

# DIFFERENT LEAF SENSING APPROACHES FOR THE ESTIMATION OF WINTER WHEAT NITROGEN STATUS

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## ABSTRACT

The objective of this study was to evaluate different methods of using SPAD and Dualex 4 for the assessment of winter wheat nitrogen status. The experiments were conducted at Quzhou Experimental Station of China Agricultural University (CAU) in the North China Plain. The chlorophyll meter readings of the first and second fully expanded leaves were collected at Feekes growth stage 6 and 10.5 in 2010 and 2011 and the Dualex reading of the first fully expanded leaf were collected at Feekes growth stage 6 and 10.5 in 2011. The preliminary results of this study indicated that the SPAD-NSI approach was a reliable approach for diagnosing N status of winter wheat in North China Plain, while the SPAD value ratio between the second and first fully expanded leaf did not work well. The nitrogen sufficiency index calculated with nitrogen balance index (NSI-NBI) obtained with the Dualex sensor was more sensitive to N status than NSI-based on SPAD readings. More studies are needed to determine the threshold values for NSI-NBI.

**Keywords:** Chlorophyll meter; Dualex 4 sensor; Nitrogen balance index; Nitrogen sufficiency index; Nitrogen nutrition index; Winter wheat

## INTRODUCTION

Nitrogen is an important variable influencing crop yield and quality (Bhatia and Rabson, 1976; Everaarts, 1993; Algerbo and Thylen, 1998; Elia et al., 1998; Samonte et al., 2006). Plant-based diagnostic methods of N deficiency are used to improve N management and maintain environmental sustainability for crops.

Nitrogen nutrition index (NNI) has been proposed as a reliable method to diagnose crop N status. However, this method is time-consuming, labor-intensive and financially expensive for large area applications. Remote sensing, as a rapid and non-destructive method, has been widely used in estimating crop nitrogen status and offers a promising practical approach for in-season N diagnosis and management.

Chlorophyll meter (e.g. SPAD meter) has been proven as an effective tool to determine the N status of many crops, including spring wheat (Follett et al., 1992; Vidal et al., 1999; Arregui et al., 2006), rice (Peng et al., 1993; Ladha et al., 1998), potato (Minotti et al., 1994), and corn (Piekielek and Fox, 1992; Schepers et al., 1992; Dwyer et al., 1995). However, some studies have reported limitations of the SPAD because the readings are affected by many factors such as variety, environmental conditions, and insect damage (Campbell et al., 1990; Schepers et al., 1992; Monje and Bugbee, 1992), thus it is difficult to set up threshold values. Persons et al. (1993) proposed establishing reference strips which received sufficient levels of nitrogen fertilizer. Then the Nitrogen Sufficiency Index (NSI) can be obtained through the ratio between average meter readings from the fields to be fertilized and reference area. A  $NSI < 0.95$  indicates N deficiency and the need for additional N application. To improve N diagnosis, Shen et al. (2002) of Zhejiang University put forward a ratio index using chlorophyll meter readings of two different position leaves, based on the principle that nitrogen of lower leaves will transfer to upper leaves when the plant has nitrogen deficiency. In their study, they found that, while the chlorophyll meter readings of two rice varieties were different, the ratios of two varieties were all less than 1 if the rice plant was nitrogen deficient.

The Dualex as a new leaf-clip instrument can also be used to estimate the crop nitrogen status. It is based on the measurement of polyphenolics (Phen), which are secondary metabolites playing multiple roles in leaves (Caldwell et al., 1983; Hahlbrock and Scheel, 1989; Kiraly, 1964). Nitrogen deficiency induces a decrease in leaf Chl and an increase in leaf Phen contents (Cartelat et al., 2005). Therefore, crop N status can also be assessed through the detection of leaf Phen content. Cartelat et al. (2005) suggested that the Nitrogen Balance Index (NBI, Chl/Phen ratio) was a good indicator of leaf N concentration at the canopy level and that this ratio would alleviate, at least partially, the problem of Chl and Phen gradients along leaves.

Cartelat et al. (2005) suggested that since the Dualex readings on adaxial side and the Dualex readings on abaxial side are highly correlated, it would be possible to use measurements from only one side for agricultural purposes.

Tremblay et al. (2007) also suggested that single side Dualex measurements are sufficient for N diagnosis in corn.

Studies are limited to compare these different N diagnosis methods, especially for winter wheat. Therefore, our main objective of this study was to evaluate different SPAD-based and Dualex-based N diagnosis methods for winter wheat in North China Plain.

## **MATERIALS AND METHODS**

### **Experimental design**

Two N fertilization experiments were conducted in North China Plain on winter wheat crops.

Experiment 1 is a long-term experiment (Exp.1) in Quzhou county (36.9°N, 115.0°E) of Hebei province with five N treatments: no N as a control (CK), Opt., Farmer's N practice, 50% and 150% of Opt. The Farmer's N practice was set at 300 kg N ha<sup>-1</sup> (150 kg N ha<sup>-1</sup> applied before planting and 150 kg N ha<sup>-1</sup> applied at stage Feekes 6), which is typical for Hebei province. The Opt. was determined according to an in-season root-zone N-management strategy (Cui et al., 2008). The experiment was four replications with randomized complete block design. The variety was Liangxing99.

Experiment 2 is a nitrogen rate experiment (Exp.2) in 2010/2011 at Quzhou county (36.9°, 115.0°E) in Hebei province. This experiment had seven N treatments: 0, 60, 120, 180, 240, 300, 360 kg N ha<sup>-1</sup>, with 50%N being applied as basal N (before sowing), and 50% applied as topdressing N (regreening-shooting stage). The experiment had three replications with randomized complete block design. The variety was Kenong9204.

### **Dualex and SPAD measurement**

A Dualex (Force-A, Orsay, France) was used to obtain Dualex values (NBI) on the first fully expanded leaf at Field 1 and 2 in 2010/2011. The NBI readings were taken at the midpoint of each adaxial side of leaf blade, 30mm apart, on one side of the midrib. NBI readings were taken at Feekes 6 and Feekes 10.5 stages.

A chlorophyll meter (SPAD-502, Soil-Plant Analysis Development Section, Minolta Camera Co., Osaka, Japan) was used to obtain SPAD values on the first and second fully expanded leaf at Field 1 in 2009/2010 and 2010/2011 and on the first fully expanded leaf at Field 2 in 2010/2011. The SPAD readings were taken at the midpoint of each leaf blade, 30mm apart, on one side of the midrib. SPAD readings were taken at Feekes 6 and Feekes 10.5 stages.

### **Plant measurement**

Aboveground biomass was destructively sampled by randomly cutting 100 by 30 cm vegetation in each plot. All plant samples were oven dried at 70°C to constant weight and then weighted, ground, and their Kjeldahl-N determined. Biomass was collected at Feekes growth stage 6 and 10.5.

### **Data analysis**

All data were analyzed using Statistical Product and Service Solutions (SPSS 13.0), SAS software (SAS Inst.,1990) and Excel 2003.

## **RESULTS AND DISCUSSION**

### **Sensitivity of Different Diagnosis Methods for the Assessment of Winter Wheat N Status in North China Plain**

In 2010 at Feekes 6. The NSI values based on either the top 1 or 2 fully expanded leaf all indicated that the 0 and 75 kg ha<sup>-1</sup> treatments were deficient in N, but the ratio index (2LFT/1FLT) did not detect any treatment as N deficient. The NSI approach detected the same two treatments at Feekes 10.5, but the ratio approach only detected the 0 kg ha<sup>-1</sup> treatment (Table 1). This result indicated that the ratio approach did not work well with winter wheat.

In 2011, the NSI approach had similar results as in 2010, but the ratio index values were all less than 1 for the five N treatments, even the 300 kg ha<sup>-1</sup> treatment, confirming the conclusion we drew for the 2010 results.

NBI values were more sensitive to N rates. The 300 and 140 kg ha<sup>-1</sup> treatments had significantly higher NBI values than the 106 kg ha<sup>-1</sup> treatment, which was significantly higher than the 75 and 0 kg ha<sup>-1</sup> treatments. The NSI calculated using NBI even indicated that the 140 kg ha<sup>-1</sup> treatment was deficient if we used 0.95 as the threshold values. It seems we should use 0.8 as the threshold value for NBI-based NSI. This needs to be further evaluated using more site-year's data, but we can draw the conclusion that NBI is more sensitive to SPAD values, and has a good potential for N status diagnosis in North China Plain.

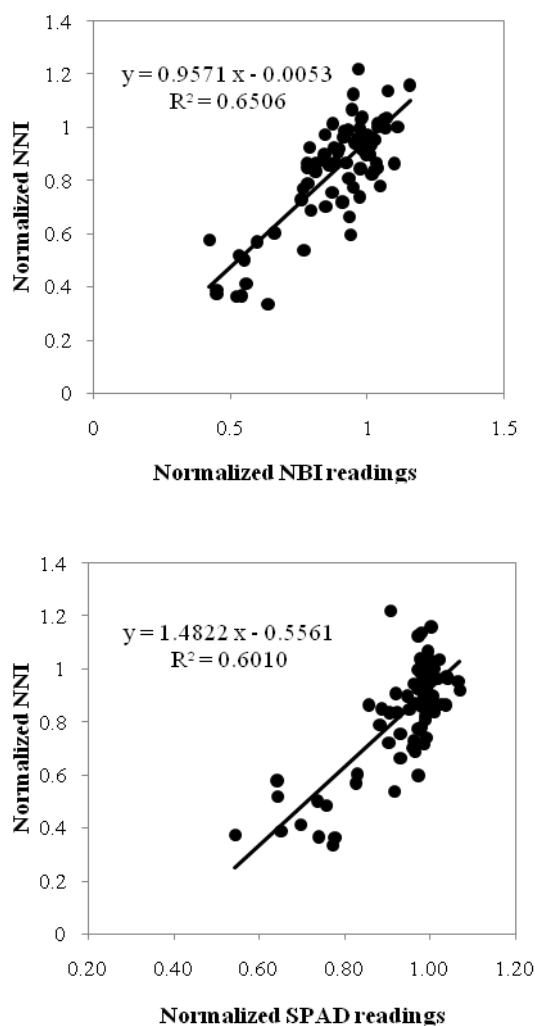
### **Relationships between Normalized Dualex, SPAD readings and Nitrogen Nutrition Index**

Normalized NBI readings and SPAD readings were significantly correlated with normalized NNI (R<sup>2</sup>=0.65 and 0.60, respectively, Fig. 1) across growth stages and experiments. This results indicated that it is possible to use Dualex and SPAD to estimate NNI. More studies are needed to confirmed these results.

**Table1.** Influence of N rates on different N status indicators in Exp.1 of 2010 and 2011

Growth Stage	N rate	2LFT/1LFT	NSI 1LFT	NSI 2LFT	NBI	NBI-NSI
Feekes 6 (2010)	0	1.04a	0.68c	0.67c		
	75	1.04a	0.85b	0.83b		
	106	1.06a	0.99a	0.99a		
	140	1.03a	1.01a	0.98a		
	300	1.06a	1.00a	1.00a		
Feekes10.5 (2010)	0	0.96b	0.79c	0.75c		
	75	1.01a	0.91b	0.91b		
	106	1.00a	1.00a	0.99a		
	140	1.02a	1.01a	1.01a		
	300	1.02a	1.00a	1.00a		
Feekes 6 (2011)	0	0.94a	0.63c	0.62b	21.56c	0.47c
	75	0.92a	0.76b	0.72b	25.50c	0.55c
	168	0.99a	0.98a	1.00a	38.36b	0.83b
	218	0.99a	1.00a	1.01a	43.53a	0.94a
	300	0.97a	1.00a	1.00a	46.09a	1.00a
Feekes10.5 (2011)	0	0.98b	0.75c	0.72c	23.26c	0.56c
	75	1.05a	0.95b	0.96b	37.16b	0.90b
	168	1.02a	0.99ab	0.98ab	41.06ab	0.99ab
	218	1.04a	0.99ab	0.99ab	43.19a	1.04a
	300	1.04a	1.00a	1.00a	41.50ab	1.00ab

Different letters a–c indicate that the readings are significantly different at the 0.05 probability level.



**Fig.1** Relationships between normalized NBI readings, normalized SPAD readings and normalized Yield, normalized NNI across growth stages and Exp.1 and 2 in 2011.

## CONCLUSIONS

The preliminary results of this study indicated that the SPAD-NSI approach was a reliable approach for diagnosing N status of winter wheat in North China Plain, while the SPAD value ratio between the second and first fully expanded leaf did not work well. The nitrogen sufficiency index calculated with nitrogen balance index (NSI-NBI) obtained with the Dualex sensor was more sensitive to N status than NSI-based on SPAD readings. More studies are needed to determine the threshold values for NSI-NBI.

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