EVALUATING LEAF FLUORESCENCE SENSOR DUALEX 4 FOR ESTIMATING RICE NITROGEN STATUS IN NORTHEAST CHINA

Weifeng Yu, Yuxin Miao*, Shanshan Hu, Jianning Shen, Hongye Wang

International Center for Agro-Informatics and Sustainable Development
College of Resources and Environmental Sciences
China Agricultural University
Beijing, China
*ymiao@cau.edu.cn

ABSTRACT

Chlorophyll meter (CM) has been commonly used to non-destructively estimate crop leaf chlorophyll concentration, and indirectly estimate crop N status. Dualex 4 is a newly developed leaf fluorescence sensor that can estimate both leaf chlorophyll concentration and polyphenolics, especially flavonoids. The ratio of chlorophyll and flavonoid concentration, which is termed N balance index (NBI), has been reported to more sensitive to crop N status than SPAD meter readings. The objective of this study was to compare the accuracy of Dualex 4 sensor and chlorophyll meter SPAD-502 for estimating the N nutritional status of rice in Northeast China. A field experiment involving five N application rates (0, 70, 100, 130, 160 kg N ha^{-1}), two cultivars (Longjing 21 and Kongyu 131) and three replications was conducted in Jiansanjiang, Heilongjiang Province, China in 2013. The preliminary results indicated that both instruments could explain over 80% of variation of leaf chlorophyll concentration. The new index NBI (Chlorophyll/Flavonoid) did not significantly improve the accuracy of estimating leaf N concentration as compared with SPAD meter. More studies are needed to further evaluate the Dualex 4 leaf fluorescence sensor for estimating rice N status and determine its potential benefits over SPAD meter.

Keywords: Leaf fluorescence sensor; Chlorophyll meter; Nitrogen status; Chlorophyll concentration; Nitrogen balance index; Flavonoid; Precision nitrogen management

INTRODUCTION

Leaf chlorophyll (Chl) content is key indicator in rice N status, since rice plant allocated about 65-70% of nitrogen to leaf and more than 80% of leaf nitrogen to chloroplast (Mae and Ohira, 1981). The Minolta SPAD-502

(Minolta Camera Co., Ltd., Japan) chlorophyll meter is one of the widely used tools. It measures leaf transmittance at two wavelengths in the red (around 660 nm) and near infrared (around 940 nm), and then uses a conversion equation to determine leaf greenness (Minolta, 1989). Dualex is a newly developed leaf-clip meter that uses leaf chlorophyll fluorescence to measure leaf polyphenols (Phen). Studies showed that combined index of SPAD and Phen was more accurate in determining plant N status (Tremblay et al., 2012). Newest version of Dualex, Dualex 4, has added a new index to measure leaf chlorophyll concentration.

So far, studies about Dualex were all concentrated on wheat and maize, but no studies reported are based on rice. Therefore, the objective of this study was to evaluate the ability of Dualex sensor in estimating the N nutritional status of rice and compare the estimating accuracy with chlorophyll meter SPAD-502.

MATERIALS AND METHODS

Two experiments were carried out in experimental station of China Agriculture University in Jansanjiang, Heilongjiang province.

Experiment 1 was a calibration experiment with 220 rice leaves (total seven dataset) sampled at three growth stages in 2013. These leaves came from five rice cultivars, i.e. Kongyu 131 (KY131), Longjing 21 (LJ21), Longjing 31 (LJ31), Longjing 36 (LJ36) and Longjing 40 (LJ40). Three key growth stages selected were mid-tillering (MT), panicle initiation (PI) and stem elongation (SE). KY131 sampled at all three growth stages, LJ21 sampled at stem elongation, and other cultivars sampled at panicle initiation. Rice leaves were carefully chosen to ensure a color range from light green to deep green. Ten readings acquired by using SPAD-502 and Dualex from each leaf were averaged as an estimation of leaf chlorophyll content. After that, we used a disc of 5 mm diameter to collect 10 leaf discs evenly along the leaf blade, which were later analyzed for leaf chlorophyll concentration (Arnon, 1949).

Experiment 2 was an N rate experiment with five N rates (0, 70, 100, 130, 160 kg N ha^{-1}) using a randomized block design with three replicates. Each treatment was also applied 50 kg P_2O_5 ha^{-1} and 105 kg K_2O ha^{-1}. Two rice cultivars Kongyu 131 and Longjing 21 were used in this experiment. Meter readings were taken from first newly and fully expanded leaves of 20 rice plant in each plot at three growth stages (panicle initiation, stem elongation and heading stage). After measurement, three hills of rice (each had 4-6 rice plants) were sampled to determine rice leaf nitrogen concentration using standard Kjeldahl method.

RESULTS AND DISCUSSIONS

Meter readings and chlorophyll concentration

Relationship between meter readings and laboratory-determined chlorophyll concentration of all leaves is presented in Fig.1. Both SPAD and Dualex 4 show a good linear relationship with chlorophyll concentration. The coefficient of determination (R^2) for the fitted models are 0.827 and 0.865, and root mean square error are 3.469 μg cm^{-2} and 3.063μg cm^{-2}, respectively. From Fig.1, we can see that SPAD has a better performance than Dualex in chlorophyll estimation. The R^2s of linear models of SPAD are more significant and RMSE are much smaller. Despite these differences, Dualex is also a good index in non-destructively estimating rice leaf Chl concentration.

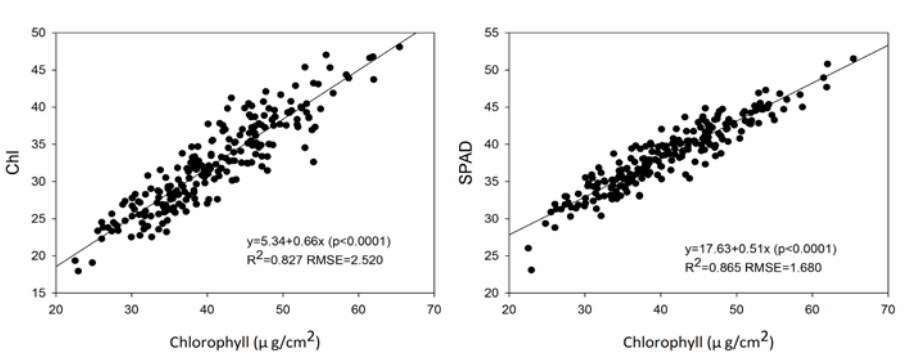
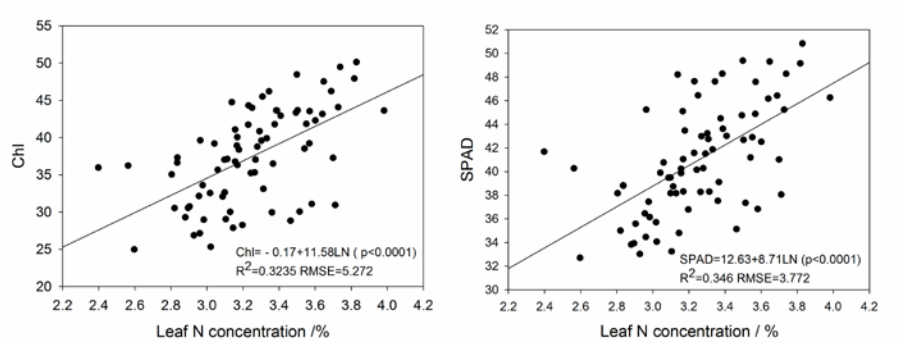


Fig 1. Relationships between meter readings and chlorophyll concentration

Meter readings and leaf nitrogen concentration

Meter readings were influenced by rice cultivars and growth stages. At each growth stage, meter readings (SPAD and Chl) showed a significant relationship with leaf nitrogen concentration. The R^2 ranged from 0.553 to 0.838 for Dualex (Chl) and from 0.499 to 0.839 for SPAD-502 (SPAD). The estimation of Flavonoids (Flav) showed a significant negative relationship with leaf nitrogen concentration at Stem Elongation and Heading stages but not Panicle Initiation stage. Besides, new index NBI (Chl/Flav) did not improve the relationship with leaf nitrogen concentration. Combined all the datasets, SPAD, Chl and NBI showed significant positive relationship with leaf N concentration and Flav negatively correlated with leaf N concentration. Flav can only explained 17.2% variation of leaf N concentration (Fig.2).



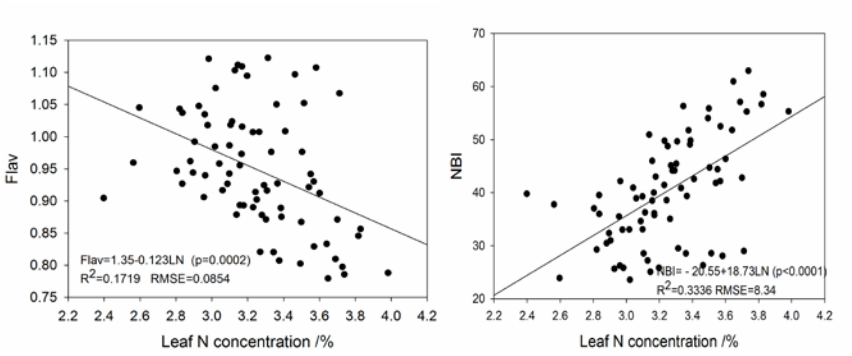


Fig. 2. Relationships between different meter readings and leaf nitrogen concentration

CONCLUSION

The preliminary results indicated that both instruments could explain over 80% variation of rice leaf chlorophyll concentration. Rice cultivars and growth stages influenced their ability to estimate leaf N concentration. Across cultivars and growth stages, Dualex 4 and SPAD meter-based indices only explained about 30% of variation of leaf N concentration. Although flavonoid was negatively correlated with leaf N concentration (R^2=0.172, p=0.0002), the new index NBI did not significantly improve the accuracy of estimating leaf N concentration as compared with SPAD meter. More studies are needed to further evaluate the Dualex 4 leaf fluorescence sensor for estimating rice N status and determine its potential benefits over SPAD meter.

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REFERENCES

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts, poluphenoloxidase in beta vulgaris. Plant Physiol. 24(1): 1–15Available at http://www.plantphysiol.org/cgi/doi/10.1104/pp.24.1.1 (verified 25 May 2013).

Cerovic, Z.G., G. Masdoumier, N. Ben Ghozlen, and G. Latouche. 2012. A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf chlorophyll and epidermal flavonoids. Physiol. Plant. 146(3): 251–60Available at http://www.ncbi.nlm.nih.gov/pubmed/22568678 (verified 29 January 2013).

Hamilton, J.G., A.R. Zangerl, E.H. Delucia, and M.R. Bernebrum. 2001. The carbon-nutrient balance hypothesis: its rise and fall. Ecol. Lett. 4: 86–95.

Li, S., P. He, and J. Jin. 2013. Nitrogen use efficiency in grain production and the estimated nitrogen input/output balance in China agriculture. J. Sci. Food Agric. 93(5): 1191–1197Available at http://www.ncbi.nlm.nih.gov/pubmed/22987539 (verified 15 May 2013).

Mae, T., and K. Ohira. 1981. The Remobilization of Nitrogen Related to Leaf Growth and Senescence in Rice Plants. Plant Cell Physiol 22(6): 1067–1074.

Minolta. 1989. Manual for chlorophyll meter SPAD-502. Japan.

Tremblay, N., Z. Wang, and C. Bélec. 2007. Evaluation of the Dualex for the Assessment of Corn Nitrogen Status. J. Plant Nutr. 30(9): 1355–1369Available at http://www.tandfonline.com/doi/abs/10.1080/01904160701555689 (verified 16 December 2013).

Tremblay, N., Z. Wang, and Z.G. Cerovic. 2012. Sensing crop nitrogen status with fluorescence indicators. A review. Agron. Sustain. Dev. 32(2): 451–464.

Uddling, J., J. Gelang-Alfredsson, K. Piikki, and H. Pleijel. 2007. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. Photosynth. Res. 91(1): 37–46Available at http://www.ncbi.nlm.nih.gov/pubmed/17342446 (verified 28 February 2013).

Zhang, F., W. Jiqing, Z. Weifeng, C. Zhenling, M. Wenqi, C. Xinping, and R. Jiang. 2008. Nutrient use efficiencies of major cereal crops in China and measures for improvement (In Chinese). ACTA Pedol. Sin. 45(5): 915–924.