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Evaluating the Potential of Improving In-season Nitrogen Status Diagnosis of Potato Using Leaf Fluorescence Sensors and Machine Learning

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Abstract.

The Best Management Practices (BMPs) guideline recommends potato growers split-apply nitrogen (N) fertilizer according to the results of petiole nitrate-nitrogen (PNN) test for improved profitability and sustainability. However, the PNN test is a wet chemistry analysis which suffers from destructive and laborious sampling campaign, high laboratory analysis cost, and long laboratory turnaround time against a short window for responsive in-season N management. A latest leaf sensor, Dualex Scientific (Dualex), is expected to overcome the disadvantages of the PNN test. The objectives of this study are 1) to investigate how well Dualex can estimate the PNN concentrations across different genetic, environmental, and management (GxExM) conditions, and 2) to evaluate the PNN concentration-based potato N status classification accuracy, and 3) to identify the best model for the PNN concentration estimation. The study was conducted at the Sand Plain Research Farm, Becker, Minnesota in 2018 and 2019 using a randomized complete block design with three replications. Six cultivars and three N rates were included. This study showed that Dualex could identify in-season potato N status non-destructively at 76% accuracy by estimating PNN concentrations with GxExM information using random forest regression. It is important to note that accumulated growing degree days and as-applied N rates were selected as two of the most important variables for PNN prediction using the random forest regression. Dualex was also compared to a traditional leaf sensor, SPAD-

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502, in the capability of the PNN concentration estimation, and they were shown to be equally capable. Further analyses and research are required to evaluate Dualex sensor under diverse on-farm conditions and develop in-season site-specific N management strategies.

Keywords.
Dualex Scientific, In-season N management, Petiole Nitrate-N, GxExM, Random Forest, Support Vector, SPAD-502

Abbreviations.
Anth, anthocyanin; BMPs, best management practices; Chl, chlorophyll; CV, coefficient of variation; Dualex, Dualex Scientific; Dualex Best MLR, the best Dualex-based multivariate linear regression model; Dualex MLR, multivariate linear regression model developed using Dualex Chl, Flav, and Anth; ESN, Environmentally Smart Nitrogen; Flav, flavonol; GDDs, growing degree days; GxExM, genetic, environmental, and management; ML, machine learning; MLR, multivariate linear regression; N, nitrogen; NBI, nitrogen balance index; NNI, nitrogen nutrition index; P, phosphorus; PE, percent error; PNN, petiole nitrate-nitrogen; R^2, coefficient of determination; radial, radial basis function; RF, random forest; RMSE, root mean square error; rSV, support vector regression with radial basis function kernel; SPAD, SPAD-502; SR, simple regression; SV, support vector

Introduction

Potatoes are one of the highest-yielding staple food crops and are also characterized by the strong resistance to various environmental conditions including cold, drought, and low soil fertility (Batool et al., 2020). These characteristics make potatoes the world's fourth most productive staple food crop and crucial for global food security (Eid et al., 2020). High nitrogen (N) fertilizer application rate is often necessary to achieve potato's high yield. Poor management of N fertilizer application will likely result in low N use efficiency, especially due to potato's shallow root system and preference to coarse-textured soils, leading to yield losses and ground water contamination through nitrate leaching (Sun et al., 2019). In order to manage N fertilizer application properly for improved profitability and sustainability, the Best Management Practices (BMPs) guideline recommends potato growers split-apply N fertilizer at key growth stages according to the results of the conventional wet-chemistry analysis called petiole nitrate-N (PNN) test (Rosen and Bierman, 2008). Petioles, specifically the fourth petiole from the shoot tip, are collected to diagnose potato N status (Rosen, 2021). However, this approach suffers from disadvantages including destructive and laborious sampling campaign, high laboratory analysis cost, and long laboratory turnaround time against a short window for responsive in-season N management.

The use of proximal sensing technologies is expected to help growers overcome some of these problems. SPAD-502 (SPAD) is a hand-held leaf-clip chlorophyll (Chl) meter released from Konica Minolta Inc. (Konica Minolta, Osaka, Japan). Chl is the primary component of chloroplast and essential for plant growth by photosynthesis. Studies have shown promising results for in-season non-destructive N status diagnosis using SPAD on many crops including potatoes (Giletto and Echeverría, 2013; Goffart et al., 2008; ZHENG et al., 2015). Dualex Scientific (Dualex), which is currently owned by Pessl Instruments GmbH (Pessl Instruments GmbH, Weiz, Austria), is another hand-held leaf-clip sensor and is capable of leaf Chl, flavonol (Flav), and anthocyanin (Anth) measurements. Flav and Anth are the substances produced in response to plant N and phosphorus (P) deficiencies, respectively. Dualex also calculates Chl/Flav and displays it as Nitrogen Balance Index (NBI), which is supposed to be more sensitive to crop N status (Cerovic et al., 2012). In addition to these additional measurements, Dualex is advertised for the linear relationship between leaf Chl and sensor readings in the units of $\mu g/cm^2$, and thus no measurement saturation. Regardless of these presumed improvements of Dualex over SPAD, there have been only a few studies on in-season non-destructive potato N status diagnosis using Dualex as this proximal sensor is still relatively new (Ben Abdallah et al., 2018).

Because N in the petiole will eventually be stored in the leaflets, the PNN concentrations should correlate with SPAD and Dualex readings. Given this assumption, the objectives of this research are 1) to investigate how well SPAD and Dualex can estimate the PNN concentrations across different genetic, environmental, and management (GxExM) conditions, and 2) to evaluate the accuracy of PNN concentration-based potato N status classification, and 3) to identify the best model for the PNN concentration estimation. Given the novelty of Dualex, more emphasis will be placed on Dualex, and SPAD will be used as a comparison in this study. The research results are expected to overcome the disadvantages of the PNN test but still allow use of the same index, PNN concentration, for in-season potato N status diagnosis.

Materials and Methods

Study Design

Small plot experiments were conducted at the Sand Plain Research Farm, Becker, Minnesota on a Hubbard loamy sand soil (Sandy, mixed, frigid Entic Hapludolls) in 2018 and 2019 (Figure 1). The experiments were conducted in two site-years. A randomized complete block design was used with three replications. Six cultivars with varying maturity were selected and managed with three N treatments: 135, 269, or 404 kg N/ha. Diammonium phosphate (18-46-0) was band-applied 8 cm to the side and 5 cm below the seed tuber to supply 45 kg N/ha for all the treatments at planting. At emergence, Environmentally Smart Nitrogen (ESN, 44-0-0) (Nutrient, Inc., Calgary, AB, Canada) was side-dressed and hilled in to supply 90, 180, or 269 kg N/ha at each N rate treatment, respectively. The rest of 45 or 90 kg N/ha for the 269 or 404 kg N/ha treatments were split-applied four times each with 11 or 22.5 kg N/ha as urea and ammonium nitrate (28-0-0), respectively. The information on cultivars and as-applied N rates is summarized in Table 1. Note that the as-applied N rates were used in the units of lbs/acre in the model development. More detailed description of the experimental field management is given by the Minnesota Area II Potato Research and Promotion Council and Northern Plains Potato Growers Association 2019 & 2020 reports (Gupta and Rosen, 2018 and 2019). SPAD and Dualex measurements were taken from 20 terminal leaflets on the 4th leaf from the shoot tip and 15 terminal leaflets on the top fully expanded leaf in each plot on four dates, respectively. These sensor measurements were then averaged for each plot and date. 20 destructive petiole samples were collected in each plot on the corresponding dates to measure the water-extractable PNN concentration in the laboratory. Daily maximum and minimum temperatures were also recorded to calculate growing degree days (GDDs) with the base temperature of 7 degrees Celsius (Worthington and Hutchinson, 2005). Sensor data and sample collection dates, and accumulated GGDs on the corresponding dates are also summarized in Table 1.

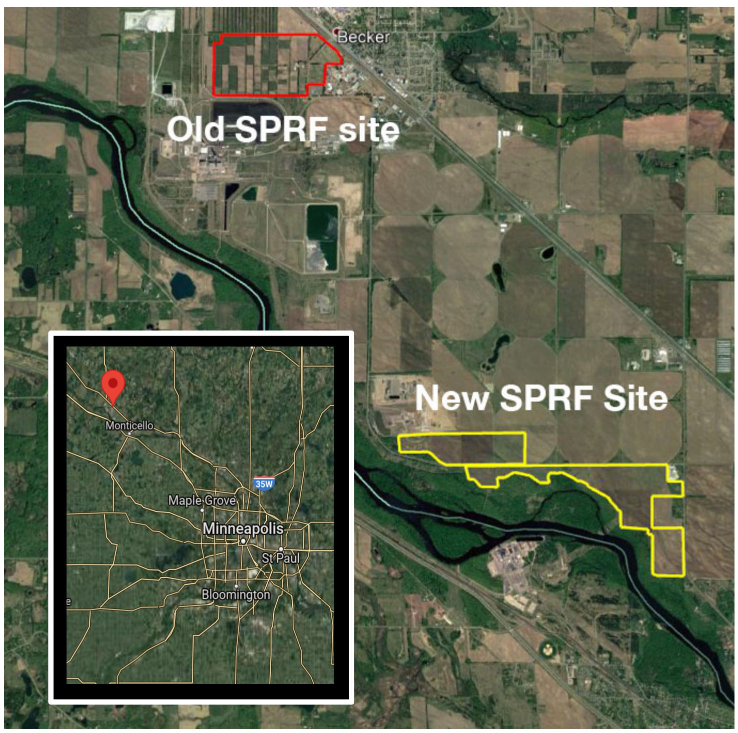

Figure 1. The old and new locations of the Becker Sand Plain Research Farm. The pin in the inset map shows the location of Becker, MN.

Table 1. Information on the details of the experiments conducted in 2018 and 2019.

Year	Cultivars	Maturity	Planting date	Harvest date	Side-dressing date	Sampling & Sensing date	Accumulated GDDs	As-applied N rates (kg/ha)		
2018	Clearwater Ivory Russet Russet Burbank Umatilla	Medium to late Early to medium Late Medium to late	May 14	Sep 25	July 9 July 16 July 23 July 30	June 26 July 10 July 18 August1	661 916 1054 1256	135 135 135 135	224 235 247 269	314 336 359 404
2019	Clearwater Lamoka MN13142 Russet Burbank Umatilla	Medium to late Medium to late Early to medium Late Medium to late	May 6	Sep 27	July 8 July 15 July 30 August 5	June 26 July 11 July 24 August7	437 667 876 1081	135 135 135 135	224 235 247 269	314 336 359 404

Note: GDDs, growing degree days

Development and Evaluation of the Regression Models

The obtained dataset was divided into the calibration and validation datasets. Data from two of the three replications were randomly assigned to the calibration dataset (238 observations) and used to train the models. The remaining data were assigned to the validation dataset (119 observations) and used to evaluate the developed models. Three observations (two from calibration, one from validation) were removed due to the absence of data. In the model development process, simple regression (SR) models using linear, quadratic, exponential, and power functions were first developed with sensor readings or GDDs-normalized sensor readings (sensor readings/accumulated GDDs) as the independent variable. Secondly, multivariate linear regression (MLR) models with sensor readings, accumulated GDDs, and as-applied N rates as the independent variables were explored. Finally, two machine learning (ML) models, support vector (SV) and random forest (RF) regressions, were developed using sensor readings, cultivar information, accumulated GDDs, and as-applied N rates. Three SV kernel functions used in this study are linear, polynomial, and radial basis function (radial). Important variables, and their order of importance in the ML regression models were also determined using the Boruta package in R software to better understand the regression models and attempt to improve the model performance accordingly. In the MLR and ML regression models, NBI from Dualex was excluded from the model development process to prevent multicollinearity. All the variables used in the MLRs were square-root transformed to improve the compliance with the assumptions of linear regressions. The parameters for the SV regressions were optimized by repeating ten-fold cross validation three times. For the RF regressions, the number of trees was set to 500, and the number of variables used in each node was optimized. In both SV and RF regressions, the independent variables were scaled and centered. Cultivar information was turned into dummy variables for the use in the SV regressions. To evaluate the developed regression models, the coefficient of determination (R^2), root mean square error (RMSE), percent error (PE), and Kappa statistic were calculated using the validation dataset. The estimated PNN concentrations were separated into deficient, sufficient, and excessive categories according to Rosen and Bierman (2008), and the potato N status classification accuracy was evaluated using confusion matrices accompanied by the accuracy statistics. R software was used to conduct all the statistical analyses, and Excel software was used to produce figures.

Results and Discussion

Simple Regression Models Only Using Sensor Data

The summary statistics including maximum, minimum, mean, and coefficient of variation (CV) for the variables of interest are shown in Table 2. The best Dualex-based SR model for the PNN concentration estimation was the quadratic regression using NBI with validation R^2 0.61, RMSE 5042.13 ppm, PE 48.10%, and Kappa statistic 0.28 (fair agreement). Unlike SPAD, Dualex is also capable of developing MLR models using sensor measurements. The best MLR model solely developed by Dualex used Chl, Flav, and Anth (Dualex MLR) and showed validation R^2 0.61, RMSE 4958.75 ppm, PE 57.25%, and Kappa statistic 0.31 (fair agreement). In contrast, the best SPAD-based SR model for the PNN concentration estimation was the power regression and showed validation R^2 0.24, RMSE 10532.13 ppm, PE 68.39%, and Kappa statistic 0.32 (fair agreement). The validation R^2 was low due to overfitting. These results are summarized in Table 3. The SPAD-based SR model classified the potato N status slightly better than Dualex MLR, which were also indicated by the respective accuracy statistics of 0.58 and 0.55 produced with their confusion matrices. This result might have come from the different wavelengths used for Chl measurement. SPAD uses the red (650 nm) and near-infrared (940 nm) lights to measure leaf Chl by comparing their transmissions. Dualex uses the far-red (710 nm) and near-infrared (850 nm) lights to avoid saturation at higher readings and to achieve a linear relationship between leaf Chl and sensor readings in the units of µg/cm2. As Yamada and Fujimura (1991) wrote, a wavelength with a higher Chl absorption coefficient such as the red wavelength of SPAD in this case is more accurate at lower leaf Chl contents. Table 2 shows that the ranges of SPAD readings both in the calibration and validation datasets were approximately between 20 and 50, which may be considered low enough to favor the SPAD performance. Yet, in other words, Dualex achieved a similar level of accuracy to SPAD using Chl, Flav, and Anth. As Cerovic et al. (2012) noted, the use of 710 nm by Dualex, which has a smaller “sieve effect” than 650 nm (Vogelmann, 1993), may also have been conducive to Dualex achieving the equivalent level of accuracy to SPAD.

Table 2. Summary statistics of the variables of interest in the calibration and validation datasets.

Measurements	Max	Min	Mean	CV
Calibration				
Petiole NO_3-N (ppm)	31410	5	11289	0.70
Dualex Chl (μg/cm^{-2})	40.42	14.00	26.02	0.17
Dualex Flav	2.14	0.94	1.44	0.18
Dualex Anth	0.27	0.05	0.12	0.28
Dualex NBI	31.80	6.77	19.50	0.27
SPAD	52.20	21.60	40.08	0.12
Validation				
Petiole NO_3-N (ppm)	29298	18	11155	0.72
Dualex Chl (μg/cm^{-2})	40.46	16.14	25.99	0.18
Dualex Flav	2.15	1.01	1.46	0.17
Dualex Anth	0.22	0.05	0.12	0.29
Dualex NBI	31.84	7.95	19.08	0.27
SPAD	52.10	22.70	40.13	0.13

Table 3. The results of PNN prediction only using Dualex or SPAD readings.

Regression Equations	Validation R^2	RMSE (ppm)	PE (%)	Kappa
y =1448.796*Du_NBI - 7.739*Du_NBI^2 - 13802.023	0.61	5042.13	48.10	0.28
y = 590.7*Du_Chl - 12768.7*Du_Flav - 49540.5*Du_Anth + 20291.9	0.61	4958.75	67827.86 *(57.25)	0.31
log y = 9.5852*log(Main_SPAD) - 26.5916	0.24	10532.13	68.39	0.32

*One near-zero estimated PNN concentration value was removed

Note: Du_Anth, Dualex anthocyanin reading; Du_Chl, Dualex chlorophyll reading; Du_Flav, Dualex flavonol reading; Du_NBI, Dualex NBI reading; Main_SPAD, SPAD reading

Multivariate Linear Regressions Using Sensor, Environmental, and Management Data

The environmental and management information was used along with Dualex measurements to make the MLR models. There were two MLR models with the highest calibration adjusted R^2 of 0.75, one used Chl, Anth, accumulated GDDs, and as-applied N rates and the other used Chl, Flav, Anth, accumulated GDDs, and as-applied N rates. Because Flav readings add the benefit of accounting for leaf mass area through their high correlation and enable better prediction of mass-based N content such as N concentrations (Meyer et al. 2006), the latter was chosen as the best MLR model (Dualex Best MLR) for the PNN concentration estimation. Dualex Best MLR showed validation R^2 0.75, RMSE 3925.54 ppm, PE 112.22%, and Kappa statistic 0.41 (moderate agreement). Compared with both of the abovementioned estimation models using the Dualex readings alone, the incorporation of the environmental and management information significantly improved the model performance. Figure 2 shows the measured PNN concentrations on the x-axis and the PPN concentrations estimated by Dualex MLR (left) or Dualex Best MLR (right) on the y-axis. This figure visually shows the model performance improvement by incorporating the environmental and management information. The confusion matrices of Dualex MLR and Dualex Best MLR in Table 4 indicate the misclassifications in the Prediction Deficient and Reference Excessive intersection reduced noticeably. The accuracy statistics also improved from 0.55 with Dualex MLR to 0.61 with Dualex Best MLR. The MLR model using the SPAD readings and the environmental and management information showed lower accuracy in PNN concentration estimation but higher N status classification accuracy again with validation R^2 0.71, RMSE 4306.31 ppm, PE 49.03%, and Kappa statistic 0.46 (moderate agreement). These results are summarized in Table 5. It is important to mention that the effects of sensor readings on the model performance improvements are somewhat marginal in the MLR models. The MLR models only using the environmental and management information showed calibration adjusted R^2 0.69, and the addition of Dualex Chl, Flav, and Anth increased calibration adjusted R^2 to 0.75. This is even more marginal in the MLR models using SPAD than Dualex because the addition of SPAD readings increased calibration adjusted R^2 only to 0.70.

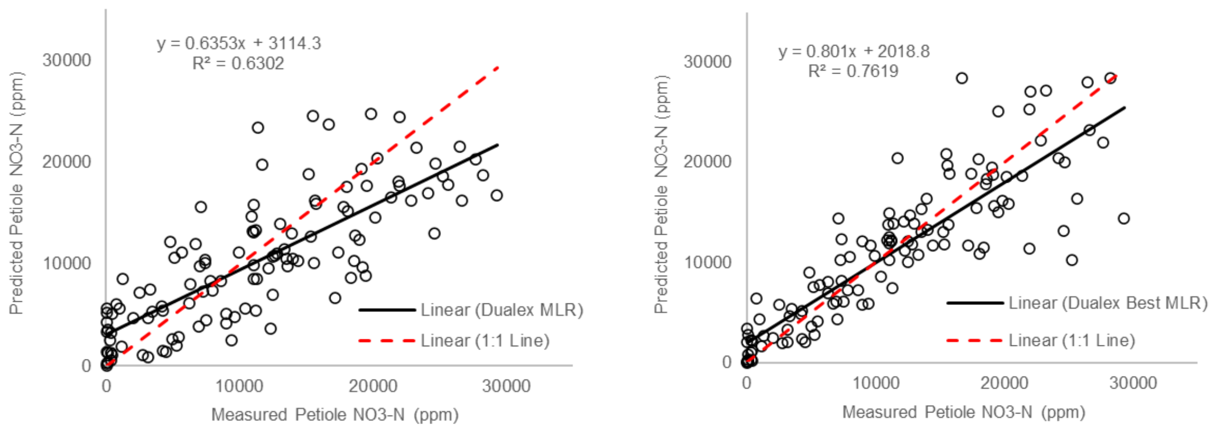

Figure. 2. The correlations between the measured petiole nitrate-N concentrations (ppm) and the petiole nitrate-N concentrations (ppm) predicted by Dualex MLR (left) or Dualex Best MLR (right).

Table 4. The confusion matrices and corresponding accuracy statistics for Dualex MLR (left) or Dualex Best MLR (right).

	Reference		
Dualex MLR	Deficient	Sufficient	Excessive
Deficient	41	9	13
Sufficient	6	13	17
Excessive	4	4	12
	Accuracy: 0.55		

	Reference		
Dualex Best MLR	Deficient	Sufficient	Excessive
Deficient	45	8	5
Sufficient	5	14	23
Excessive	1	4	14
	Accuracy: 0.61		

Table 5. The results of PNN concentration prediction using the environmental and management information along with Dualex or SPAD readings in MLRs.

Regression Equations	Validation R^2	RMSE (ppm)	PE (%)	Kappa
y = 290.045*Du_Chl + 2424.748*Du_Flav - 33301.276*Du_Anth + 45.835*as-applied - 19.596*GDDs + 10940.451	0.75	3925.54	112.22	0.41
y = 286.655*Main_SPAD + 43.335*As_applied - 19.518*Accumulated_GDDs +6898.939	0.71	4306.31	49.03	0.46

Note: Accumulated_GDDs, accumulated growing degree days; As-apploed, as-applied N rates; Du_Anth, Dualex anthocyanin reading; Du_Chl, Dualex chlorophyll reading; Du_Flav, Dualex flavonol reading; Du_NBI, Dualex NBI reading; Main_SPAD, SPAD reading

Machine Learning Regressions Using Sensor, and GxExM Data

Two machine learning regressions, SV and RF regressions, were trained and tested using the GxExM information along with the SPAD or Dualex readings. The best Dualex-based SV regression used the radial kernel (rSV) and showed validation R^2 0.91, RMSE 2410.51 ppm, PE 36.91%, and Kappa statistic 0.62 (substantial agreement). The Dualex-based RF regression showed validation R^2 0.91, RMSE 2407.19 ppm, PE 26.05%, and Kappa statistic 0.64 (substantial agreement). Thus, the best Dualex-based ML regression model, RF regression, showed a significant improvement from Dualex Best MLR. This model performance improvement can be attributed to the incorporation of genetic information and the consideration of non-linear relationships. The importance analysis on variables revealed that all the variables were considered important, yet in the following order as shown in Figure 3, as_applied N rates, accumulated GDDs, Flav, Chl, Anth, and cultivars. As a result, the consideration of non-linearity of data seems to have contributed to much of the model performance improvement. Figure 4 shows the measured PNN concentrations on the x-axis and the PPN concentrations predicted by Dualex Best MLR (left) or Dualex RF (right) on the y-axis. This figure visually shows the model performance improvement by incorporating the genetic information and considering non-linearity. The confusion matrices of Dualex Best MLR and Dualex RF in Table 6 indicate there are few misclassifications between the Deficient and Excessive categories. The accuracy statistics further improved from 0.61 with Dualex Best MLR to 0.76 with Dualex RF. In contrast, the best SPAD-based SV regression used the radial kernel showing validation R^2 0.84, RMSE 3224.12 ppm, PE 28.38%, and Kappa statistic 0.53 (moderate agreement). The SPAD-based RF regression model showed validation R^2 0.92, RMSE 2319.03 ppm, PE 24.16%, and Kappa statistic 0.66 (substantial agreement). These results are summarized in Table 7. In short, the best ML regression models, RF regressions, using Dualex or SPAD readings showed equivalent model performance. This is understandable given that the impact of sensor measurements becomes more marginal in the complex models when supporting variables are added. Nevertheless, the RF regressions using the Dualex readings along with the GxExM information estimated the PNN concentrations most accurately and showed its potential for in-season non-destructive potato N status diagnosis.

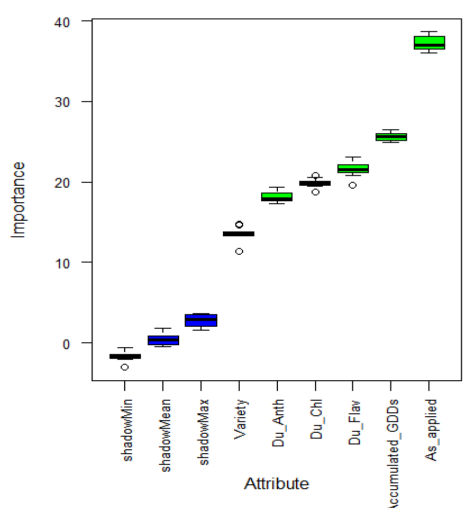

Figure. 3. The result of importance analysis on variables using the Boruta package in R software.

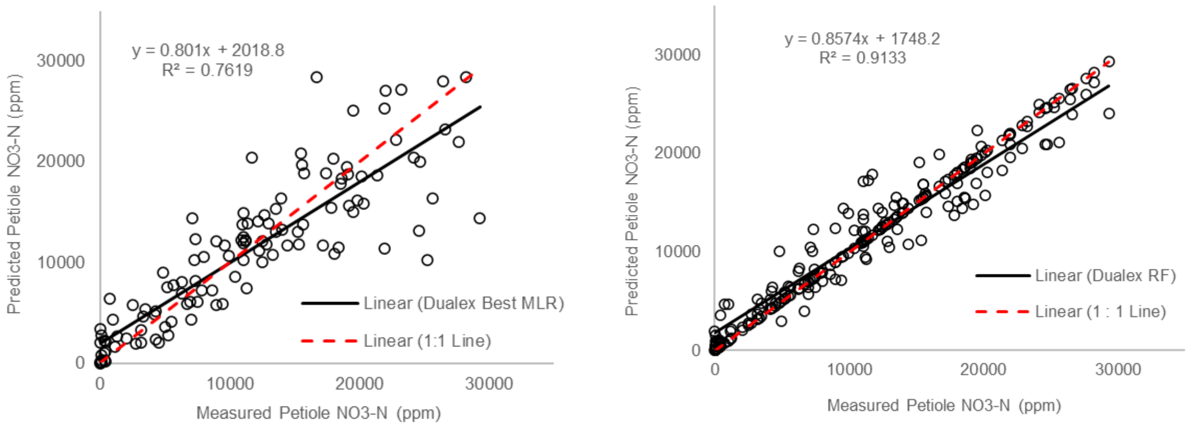

Figure. 4. The correlations between the measured petiole nitrate-N concentrations (ppm) and the petiole nitrate-N concentrations (ppm) predicted by Dualex Best MLR (left) or Dualex RF (left).

Table 6. The confusion matrices and corresponding accuracy statistics for Dualex Best MLR (left) or Dualex RF (right).

	Reference				Reference		
Dualex Best MLR	Deficient	Sufficient	Excessive	Dualex RF	Deficient	Sufficient	Excessive
Deficient	45	8	5	Deficient	41	6	0
Sufficient	5	14	23	Sufficient	9	17	9
Excessive	1	4	14	Excessive	1	3	33
	Accuracy: 0.61				Accuracy: 0.76		

Table 7. The results of PNN concentration prediction using the GxExM information along with Dualex or SPAD readings in ML models.

Types	Validation R^2	RMSE (ppm)	PE (%)	Kappa
Dualex rSV	0.91	2410.51	36.91	0.62
Dualex RF	0.91	2407.19	26.05	0.64
SPAD rSV	0.84	3224.12	28.38	0.53
SPAD RF	0.92	2319.03	24.16	0.66

Note: rSV, support vector regression with radial kernel; RF, random forest regression

Future Studies and Limitations

There are a few points that need to be addressed in future studies. The Dualex-based regression models at each growth stage should be explored and compared with the corresponding SPAD-based regression models to determine if there is any difference in their model performance. One sensor might detect a difference in the potato N status earlier than the other, which will help the management of split N fertilizer application better. Furthermore, the use of these sensors should be considered in terms of guiding variable rate N application. Rosen and Bierman (2008) suggested in the BMPs guideline 22.5 to 45 kg/ha of N fertilizer be injected responsively if the PNN concentration is below the sufficiency range. Thus, the best PNN concentration estimation models using SPAD or Dualex can also be used according to this BMPs guideline. However, more ideally, each in-season N fertilizer application rate should be varied using SPAD or Dualex. Because the PNN concentrations tend to temporally fluctuate due to ambient conditions of plants, changing each in-season N application rate according to the PNN concentrations can be challenging. Crops such as corn and wheat, whose harvest parts are born aboveground, tend to show a stronger correlation between their yield and sensor measurements. As a result, the Oklahoma State University algorithm (Franzen et al., 2016) using the N rich strips or ramp calibration strip technique is suitable for guiding variable rate N applications. Meanwhile, the potato yield estimation is not as straightforward and promising because the aboveground biomass might be stressed due to a lack of available nutrients or nutrient translocation to the tubers. So, the efforts to find a correlation between sensor measurements and potato yield and to use it in the abovementioned algorithm require more studies. Li et al. (2021) predicted potato yield using selected vegetation indices calculated from UAV remote sensing data along with the potato cultivar information and accumulated GDDs in the ML regressions. Based on the findings of this study, the ML prediction could be improved by incorporating the as-applied N rate information. Similar ML regressions developed using the proximal sensing technologies such as SPAD and Dualex might be more accurate and could serve as reference data. Estimating N nutrition index (NNI) using SPAD or Dualex may also be effective in diagnosing potato N status and guiding variable rate N application. However, it should be noted that the aboveground biomass must be estimated by other sensors or destructive sampling to obtain plant N uptake and to calculate N fertilizer application rates. Further research is required to make progress in this area.

Conclusion

This study showed Dualex Scientific could identify in-season potato N status non-destructively at 76% accuracy by estimating petiole nitrate-N concentrations with the help of the genetic, environmental, and management information in the random forest model. The environmental and management information was especially important for the performance of the random forest regression. When only sensor data were used in simple regression models, Dualex sensor performed better than SPAD meter in estimating petiole nitrate-N concentrations. When the genetic, environmental and management information was used together with the sensor data, the difference in the two sensor-based models was negligible. More research is needed to evaluate the two sensors for early detection of potato N status diagnosis and develop in-season N recommendation strategies to improve N use efficiency in potato production.

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