaves under three nitrogen treatments in two cultivars. The results showed that nitrogen levels could affect the maximum leaf SPAD reading ($SPAD_{max}$) and the duration of high SPAD reading. A rational model was used to measure the changes in SPAD readings over time in single leaves. This model was suitable for predicting the dynamics of the nutrient status for each leaf position under different nitrogen treatments, and model parameter values were position dependent. $SPAD_{max}$ at each leaf decreased with the reduction of nitrogen supply. Leaves at different positions in both cultivars responded differently to higher nitrogen rates. Lower leaves (8th–10th positions) were more sensitive than the other leaves in response to nitrogen. Monitoring the SPAD reading dynamic of lower leaves could accurately characterize and assess the nitrogen supply in plants. The lower leaves in nitrogen-deficient plants had a shorter duration of high SPAD readings compared to nitrogen-sufficient plants; this physiological mechanism should be studied further. In summary, the spatio-temporal variation of plant nitrogen status in maize was analysed to determine critical leaf positions for potentially assisting in the identification of appropriate agronomic management practices, such as the adjustment of nitrogen rates in late fertilization.

Introduction

Under field conditions, visual indications of plant nitrogen supply typically manifest as leaf physiological traits. Nitrogen deficiency in maize is often visually apparent via loss of green colour and reduction in leaf area (Ciampitti and Vyn, 2011). Green leaves serve as the main photosynthetic structures of crop plants, containing the majority of chlorophyll (Piazza *et al*., 2005; Borhan *et al*., 2017), the functional traits of which directly affect photo-assimilate production and grain yield. Leaf chlorophyll content, a key factor in determining leaf photosynthetic rates, can be used as a proxy for the photosynthetically active nitrogen pool (Gu *et al*., 2017; Zhang *et al*., 2021). Therefore, accurate assessment of leaf chlorophyll content is of great significance in characterizing plant nutritional status and evaluating leaf photosynthetic capacity. Monitoring crop nutritional status in real time makes it possible to adapt plant nitrogen uptake based on the target yield by optimizing the timing and amount of nitrogen fertilization, thus enhancing nitrogen fertilizer use efficiency (Lemaire *et al*., 2008; Yang *et al*., 2018). Chlorophyll levels and green leaf area are important indicators of leaf photosynthetic activity and are readily measurable functional traits. Leaf chlorophyll content can be rapidly estimated with a soil-plant analysis development (SPAD) meter (Li *et al*., 2009), which takes measurements that can reflect plant functional status at different growth stages. Temporal changes in the vertical profile of canopy leaf functional status can be seen in the spatio-temporal variation in leaf SPAD readings. Therefore, accurate retrieval of SPAD readings at different spatio-temporal scales is crucial for effectively monitoring the physiological condition of leaves (Ciganda *et al*., 2008).

SPAD readings reflect the leaf growth status and can be used to quantitatively study the leaf chlorophyll status at different plant densities (Samborski *et al*., 2009; Yan *et al*., 2016; Yuan *et al*., 2016*a*). Plants under crowding stress may have a low chlorophyll content and nitrogen concentration in the leaf blades (Bonelli and Andrade, 2020). Additionally, SPAD readings have previously been used to characterize chlorophyll content responses to nitrogen fertilization, with the ultimate goal of developing strategies such as late N fertilization (Fernandez *et al*., 2020) to manage crop nutrition and match N supply with crop demand during the growing season (Lemaire *et al*., 2008; Yang *et al*., 2014*b*). In rice, increasing

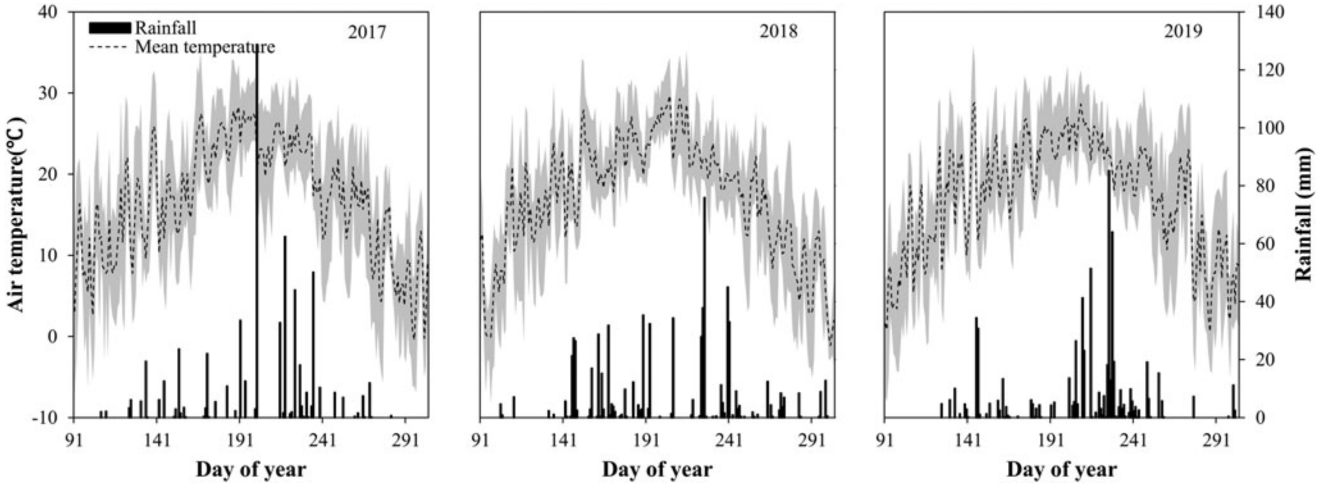

Fig. 1. Daily air temperatures and rainfall for the experimental years. The grey shadow represents the range of daily air temperature.

nitrogen fertilizer increased canopy photosynthesis by manipulating the temporal and vertical distribution of leaf chlorophyll content, resulting in an improved grain yield (Gu *et al*., 2017). Chlorophyll content increases with leaf growth and increases at a faster rate with an increased nitrogen supply. During the leaf senescence phase, chloroplasts become degraded, and chlorophyll content decreases significantly with reduced N supply (Vos *et al*., 2005; Lim *et al*., 2007; Kitonyo *et al*., 2018). These dynamics of chlorophyll content with leaf age, which can be quantified with SPAD readings, decrease the duration of leaf photosynthesis, photo-assimilate production and grain yield. Because the longevity and photosynthetic capacity of a leaf are related to its chlorophyll status, it is important to understand the regulation of high-functional trait duration, especially functional differences in response to nitrogen fertilizer levels along a vertical distribution, in order to prolong longevity and increase photosynthetic capacity (Gu *et al*., 2017; Li *et al*., 2019). The vertical functional distribution is defined as changes in patterns of functional traits such as photosynthetic activity, chlorophyll and nitrogen content along the height of a plant; it is an important factor that requires investigation (Hikosaka *et al*., 2016; Li *et al*., 2019). Changes in leaf greenness in response to environmental factors vary between leaves in different positions on a plant. Previous studies in rice have divided the leaves into three layers to analyse differences based on vertical height, and reported that lower leaves are much more sensitive to increased nitrogen rates compared to upper leaves (Zhou and Wang, 2003; Wang *et al*., 2006; Li *et al*., 2009; Zhao *et al*., 2016). The effect of N supply on SPAD readings has also been shown to manifest more distinctly with leaf age (Li *et al*., 2009).

The studies discussed above regarding spatial distribution of leaf SPAD readings mostly focused on upper, middle and lower leaf layers, failing to consider the vertical distribution of leaves at each leaf position. Thus, more information is needed regarding leaf positions for the diagnosis of plant nutritional status. The current paper obtained spatially distributed SPAD readings more systematically and accurately through detailed measurements of the main growing leaves from the top to the bottom of the plant and analysed the effects of different nitrogen application rates and time on crop nutritional status. The aims were to: (1) quantitatively characterize and evaluate the spatio-temporal dynamics of leaf SPAD readings under different nitrogen application rates and (2) clarify the effect of positional differences on leaf nutritional status sensitivity to nitrogen. This quantitative analysis of leaf SPAD reading dynamics under different nitrogen application rates and time is of great significance in monitoring crop nutritional status and in diagnostic research of nitrogen fertilizer management.

Materials and methods

Experimental design

The experiments were conducted in 2017–2019 at the Gongzhuling Experimental Station of the Chinese Academy of Agricultural Science (43°53′N, 124°81′E), Gongzhuling county, Jilin province, China. The area was a typical rain-fed spring maize area with ridge planting and was harvested once per year. The daily maximum, minimum and mean air temperatures at the experimental site during the growth period are shown in Fig. 1. The average annual conditions were as follows: maximum temperature, 35.4°C; minimum temperature, −27.0°C; rainfall, 645.6 mm and frost-free period, 161 days. The monthly meteorological data for 2017, 2018 and 2019 and the mean values over a period of 20 years (2000–2019) are listed in Tables 1 and 2, including average temperature and precipitation. The average temperature in July was 1.4°C higher in 2018 than in any other year included. The precipitation levels in August 2017, August 2018 and August 2019 were also significantly higher than others in the past 20 years. In total, the average temperature and precipitation were relatively consistent throughout the growing season in the years these experiments were conducted.

The study was conducted in a field that was fertilized with nitrogen over a long-term period, from 2009 to 2019. Experiments were laid out as a split plot design with three replications. Nitrogen fertilization formed the main plots, maize cultivars formed the sub-plots. The size of the sub-plot was 6.5 m × 10 m. The row spacing was 0.65 m, and there were ten rows in each plot. Two maize cultivars widely cultivated in China were used: Zhengdan958 (ZD958) and Xianyu335 (XY335). These cultivars have different senescence behaviours: ZD958 is a stay-green variety whereas XY335 is a standard/not stay-green variety. Seeds were planted at a density of 67 500 plants/ha. Three N fertilization treatments were used: 300, 150

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Table 1. Average temperature during the maize growing seasons in 2017–2019

	Average temperature (°C)			
Month	2017	2018	2019	2000–2019
April	10.2^{ns}	10.4^{ns}	9.3^{ns}	9.5
May	16.5^{ns}	17.1^{ns}	18.0^{ns}	17.1
June	20.8^{ns}	22.3^{ns}	20.8^{ns}	21.9
July	24.7^{ns}	25.5**	24.7^{ns}	24.1
August	21.8^{ns}	21.6^{ns}	21.7^{ns}	22.4
September	16.2^{ns}	15.5^{ns}	17.8^{ns}	16.8
October	7.6^{ns}	7.6^{ns}	9.0^{ns}	8.2
Average	16.8	17.2	17.3	17.2

A *t* test was conducted for each month to test for differences between each experimental year and the past 20 years.
**$P < 0.01$; ns, not significant.

Table 2. Monthly total precipitation during the maize growing seasons in 2017–2019

	Precipitation (mm)				
Month	2017	2018	2019	2000–2019	
April	4.1	12.5	0.0	24.3	*
May	53.3	87.1	95.7	61.3	ns
June	62.8	121.9	43.5	96.3	ns
July	190.8	128.1	135.5	131.1	ns
August	239.5	246.7	317.5	142.5	**
September	37.1	51.4	49.5	38.4	ns
October	0.6	38.0	21.1	24.1	ns
Total	588.2	685.7	662.8	517.7	

A *t* test was conducted for each month to test for differences between 2017–2019 and the past 20 years. **$P < 0.01$; *$P < 0.05$; ns, not significant.

and 0 kg N/ha/year (referred to as N3, N1 and N0 treatments, respectively). For the N3 treatment, N (urea) was applied at 150 kg N/ha/year before sowing, then top-dressed at the V8 and VT stages (75 kg N/ha/year each time). For the N1 treatment, N was applied before sowing as described for the N3 treatment. For the N0 treatment, no N fertilizer was applied. The averages for soil composition in 2017–2019 were measured prior to application of N fertilizer; organic matter content was 29.7 g/kg, available N was 123.8 mg/kg, available P was 28.2 mg/kg, available K was 240.3 mg/kg and the total nitrogen content was 1.13, 0.95 and 0.74 g/kg for the N3, N1 and N0 treatments, respectively.

Spatial and temporal dynamics of leaf SPAD readings

An SPAD-502 chlorophyll meter (Minolta Camera Co., Osaka, Japan) was used to measure chlorophyll levels in leaves. Spatio-temporal variation was analysed to determine how chlorophyll content changed in the canopy profile and throughout the growing period. Five representative plants with stable growth status were selected at the vegetative fourth leaf (V4) stage for long-term observation and measurement. Leaves were numbered from the bottom to the top. At the vegetative sixth leaf (V6) stage, the leaves were marked with red spray paint to ensure correct identification throughout the growing period. To observe temporal dynamics at each leaf position, we took SPAD readings from leaf emergence to senescence. Specifically, SPAD readings were taken to measure chlorophyll in the 6th–22nd leaves on tagged plants. The dates on which measurements were taken for each leaf position are shown in Fig. 2. On each sampling date, three SPAD readings were taken around the midpoint of each leaf blade on each plant, and SPAD readings of the five representative plants were averaged to obtain the mean SPAD value for that leaf position. The SPAD meter could not measure completely chlorotic (yellow and dry) leaves; therefore, we considered SPAD values of 0 for such leaves. The leaves at different positions develop at different times during the season and therefore under different temperature conditions. Thus, leaf life span from the date of leaf tip visible to the sample date can be calculated using growing degree day (GDD) accumulation. Daily GDD is calculated as follows:

$$GDD = \frac{T_{\max} + T_{\min}}{2} - 8 \tag{1}$$

where $T_{\max}$ is the maximum daily air temperature, $T_{\min}$ is the minimum daily air temperature and 8°C is the base temperature.

We generated an example curve of SPAD readings *v*. GDD to analyse the dynamics of SPAD readings over time and describe the derivative indicators of leaf SPAD readings (Fig. 3). $SPAD_{\text{expand}}$ is the SPAD reading when the leaf is fully expanded; $SPAD_{\max}$ is the maximum SPAD reading during leaf growth and $SPAD_{\text{function}}$ is the SPAD reading when the leaf enters the functional stage. The day of full leaf expansion was the onset for measuring leaf longevity. After a leaf is fully expanded, it has the ability to perform photosynthesis and provide photo-assimilate production to reproductive organs. Therefore, the current study clarified the ratio of $SPAD_{\text{expand}}$ to $SPAD_{\max}$ ($SPAD_{\text{expand}}/SPAD_{\max}$) to determine the relationship between the photosynthetic ability of a leaf at full expansion and its peak photosynthetic ability. This ratio is defined as the percentage of $SPAD_{\text{function}}$ to $SPAD_{\max}$. The number of days in which SPAD readings are higher than $SPAD_{\text{function}}$ is defined as the duration of high SPAD reading.

Statistical model performance evaluation

To better clarify the dynamics of SPAD readings at the single leaf scale, a leaf SPAD reading model was established to analyse in depth the dynamics of nitrogen status in the different strata of the canopy profile. Several analysis methods were used to assess model performance, including coefficient of determination (R^2) and normalized root mean square error (NRMSE; Janssen and Heuberger, 1995). R^2 is commonly used for evaluating the goodness of fit in regression models. NRMSE quantifies the difference between an observation and prediction, and was calculated as follows:

$$\text{NRMSE} = \frac{\sqrt{\sum_{i=1}^{n}\left(SPAD_{\text{mea}} - SPAD_{\text{est}}\right)^2 / n}}{\overline{SPAD_{\text{mea}}}} \tag{2}$$

where $SPAD_{\text{mea}}$ is the measured SPAD reading, $\overline{SPAD_{\text{mea}}}$ is the average of the measured SPAD reading, $SPAD_{\text{est}}$ is the estimated SPAD reading and n is the number of samples.

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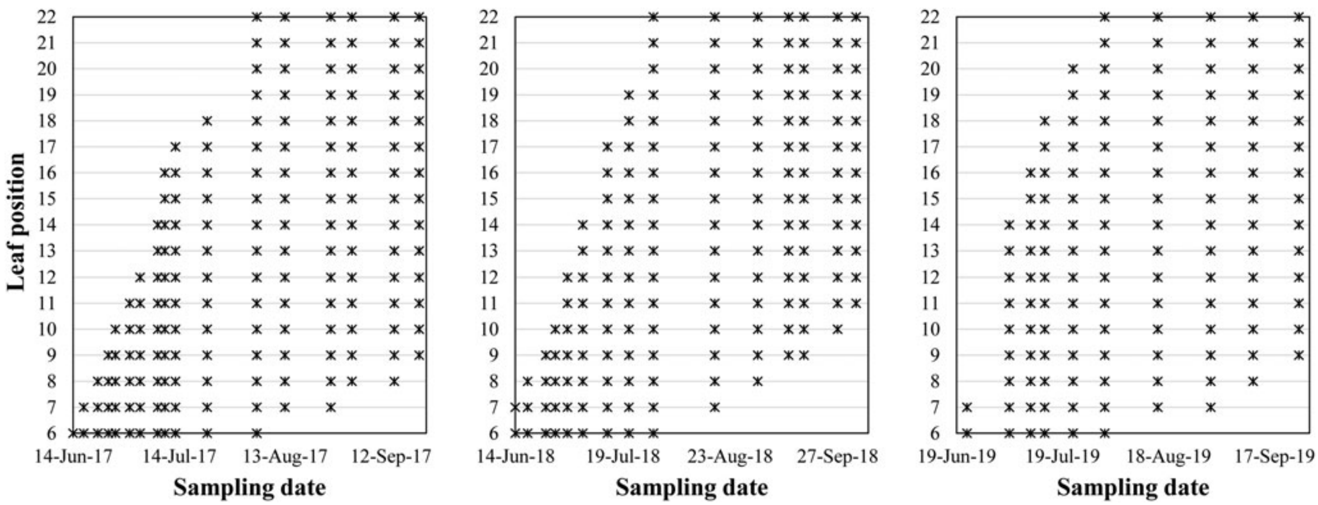

Fig. 2. Sampling dates for SPAD readings of the 6th–22nd leaves in 2017–2019. The measurement was stopped when the lower leaves completely lost their green colour.

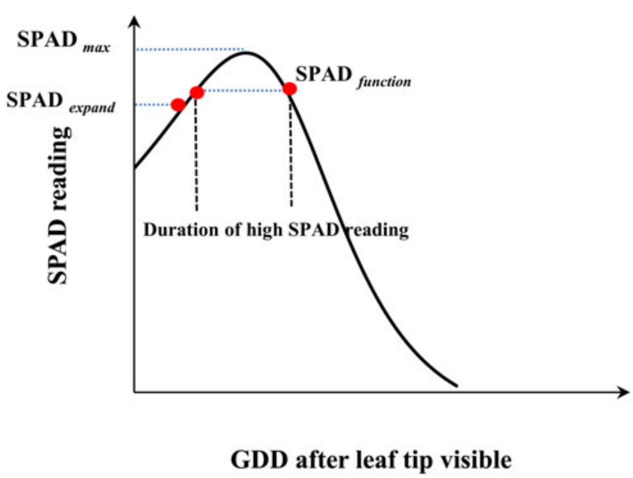

Fig. 3. (Colour online) Schematic diagram of the temporal dynamic of SPAD reading over leaf lifespan. $SPAD_{expand}$, $SPAD_{max}$ and $SPAD_{function}$ represent the trait of leaf SPAD reading. The duration of high SPAD reading represents the duration of higher than the SPAD function. GDD, growing degree days.

Data analysis

The data shown in Figs 1–10, and Tables 1 and 2 were analysed in Microsoft Excel 2010. A frequency distribution histogram (Fig. 11) was drawn and statistical analysis was conducted with *t*-test in SPSS Statistics 20.0 (SPSS Inc., 122 Chicago, IL, USA). CurveExpert Professional 2.2.0 was used to simulate the dynamic process in leaf SPAD readings in relation to GDD in a single leaf and to establish the optimal leaf SPAD reading model with biological significance. The heatmap shown in Fig. 12 was generated with Matlab 2012b (MathWorks, Natick, MA, USA).

Results

Spatio-temporal variation in leaf SPAD readings

The spatio-temporal changes in SPAD readings were used to quantify the dynamics of leaf nutritional status. The data from all three experimental years and all five representative plants per year demonstrate the canopy spatial distribution pattern of SPAD readings in each leaf position, as shown in Figs 4 and 5. For each individual leaf, the SPAD reading gradually increased after the leaf tip was visible, continued to increase while the leaf was fully expanded, entered the photosynthetically functional stage after leaf expansion reached the peak value, then decreased when beginning senescence. For each individual plant, leaf position affected the distribution of SPAD readings in the canopy. The lower and upper leaves showed large amplitudes in SPAD readings, whereas readings were relatively stable in the middle leaves. With the reduction in nitrogen application rates, the effect of positional difference on leaf SPAD readings gradually strengthened. The effects of nitrogen levels on SPAD readings for each leaf were more distinguishable over time as leaves aged. Additionally, the positional difference in the leaf SPAD readings was most prominent in the duration of high SPAD reading. From the bottom to top of the canopy, the duration of high readings generally increased, peaked around the ear-layer and then decreased. The magnitude of the response to the nitrogen level at each leaf position was different. With lower nitrogen levels, the duration of high SPAD readings decreased, especially in the lower leaves. The vertical profile and temporal variation of SPAD readings showed a similar trajectory in cultivars ZD958 and XY335.

Effects of nitrogen application rates on SPAD readings

$SPAD_{expand}$ and $SPAD_{max}$ were well correlated with leaf position (Fig. 6). From the bottom to the top of the plant, $SPAD_{expand}$ initially increased, peaked near the lower leaves, then decreased along with increasing leaf position. Among all leaf positions, cultivars and nitrogen treatments, $SPAD_{expand}$ measurements ranged from 21.1 to 62.6, increasing with higher levels of nitrogen application. Similarly, from the bottom to the top of the plant, $SPAD_{max}$ initially increased, peaked near the middle leaves, then decreased with increasing leaf position; among all leaf positions, cultivars and nitrogen treatments, $SPAD_{max}$ ranged from 32.7 to 67.8. Leaves in different positions responded differently to the increasing N levels, and there was greater variability at different leaf positions of the N0 treatment group. The largest positional differences in $SPAD_{expand}$ and $SPAD_{max}$ were observed in the top leaves.

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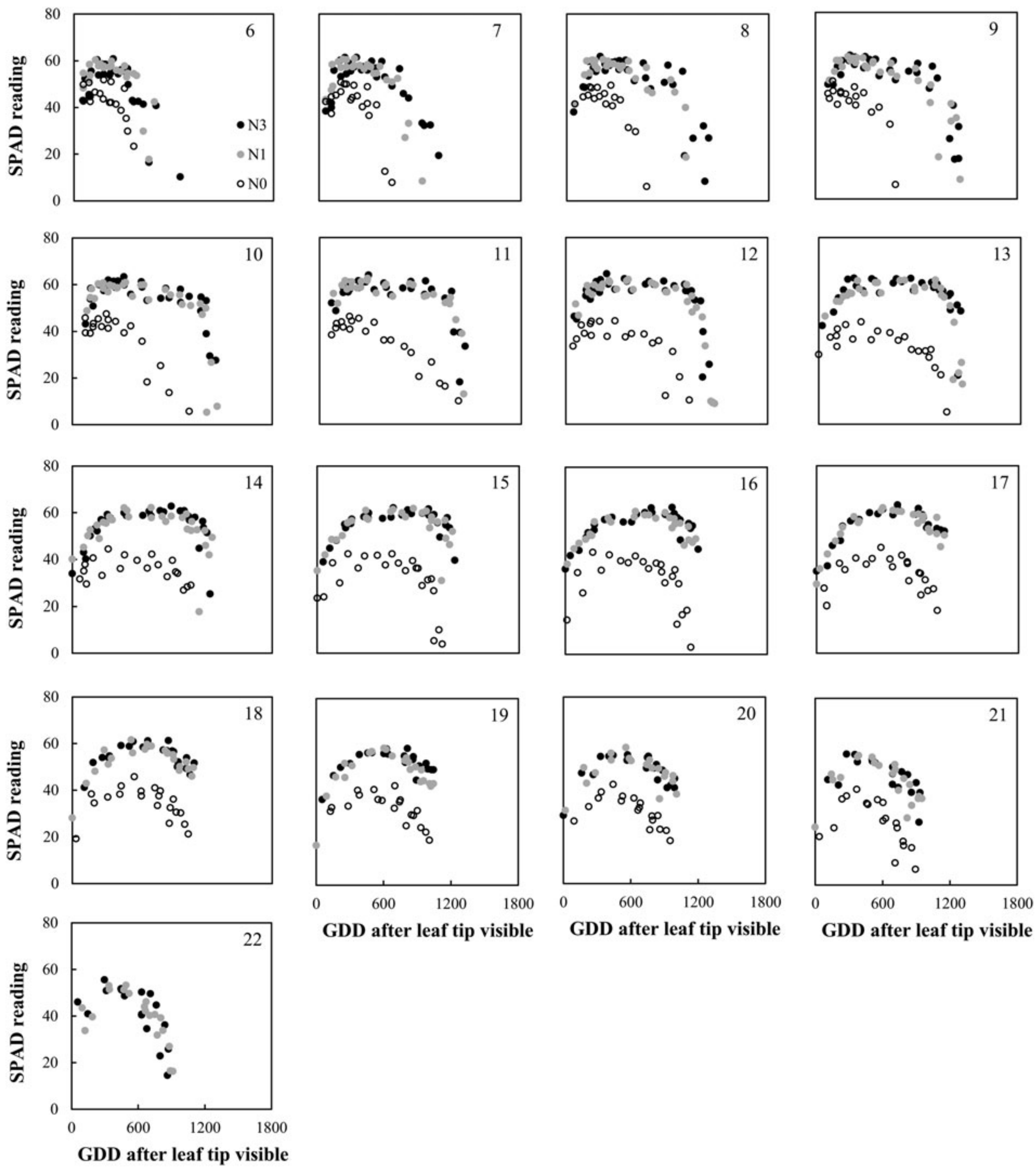

Fig. 4. Temporal dynamic of 6th–22nd leaves SPAD reading of ZD958 from all experimental years under different nitrogen application rates. The position of the ear leaf is the 16th leaf. N3, N1 and N0 indicate nitrogen application rates of 300, 150 and 0 kg N/ha/year, respectively. GDD, growing degree days.

The values of $SPAD_{expand}/SPAD_{max}$ formed a normal distribution (Fig. 11), ranging from 63.3 to 99.5%; the median was 85.4% and the mean was 85.2%. Consequently, the mean ratio 85% was clarified as the percentage of the leaf $SPAD_{function}$ to the $SPAD_{max}$. The following analyses used the $SPAD_{function}$ (85% of the $SPAD_{max}$) as the onset point of the duration of high SPAD readings.

Leaf SPAD reading model developing and fitting

To better understand the spatio-temporal dynamics of leaf SPAD readings, a rational model was used to fit the data. The equation was as follows:

$$SPAD = \frac{a + bT}{1 + cT + dT^2} \quad (3)$$

where *SPAD* is the leaf SPAD reading predicted by the equation, *a*, *b*, *c* and *d* are model parameters (the *a* values (range 19.45–57.54), the *b* values (range −0.0420 to 0.1433), the *c* values (range −0.00304 to 0.00028) and the *d* values (range 0.00000010–0.00000431) are position-dependent) and *T* is the thermal time (°C d) based on the mean daily temperature (°C, based on 8°C) starting on the emergence day.

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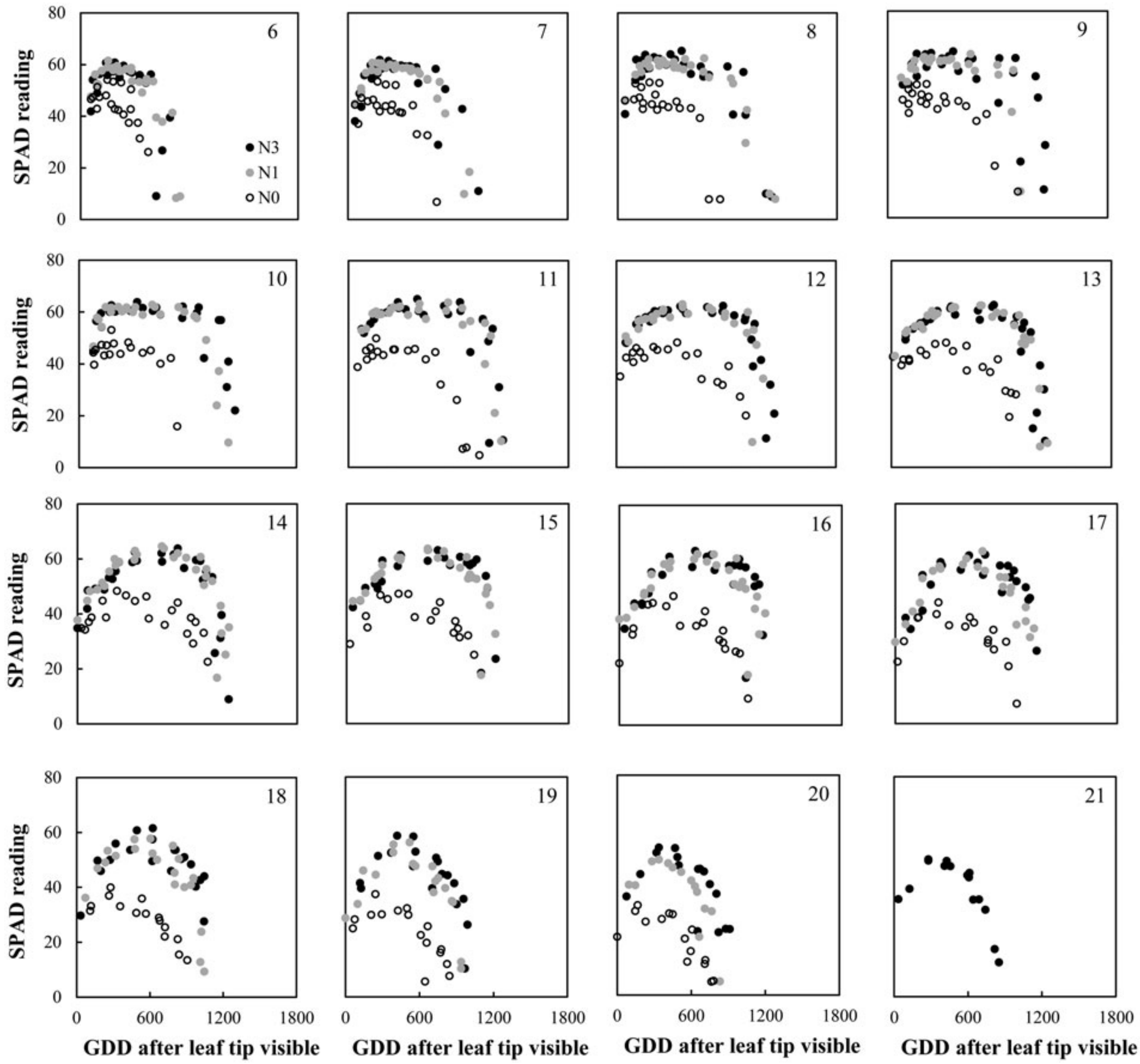

Fig. 5. Temporal dynamic of 6th–21st leaves SPAD reading of XY335 from all experimental years under different nitrogen application rates. The position of the ear leaf is the 14th leaf. N3, N1 and N0 indicate nitrogen application rates of 300, 150 and 0 kg N/ha/year, respectively. GDD, growing degree days.

The model was fitted to data from each leaf position independently. For each leaf position under different cultivars and nitrogen application rates, the SPAD readings from all years were combined to develop the leaf SPAD reading model based on the rational function for accurately estimating the duration of high SPAD reading. Leave-one-out cross-validation was used to evaluate the performance of the SPAD reading model, meaning that each year was iteratively dropped from the data set and the model was refitted using the remaining data. Results from the cross-validation analysis, statistical criteria of NRMSE, were used to compare the predicted and observed values for the model testing (Table S1). The predicted SPAD readings were relatively consistent with the observed readings, with the NRMSE values ranging from 0.030 to 0.384. Due to the positional differences in the duration of high SPAD reading, the progression of the leaf SPAD reading model followed two patterns (Fig. 7): upper and lower leaves, with a short duration of high SPAD values (*A*), e.g. the fitting equation for the 6th leaf of N3 treatment is:

$$SPAD = \frac{38.19 - 0.0332T}{1 - 0.0027T + 0.00000308T^2} \quad (R^2 = 0.932,\ \text{NRMSE} = 0.167) \tag{4}$$

and middle leaves, with a long duration of high SPAD values (*B*), e.g. the fitting equation (*B*) for the 16th leaf of N3 treatment is:

$$SPAD = \frac{38.61 + 0.0422T}{1 - 0.000458T + 0.000000891T^2} \quad (R^2 = 0.809,\ \text{NRMSE} = 0.052) \tag{5}$$

R^2 and NRMSE values for model evaluation are shown in Fig. 12; a higher R^2 indicates better performance whereas a lower NRMSE represents better performance. The data demonstrate that the rational function was suitable for fitting the SPAD reading of each leaf position. SPAD readings from the model ranged from 0 to 65.2. The R^2 values ranged from 0.52

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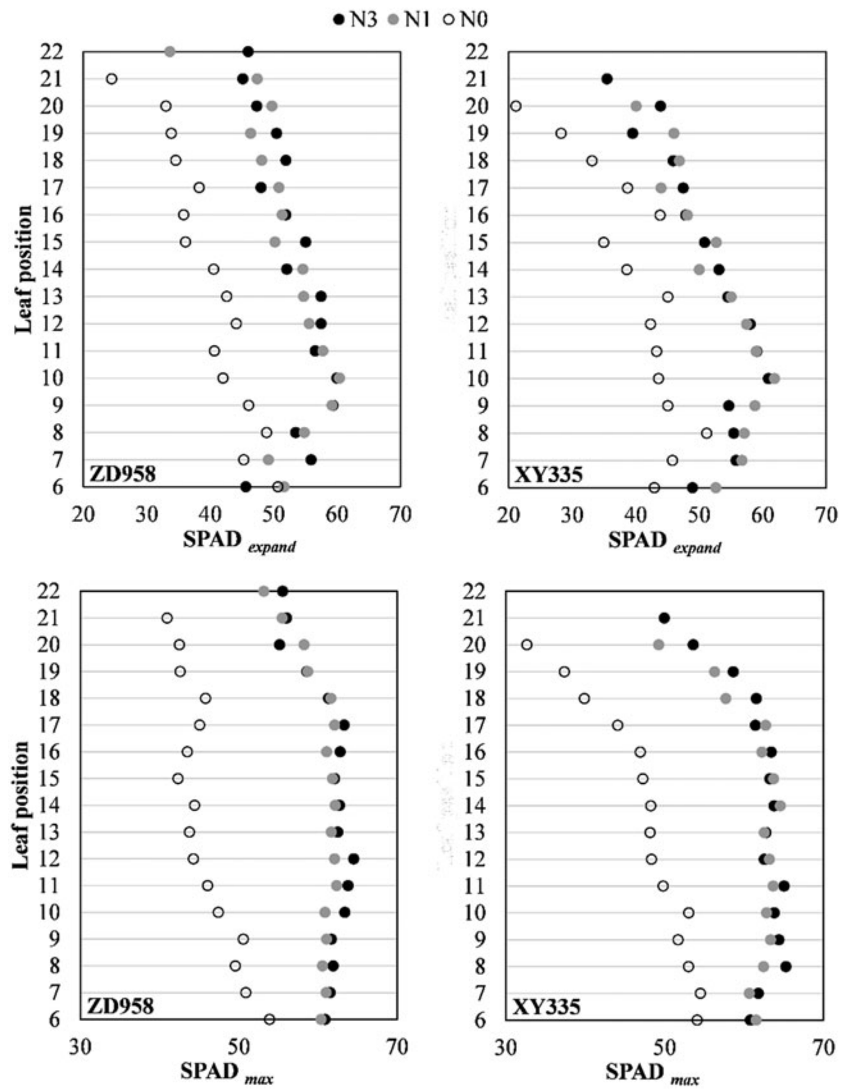

Fig. 6. Distribution pattern of $SPAD_{expand}$ and $SPAD_{max}$ under different cultivars and nitrogen application rates. The ear position leaves of ZD958 and XY335 are on the 16th and 14th leaves, respectively. N3, N1 and N0 indicate nitrogen application rates of 300, 150 and 0 kg N/ha/year, respectively.

to 0.97, with 80% of the values higher than 0.75. The NRMSE values ranged from 0.034 to 0.287, and 80% of the values were <0.197.

Positional differences in the temporal dynamic of leaf SPAD readings under different nitrogen application rates

The onset of $SPAD_{function}$ was defined as 85% of the estimated $SPAD_{max}$ to quantify the duration of high SPAD readings (Fig. 8). Within N treatment groups, the duration of high SPAD readings initially increased, then decreased with the increasing leaf position from the bottom to the top of the plant. Higher nitrogen application was associated with an increased duration of high SPAD readings, but the proportional increase varied by leaf position from the bottom to the top of the plant, an effect that gradually weakened. The duration of high SPAD readings was more sensitive to nitrogen levels in the lower leaves than that in the upper and middle leaves. Additionally, SPAD readings for leaves 6–13 showed that nitrogen deficiency reduced $SPAD_{max}$ and shortened the duration of high SPAD readings in lower leaves, with the 8th–10th leaves being the most sensitive (Figs 9 and 10). The vertical distribution pattern for the duration of high SPAD readings was the same for cultivars ZD958 and XY335.

Discussion

An SPAD-502 chlorophyll meter (Minolta, 1989; Raymond Hunt and Craig, 2014; Yuan *et al.*, 2016*a*; Borhan *et al.*, 2017) is a rapid, non-destructive, hand-held spectral device that is widely used for leaf chlorophyll measurement in the laboratory and in the field (Li *et al.*, 2009; Ling *et al.*, 2011; Xiong *et al.*, 2015; Yuan *et al.*, 2016*b*). SPAD readings can be used to quantify leaf nutrient content and reflect leaf functional status (Wang *et al.*, 2018; Yang *et al.*, 2018; Li *et al.*, 2019). The current paper systematically analysed the dynamic distribution of leaf SPAD readings between leaf positions, cultivars and nitrogen treatments (Figs 4 and 5). Leaf physiological characteristics differed greatly based on their positions on the plant; SPAD readings increased from the bottom leaves and from the top leaves, with maximal SPAD readings centred around the ear-layer. These differences in SPAD readings were due to differences in leaf age, which was reflected in leaf N status and used to quantify the real-time dynamics of functional traits such as chlorophyll content and photosynthetic rate. This is consistent with previous studies (Li *et al.*, 2009; Escobar-Gutiérrez and Combe, 2012; Yang *et al.*, 2014*a*; Li *et al.*, 2019). Each leaf position along the vertical growth of the canopy has regular and sequential characteristics (Stewart and Dwyer, 1994; Lisson *et al.*, 2000; Birch *et al.*, 2003; Ciganda

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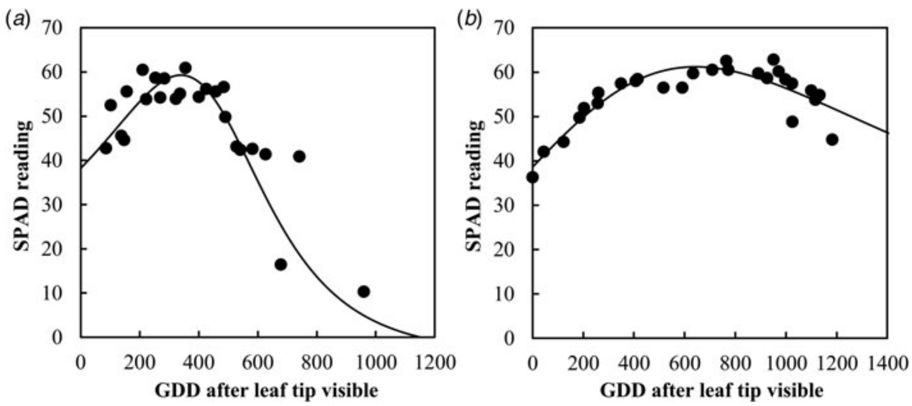

Fig. 7. Schematic diagram of the leaf SPAD reading model. The progression of leaf SPAD model includes two patterns (*a* and *b*). The circles represent the measured value, and the lines represent the fitted line. GDD, growing degree days.

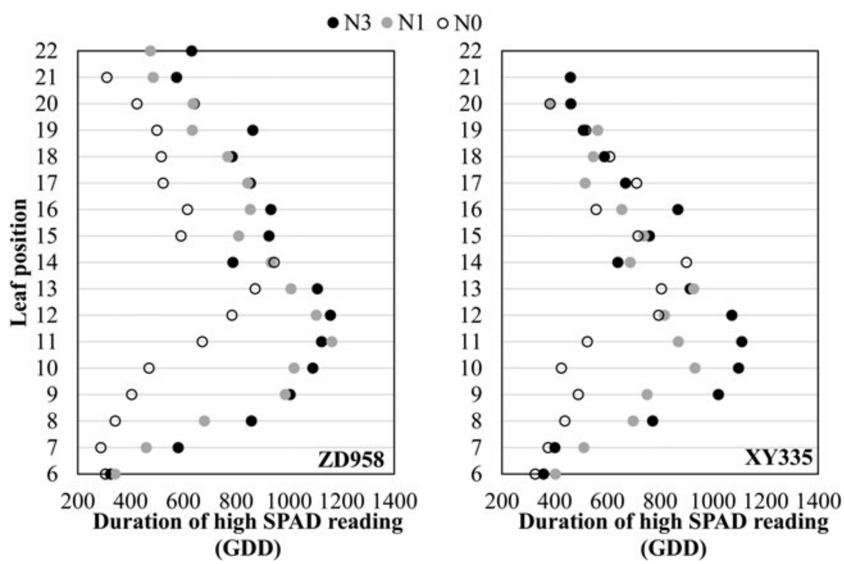

Fig. 8. Effect of nitrogen application rates on the duration of high SPAD reading. The ear position leaves of ZD958 and XY335 are on the 16th and 14th leaves, respectively. N3, N1 and N0 indicate nitrogen application rates of 300, 150 and 0 kg N/ha/year, respectively. GDD, growing degree days.

et al., 2008; Kitonyo *et al.*, 2018). The leaf expansion process, which is related to the order of leaf position, caused differences in the spatial distribution of leaf nutrient status (Biemond, 1995; Vos *et al.*, 2005). SPAD readings showed different vertical distributions along with plant height in the plant growth stages (Kitonyo *et al.*, 2018; Li *et al.*, 2019; Li *et al.*, 2020). Leaf age dramatically affects leaf chloroplast structure and therefore affects SPAD readings due to age-related effects on leaf expansion, longevity and senescence processes (Chang *et al.*, 2007; Ciganda *et al.*, 2008; Yang *et al.*, 2014*a*; Wang *et al.*, 2018).

Regardless of the nitrogen treatment group or cultivar, the dynamics of SPAD readings at different leaf positions could be accurately captured by the rational model (Fig. 7). The model developed had good applicability to the dynamic changes of SPAD readings in each leaf position (Fig. 12). In previous studies on rice, the dynamics of leaf SPAD readings were modelled with a piecewise function and divided into three phases over the lifespan of a single leaf (Chang *et al.*, 2007; Yang *et al.*, 2014*a*). Yang *et al.* (2014*a*) monitored changes in leaf SPAD readings in three stages based on a piecewise function and analysed the effect of nitrogen fertilizer on leaf SPAD readings through model parameters. However, the breakpoint has differed among leaf position and nitrogen supply under the field conditions, thus decreasing the feasibility of the piecewise function. The accurate introduction of breakpoint is very important. The discontinuity of the piecewise function affects the function value of the breakpoint and limited the applicability, whereas the prediction accuracy of the continuous function is improved. The model developed herein was a simple method to accurately predict the SPAD reading dynamics during the entire leaf lifespan without partitioning the three phases. Additionally, few systematic analyses have been attempted to quantify effects on SPAD readings in a single leaf blade (Yang *et al.*, 2014*a*; Wang *et al.*, 2018). In this study, a continuous model was established for individual leaf positions along the whole plant to accurately quantify the maximum value, evaluate the duration of high SPAD readings and analyse the response of each leaf position to the nitrogen fertilizer treatments.

High $SPAD_{max}$ values suggest maintenance of high-photosynthetic activity (Kitonyo *et al.*, 2018). Maintaining a high-photosynthetic rate over time plays an important role in the accumulation of photosynthetic products. The current study quantified the functional duration through the SPAD value, a non-destructive measurement indicator. The thermal time of the $SPAD_{function}$ is the onset of the duration of high SPAD readings. The results showed that 85% of the $SPAD_{max}$ determine the $SPAD_{function}$ (Fig. 11). The duration of high SPAD readings was longer and the rapid drop in leaf SPAD readings was suppressed

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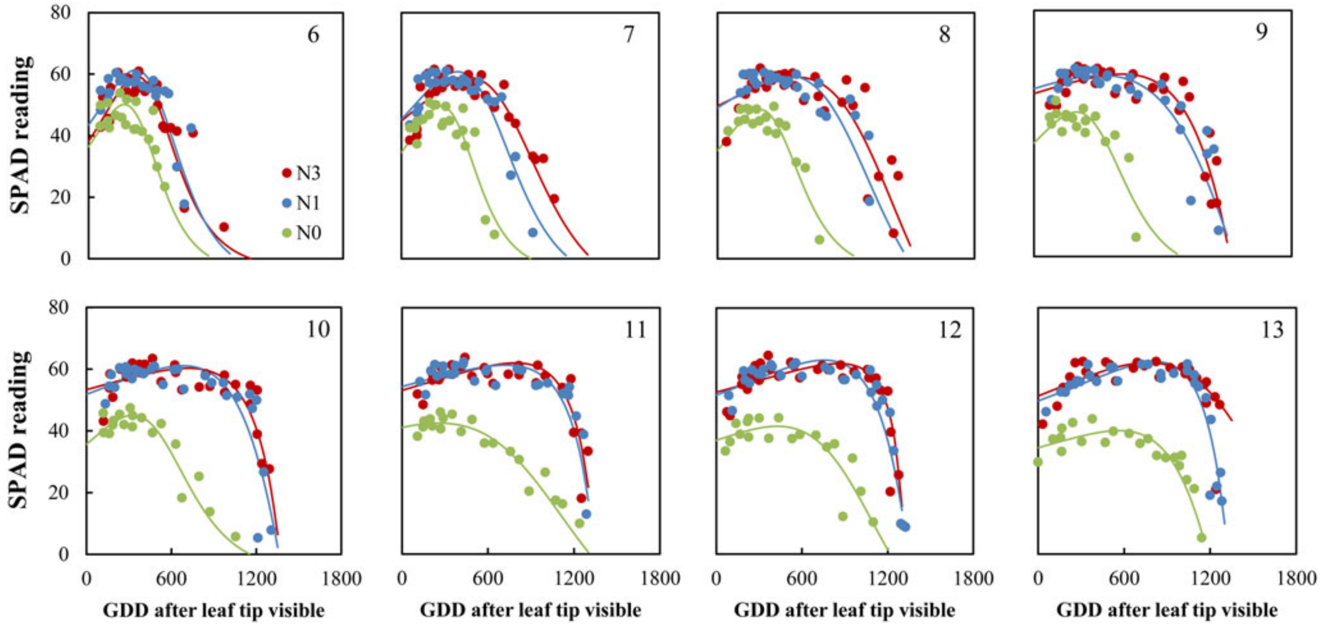

Fig. 9. (Colour online) Temporal dynamics of SPAD readings of 6th–13th leaves of ZD958 over lifespan fitted to the rational model under different nitrogen application rates. N3, N1 and N0 indicate nitrogen application rates of 300, 150 and 0 kg N/ha/year, respectively. The circles represent the measured value, and the lines represent the fitted line. GDD, growing degree days.

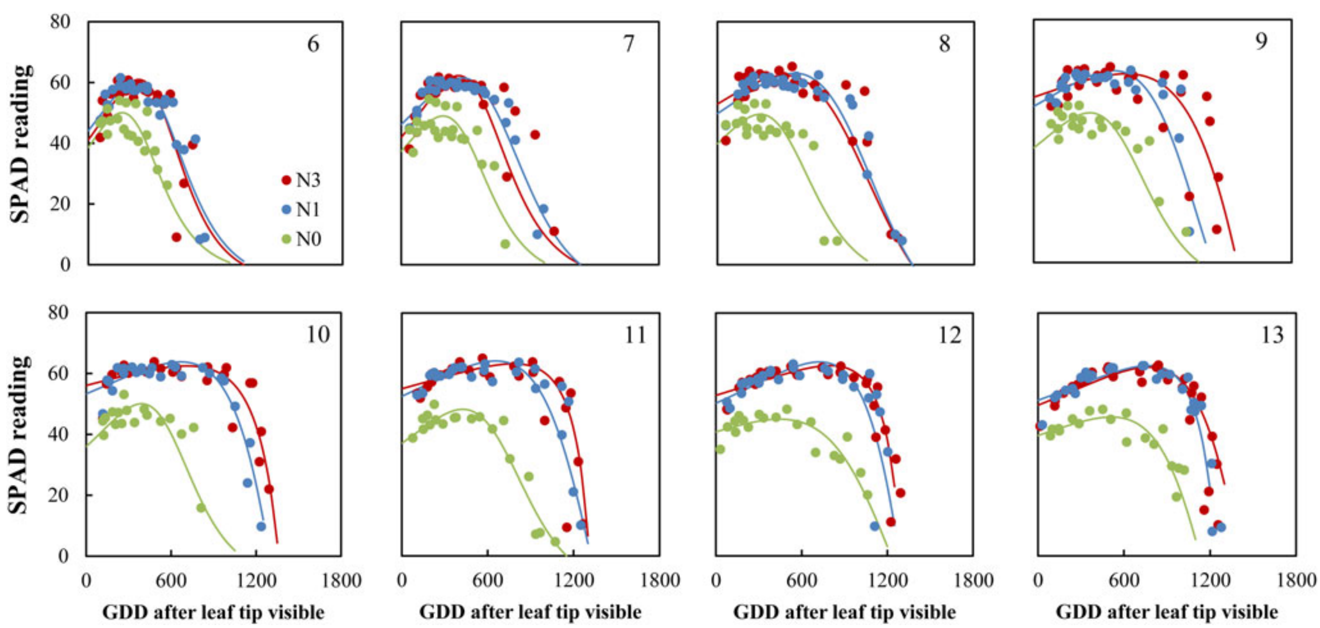

Fig. 10. (Colour online) Temporal dynamics of SPAD readings of 6th–13th leaves of XY335 over lifespan fitted to the rational model under different nitrogen application rates. N3, N1 and N0 indicate nitrogen application rates of 300, 150 and 0 kg N/ha/year, respectively. The point represents the measured value, and the line represents the fitted line. GDD, growing degree days.

in plants treated with higher nitrogen application rates. However, when nitrogen was insufficient, the leaf SPAD readings continued to decrease, and the high values lasted for a shorter period of time (Fig. 8). To summarize, nitrogen-sufficient and nitrogen-deficient plants could be identified by the length of high SPAD readings (Liu *et al.*, 2018). Previous studies have shown that the duration of high SPAD readings is closely related to the duration of leaf photosynthetic function (Cao *et al.*, 2001). The length of time when a leaf has a high-photosynthetic rate is the key for improving photosynthetic productivity (Abeledo *et al.*, 2020; Li *et al.*, 2021). Maintaining the duration of high-photosynthetic rate during the grain-filling period was one important physiological trait with implications for yield potential related to increased assimilation availability (Gu *et al.*, 2017; Wang *et al.*, 2018). Previous studies have shown that addition of nitrogen fertilizer during the silking stage sustained high-photosynthetic rates during the

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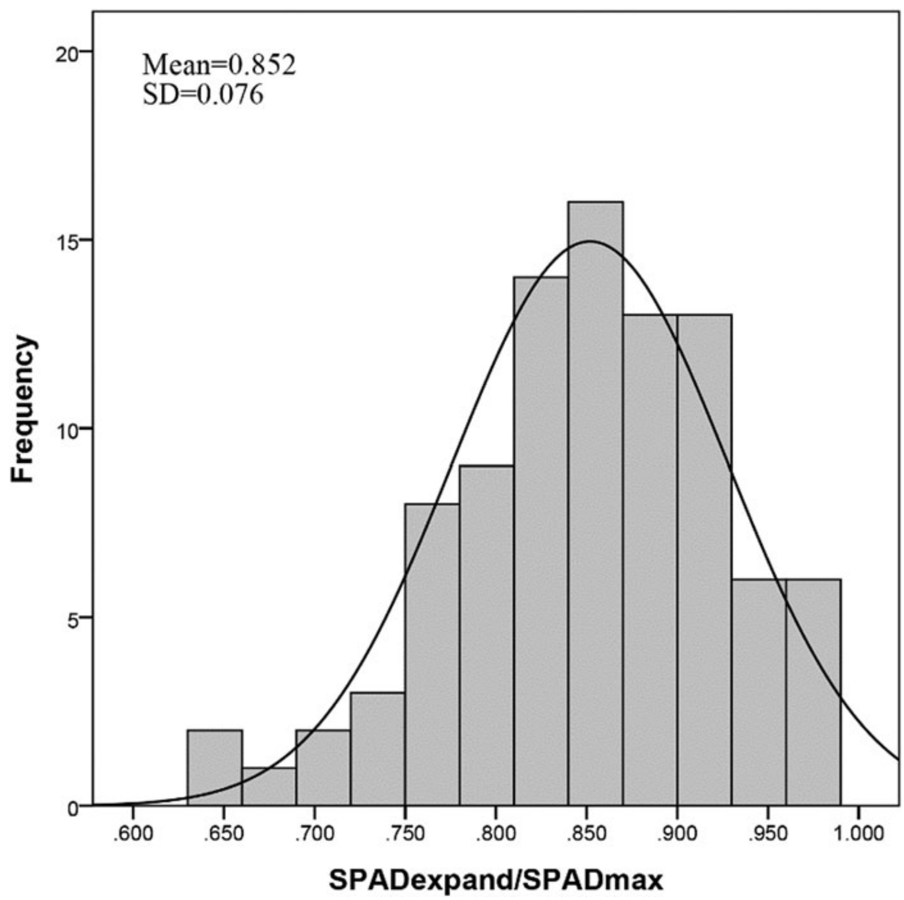

Fig. 11. Frequency distribution histogram of the ratio of $SPAD_{expand}$ to $SPAD_{max}$. The line represents the frequency density curve.

grain-filling period (Scharf *et al*., 2002; Mueller and Vyn, 2018). Therefore, frequent application of fertilizer and increasing the amount of nitrogen fertilizer applied during periods of greatest crop demand could increase fertilizer use efficiency by prolonging the leaf photosynthetic function duration while maintaining or increasing crop yield (Lemaire *et al*., 2008; Yang *et al*., 2014*b*; Mueller and Vyn, 2018).

The results of the current study showed that addition of nitrogen fertilizer prolonged the duration of high SPAD values in lower leaves. Zhou and Wang (2003), Li *et al.* (2009) and Zhang *et al.* (2020) reported that SPAD readings in the lower leaves showed a greater sensitivity than upper leaves to nitrogen rates in the canopy. Under nitrogen-deficient conditions, plant N moved easily from lower leaves to upper leaves to allow continued use, causing rapid chlorosis in the lower leaves (Joshi *et al*., 2019). However, when the supply of N was adequate, upper leaves did not require additional N, so the excess would be stored in the lower leaves. This meant that the chlorophyll content was still high in lower leaves, reducing the chlorophyll gradient between upper and lower leaf profiles (Wang *et al*., 2006; Yuan *et al*., 2016*a*). Through analysis of the spatio-temporal dynamics of SPAD readings, the current study clarified the sensitivity of different leaf positions in response to nitrogen fertilizer to prolong the duration of high leaf function and increase the accumulation of photo-assimilates through late N fertilization. These results are expected to aid in further development of nitrogen management strategies that better synchronize fertilizer nitrogen supply with crop N demand.

Measuring leaf SPAD reading variation over spatial and temporal scales is a critical method for assessing nutrient status and photosynthetic production capacity of the plant canopy, and is widely used to diagnose plant nutrient status and guide the precise management of fertilization (Croft *et al*., 2014; Rey-Caramés *et al*., 2016; Baresel *et al*., 2017). The SPAD-502 meter allows real-time, high-density and non-destructive monitoring of chlorophyll content, which can provide a vital reference for field fertilization management, crop growth performance and yield estimation (Li *et al*., 2009; Ling *et al*., 2011; Singh *et al*., 2011; Ali *et al*., 2014; Xiong *et al*., 2015; Yuan *et al*., 2016*b*). The current study used the duration of high SPAD readings to describe the duration of high leaf function and continuously monitored the SPAD readings of lower leaves to assess leaf nutrient status. Additionally, the end time of the duration of high SPAD reading and the onset time of the SPAD reading decrease were considered to be the sensitive stage of leaf senescence in response to nitrogen, which can be used for evaluation and regulation of the nitrogen status under different nitrogen application rates. Higher plant density is an important way to improve grain yield (Kuai *et al*., 2016; Li *et al*., 2017). The correlation of canopy photosynthetically active radiation and nutritional status distribution will be considered in future studies. Different nitrogen management strategies are needed for different plant densities (Qian *et al*., 2016; Yan *et al*., 2016). Selecting an optimal plant density and corresponding nitrogen management strategy is a potentially effective method for achieving a high grain yield and

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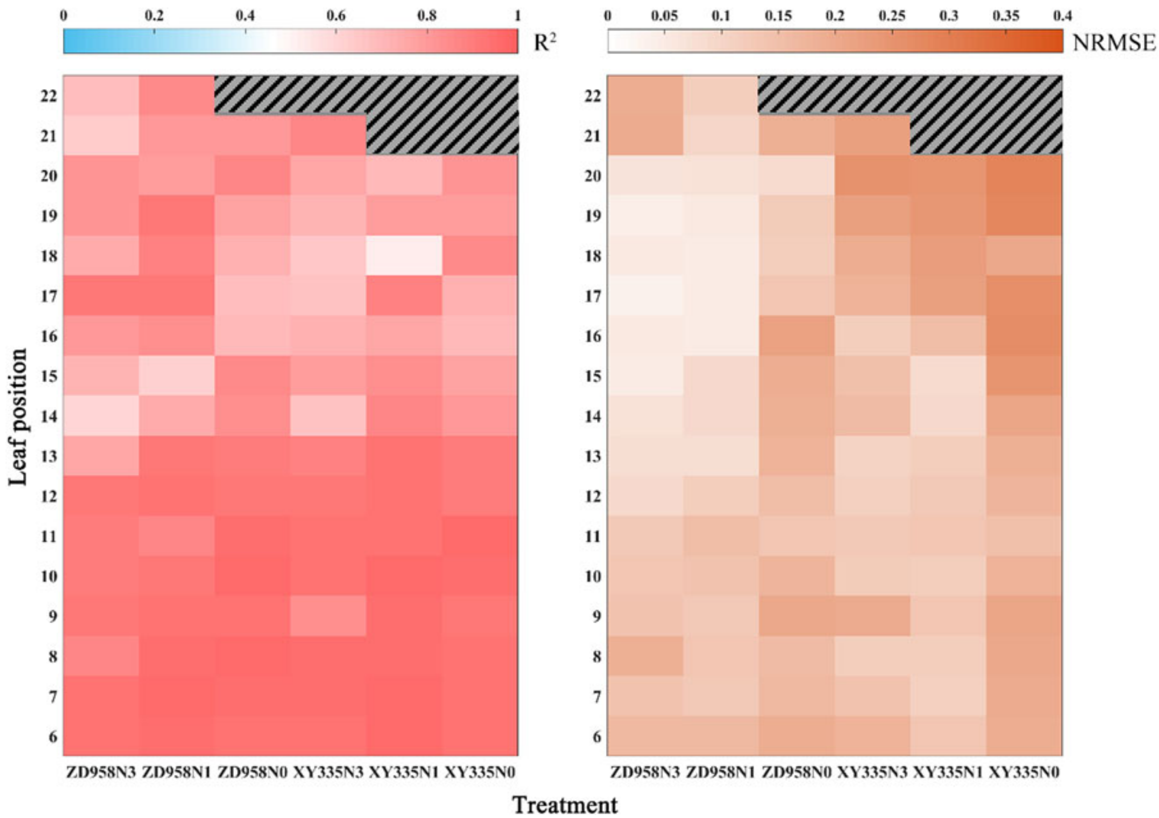

Fig. 12. (Colour online) The coefficient of determination (R^2) and NRMSE distribution results of each leaf SPAD reading model under different nitrogen application rates and cultivars. The grey hatched regions had no leaves. The depth of shading in the heatmaps indicates the degree of curve fit: the darker the shading on the left map has the better fit, the lighter the shading on the right map has the better fit.

high nitrogen use efficiency for maize production (Yan *et al*., 2016).

Conclusion

In the current study, the spatio-temporal dynamic characteristics of leaf SPAD readings were determined systematically under different nitrogen application rates, accurately quantified the time course of leaf SPAD readings through development of an SPAD reading model, and quantitatively assessed the duration of high SPAD readings. Furthermore, the positional difference in duration of leaf function response to nitrogen fertilizer was evaluated based on a model built for SPAD readings in this study. It was found that lower leaves were the most sensitive to nitrogen application rates based on the SPAD values. These findings contribute to understanding of how mechanisms that regulate crop nitrogen status are affected by agronomical practices and adds important insights to be considered in late N fertilization of maize. This study provides evidence for the usefulness of SPAD meter-guided need-based fertilizer nitrogen management technology and guides direction for the next steps to accurately assess the sensitive stage of a plant line to nitrogen fertilizer.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0021859621001052.

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Conflict of interest. The authors declare there are no conflicts of interest.

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