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Developing empirical method to estimate phosphorous in potato plants using spectroscopy-based approach

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Abstract: In thinking of deploying sensors for potato nutrient estimation, it is necessary to study the foliar spectral reflectance in relation to the chemical content of petioles rather than leaves. This study aimed to investigate the phosphorus (P) for its crucial role in potato growth. The common measurement of the amount of P is the total phosphorus content. Near infrared spectroscopy (NIRS) has been widely assessed to determine the P in plants based on leaves P content. However, no studies assessed the potential of NIRS to estimate P based on petiole chemical content. Thus, this study aimed to investigate whether there is a correlation between the P content in potato petioles and leaf spectral data assuming independence in relation to the other nutrients and whether indoor planting under controlled application can improve P estimation. A total of 40 datapoints were collected from open farms and another 20 datapoints were taken from an indoor cultivation area. The farm data underwent the standard application of nutrients, while the indoor data followed exaggerated applications of P. Samples were collected biweekly, and the chemical testing of petiole was done following the official methods of the Association of Official Analytical Chemists. The dried leaves were placed in an NIRS Analyzer to measure the reflectance between 400-2500 nm. Two datasets were developed between P content from the farm and the indoor in response to leaf spectrum based on a linear relationship. Lasso multiple linear regression modelling (Lasso MLR) for its feature imposes a shrinkage to select the most informative wavebands. Performance of the generated model was evaluated using Ratio of standard error of Prediction to standard Deviation (RPD) which showed better performance of the model generated from the farm and indoor data combined with an RPD value of 2.54. Likewise, the coefficient of determination improved at a value of 0.82. The results show that there is a correlation between the P content of potato petioles and dried leaf spectrum. Varying the range of concentrations in the dataset improved the model performance. Further work is planned to validate the significance of the developed model.

Keywords.

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Leaf spectral reflectance, petiole chemical testing, phosphorous, multiple linear regression.

Introduction

Potato growers assess nutrient deficiencies within the season using multiple assessment tools including visual diagnosis, plant tissue tests, soil tests, and cropping history (Fageria et al., 2009). Amongst them, tissue tests are recognized to be the most representative method (Motsara and Roy, 2008). Tissue tests, despite being a common method, they are considered destructive, laborious, time-consuming, and expensive (Wang et al., 2017). These drawbacks have led growers to adapt other techniques for nutrient assessment.

Near infrared spectroscopy (NIRS) for plant nutrient assessment is one of those techniques that shows potentials to provide efficient information on nutrient contents based on leaf or canopy reflectance as a cheaper, non-destructive, and more accessible tool (Prananto et al., 2020). The concept of NIRS is based on the reflectance of visible light and near infrared, which refers to the fact that molecules absorb frequencies that are characteristic of their structure. Previous research found that significant spectral bands in crops exist at the visible and very near infrared (Vis-VNIR, 400 - 1100 nm) and in short wave infrared (SWIR, 1000 - 3000 nm) (Saari et al., 2011). Based on this concept, ground-based sensors are delivered to markets to estimate plant properties using vegetation indices at specific wavelengths of canopy reflectance (Gabriel et al., 2017). However, their drawbacks include atmospheric and soil interference (Muñoz-Huerta et al., 2013). Ground-based sensors at a leaf level have been introduced to eliminate the noise coming from atmospheric and soil interferences such as in studies performed by Mahajan et al. (2021), Peng et al. (2020) and Liao et al. (2012).

Nevertheless, in potatoes, nutrient levels are estimated by conducting chemical tests for the petioles rather than in leaves (Zebarth et al., 2007). Few research has been done to study the spectrum based on petiole chemical testing rather than leaf chemical testing such as Davenport et al. (2005), Botha et al. (2006), and Cohen et al. (2010). However, these studies were done only for nitrogen and there are no studies compared the results of NIRS between leaves with other macronutrients such as phosphorus (P), calcium (Ca), magnesium (Mg) and sulfur (S).

Although all the mentioned macronutrients are substantially important for proper physiological and biochemical conditions in plants, P in particular plays a crucial role in potatoes for tuber bulking (Koch et al., 2020). The most common measurement of the amount of phosphorus in plants is the total phosphorus content (Wieczorek et al., 2022). The NIRS has been widely assessed for its potential to determine the total phosphorous in plants. Previous studies identified the significant wavebands to predict P content in the VNIR region (730 nm and 930 nm) and blue region (440 and 445 nm) in corn canopy (Osbourne et al., 2002). A combination of two-band at 1080 nm and 1460 nm wavelengths was found capable for the prediction of P content in wheat (Mahajan et al., 2014). Another study obtained high accuracy to determine P in oilseed rape leaves within the range of 380 - 1030 nm (Zhang et al., 2013). No studies assessed the P content in leaves based on petiole chemical content within the full range of 400 – 2500 nm.

The first objective of this study aimed to find correlation between P concentration in potato plant petioles and foliar spectral reflectance. Ideally, samples can be taken from commercial farms during the season. However, phosphorous application follows a specific procedure adapted by growers and depending entirely on farm data may cause overfitting of datapoints in the models. Hence, the second objective was to maximize the variation in the datapoints by growing indoor under exaggerated P application schemes to find the influence of adding indoor datapoints on building the estimation empirical models.

Materials and Methods

Sample preparation

A total of 40 datapoints were collected from two farms of Russet Burbank in NB, Canada.

Sampling was performed from late June to late September 2020 every two weeks as recommended to give the best results of crop nutrient status (Zebarth et al., 2007). Samples of petioles were collected from the 4th leaf from the apex of the shoot (Rowe, 1993). The size of the sampling area of each farm was 18 m^2 and the fertilizer application followed the standard application of nutrients by the growers in NB, Canada.

Another 20 datapoints were taken from an indoor cultivation area from September to December 2020 at the Department of Engineering in the Agriculture Campus of Dalhousie University in Truro, NS. The fertilizer application followed different fertilization schemes than the ones in the open farms. Indoor grown potatoes were planted under two different application rates of 20-20-20 and 22-0-22 NPK on weekly basis until the end of the season. Samples of petioles were collected from the 4th and 6th leaves from the top of the plant. Though, this study does not aim to assess the concentrations of P at different heights of the plant, we rather aim to maximize the variation of the dataset at different concentrations. The different application schemes also allowed us to give a variation in concentrations above, within and under the recommended range to avoid overfitting of the data when modelling.

Figure 1 shows the steps taken for sampling and analysis. Each datapoint contained 40 petioles and 40 leaves for lab chemical testing. The leaves and petioles were immediately vacuum packed into sampling bags after peeling them off and refrigerated before shipment in a box with an ice bag. The leaves and petioles were dried at 55 - 60 degree Celsius (°C) over 16 - 24 hours. Chemical testing of P was performed following the official method of the Association of Official Analytical Chemists (AOAC).

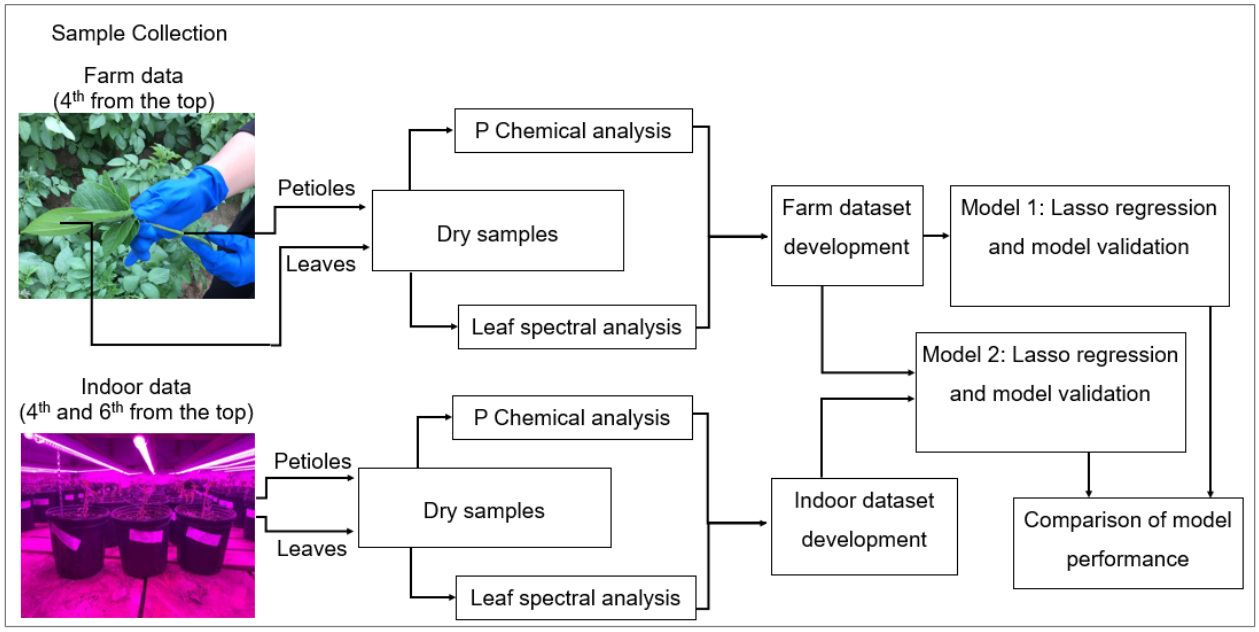

Figure 1. Steps of sample collection, chemical testing and spectral analysis, and model development.

Spectral measurements and dataset development

The leaves were analyzed for their spectral reflectance over the range of 400 – 2500 nm at 0.5 nm interval using NIRS Analyzer (DS2500, Metrohm USA Inc.) (Table 1). One reading was taken in an interval of 8 nm as a representative spectral signature, a total of 262 readings were used for data analysis using the R statistical language (R Version 4.0.2; R Core Team, 2021). A dataset was then developed between P content of the samples from the farm and leaf spectrum, hereafter called farm dataset. Another dataset was developed between P concentrations of petioles taken from the indoor cultivation area and leaf spectrum; hereafter called indoor dataset. Both datasets were based on a linear relationship, where the chemical results of petioles acted as responses and the spectral results of the leaves within the range of 404 - 2492 nm functioned as predictors. The number of predictors is larger than the number of responses, which may result in over-fitting

(Ye et al., 2020), and thus, model accuracy can be improved by following one of the subset selection methods.

Development of model and model performance

Multiple linear regression (MLR) model was built between the P chemical content of petioles and spectral results of leaves. Lasso MLR was decided to be followed for its unique features to identify the most informative, least redundant features using a complexity parameter (*λ)*, which controls the amount of shrinkage (Hastie et al., 2008). The model selects the value of λ which minimizes the root mean squared error (RMSE). Lasso was implemented using the *glmnet* and *caret* packages of the R statistical language. The model was firstly run over the farm dataset only, and then the model expanded to run over the farm and indoor datasets to compare the model performance whether improved or not after adding the variation given by the indoor dataset.

For model performance, training models were conducted using 10-fold cross validation. The coefficient of determination (r^2) between the actual P concentration and estimated P concentration were calculated as the mean across the cross-validation folds as shown in Figure 3. The model performance was evaluated by calculating the ratio of (standard error of) Prediction to (standard) Deviation know as RPD (Williams, 2019) and categorizing the performance of model based on the RPD values was classified as excellent (>2), acceptable (≥ 1.4 - 2.0) and nonreliable (< 1.40) (Mahajan et al., 2021).

Table 1. Operating specifications of NIRS DS2500 Analyzer

Item	Specification
Measurement Mode	Reflectance
Wavelength Range	400 - 2500 nm
Detectors	Silicon (400 - 1100 nm) and Lead Sulfide (1100 - 2500 nm)
Optical Bandwidth	8.75 ±0.1 nm
Spectral resolution	0.5 nm
Number of data points	4200
Wavelength Accuracy	± 0.05 nm

Results and Discussion

P Chemical content in potato petioles

Figure 2 shows the P concentrations of the data taken from the farm, and the indoor cultivation area in comparison to the recommend range of P content between 0.24 – 0.35 % (A & L Canada Laboratories Inc., Ontario). Overall, the farm and indoor data gave a range of variation in concentrations above, within and under the recommended range.

The concentrations of the farm data ranged between 0.07 – 0.51 %. The trend shows a general decline over the entire season and that might refer to the dilution phenomenon as plant biomass increases over the season (Du et al., 2020; Gómez et al., 2020). Whereas the high concentrations in the beginning of the season may refer to a large application of fertilizers at early stages to fulfill the P fertilizer requirements during plants' vegetative and reproductive stages and to avoid P deficiencies later in the season. Additionally, the high P concentrations in the early growth stages is commonly applied for its significant impact on the setting of potato tubers (Koch et al., 2020).

The indoor data were categorized into four groups based on the application rate and the position of petiole during sampling (4^{th} or 6^{th} petiole from the top). The concentrations varied between 0.37 – 0.7 % as shown in Figure 2. The application rate at 20-20-20 led to an increase in the dataset upper concentration from 0.51 % to 0.7. The petioles taken from the 4^{th} leaf from the top shows higher P concentration than the ones taken from the 6^{th} (Figure 2). Similar results were revealed by Chea et al. (2021) stating that the P concentration in young leaves increases more than to old leaves up to a certain concentration. Under P deficiency at 22-0-22, the P concentrations were apparently less in petioles, but rather, there was no significant difference in P concentrations

between the petioles taken from the 4th and the 6th leaves from the top where the first two concentrations were matching and the last one had a slightly different value (Figure 2). This would refer to the reason that the root-to-root ratio increases under P deficiency levels and that led the plant to endure into a P conservation strategy amongst the young and old leaves to improve the plant uptake (Chea et al., 2021).

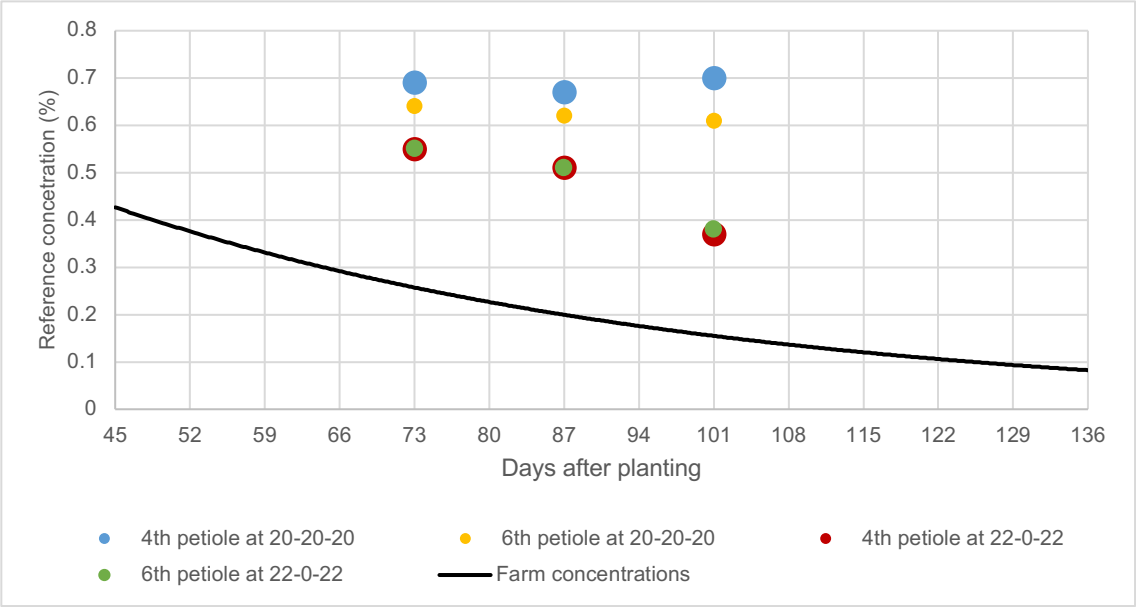

Figure 1. Temporal decline for phosphorus (P) during the growing season in farm plants. The dots show concentrations measured in plants grown indoor under the two different application schemes

Lasso MLR analysis and model performance

Table 2 shows the validation results of the two datasets; farm dataset (model 1), and farm and indoor datasets combined (model 2). Both models suggest excellent performance for estimating P based on the RPD values shown in Table 2. The slight increase in the RPD value in model 2 in comparison to model 1 shown in Table 2 may provide supporting evidence that the generated model for estimating P accounted for more of the variance in the datapoints represented by the indoor concentrations shown in Figure 2. Moreover, the r^2 value was improved by model 2 which gave another indication that varying the concentrations of the P enhances the correlation between the reference and the estimated values. Figure 3 shows the distribution of P reference concentration versus the estimated values by running the Lasso MLR over the two models. Figure 2 shows that many of the P concentrations (58%) are under the normal range, despite that, the model had reasonable estimation around the fitting line presented in Figure 3.a. The addition of the indoor data to the farm dataset increased the range of the concentration above the normal range (Figure 2), though, the estimated values were closer to the fitting line (especially after the concentration of 0.2%) than the ones by the farm data solely as shown in Figure 3.b. This could explain the increase in the correlation value of r^2 after adding the indoor data into the model.

Table 2. Validation results of the testing models

	Validation results of the testing model	
	r^2	RPD
Model 1: Farm dataset	0.74	2.42
Model 2: Farm dataset and indoor dataset combined	0.82	2.54

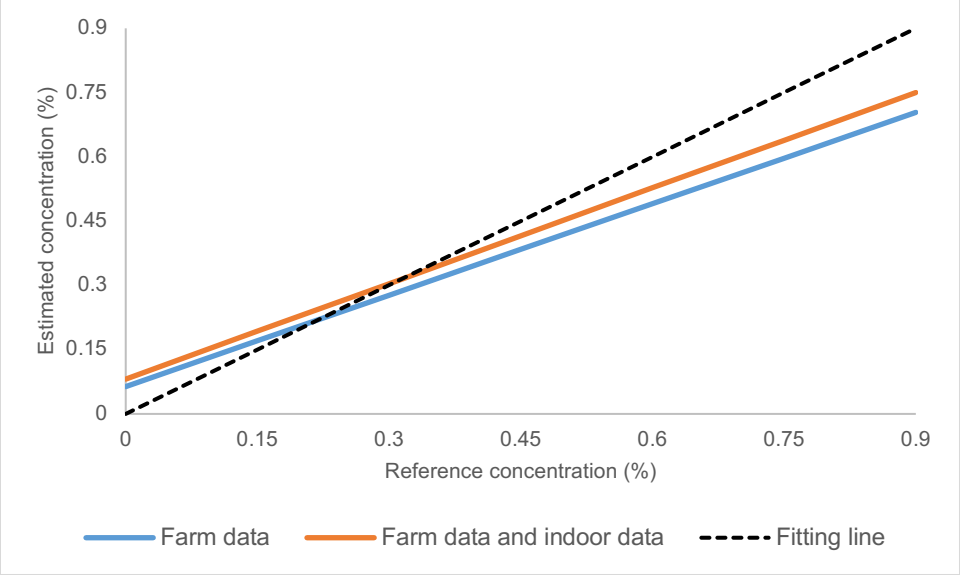

Figure 3. Validation results of the reference versus estimated phosphorous (P) concentrations

Conclusion

The preliminary results of this study show that there is a correlation between the P in potato petioles and leaf spectrum. Maximizing the variance in the concentrations of P improved the model performance and the correlation value. Further research is planned to validate the significance of the developed models using an increased number of datapoints.

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