2020

Validation and Optimization of Hyperspectral Reflectance Analysis-Based Predictive Models for the Determination of Plant Functional Traits in Cornus, Rhododendron, and Salix

Milton I. Valdiviezo
University of Central Florida

VALIDATION AND OPTIMIZATION OF HYPERSPECTRAL REFLECTANCE ANALYSIS-BASED MODELS FOR THE DETERMINATION OF PLANT FUNCTIONAL TRAITS ACROSS GENUS *CORNUS*, *RHODODENDRON*, AND *SALIX*

by

MILTON I. VALDIVIEZO

A thesis submitted in partial fulfillment of the requirements for the Honors in the Major Program in Biology
in the College of Sciences
and in the Burnett Honors College
at the University of Central Florida
Orlando, Florida

Summer Term 2020

Thesis Chair: Dr. Chase Mason

Abstract

Near infrared spectroscopy (NIR) has become increasingly widespread throughout various fields as an alternative method for efficiently phenotyping crops and plants at rates unparalleled by conventional means. With growing reliability, the convergence of NIR spectroscopy and modern machine learning represent a promising methodology offering unprecedented access to rapid, high throughput phenotyping at negligible costs, representing prospects that excite agronomists and plant physiologists alike. However, as is true of all emergent methodologies, progressive refinement towards optimization exposes potential flaws and raises questions, one of which is the cornerstone of this study. Spectroscopic determination of plant functional traits utilizes plants' morphological and biochemical properties to make predictions, and has been validated at the community (inter-family) and individual crop (intraspecific) levels alike, yielding equally reliable predictions at both scales, yet what lies amid these poles on the spectrum of taxonomic scale remains unexplored territory. In this study, we replicated the protocol used in studies of the aforementioned taxonomic scale extremes and applied it to an intermediate scale. Interestingly, we found that predictive models built upon hyperspectral reflectance data collected across three genera of woody plants: *Cornus*, *Rhododendron*, and *Salix*, yielded inconsistent predictions of varying accuracy within and across taxa. Identifying the potential cause(s) underlying variability in predictive power at this intermediate taxonomic scale may reveal novel properties of the methodology, potentially permitting further optimization through careful consideration.

Table of Contents

Abstract ii
Table of Contents iii
Introduction 1
NIR spectroscopy and hyperspectral imaging 1
Taxonomic scaling 2
PLSR vs SVM 3
Methods 5
Sample preparation 5
Spectral measurements 5
Model design 6
Results 8
Overview 8
PLSR vs SVM 10
Differences Among Genera 10
Regular, Jackknife, and Average of Coefficients Models 11
Discussion 12
Scale-Sensitive Predictive Power 12
Phenotypic Plasticity and Trait Interdependence 13
Convergent Evolution 14
Best Practices for Spectral Data Collection and Modeling 15
Conclusion 17
References 18

Introduction

A wide array of conventional characterization methods and technologies lay at the disposal of conservative researchers, offering risk-free, temporally validated methods of analysis to those who value reliability. Those willing to forfeit familiarity in exchange of calculated risk, however, may discover unprecedented efficiency among the offerings of novel methods. For instance, one of the most accurate conventional analytical methods for determining leaf nutrient content is inductively coupled plasma mass spectrometry (ICP-MS), a method that consistently yields accurate results but requires both the destruction of the sample and a high per-sample cost. Alternatively, emergent methods, namely those relying on spectroscopic techniques that leverage the optical properties of plant tissues boast the prospect of easily accessible, high throughput phenotyping at virtually no cost, and at a mere fraction of the time required by ICP-MS to obtain the same data.

NIR spectroscopy and hyperspectral imaging

Inclusion of near infrared (NIR) spectroscopy as an analytical tool to the botanical sciences and agricultural fields has revolutionized aspects of these fields requiring intensive phenotyping efforts (Araus and Cairns et al., 2014). Plants' anatomical, physiological, and biochemical attributes are largely defined by and dependent on their interaction with the electromagnetic spectrum; this is particularly the case with individual leaves, where light-responsive pigments, secondary metabolites, and photosynthetic machinery abound. Plant interactions with electromagnetic radiation often differ across taxa, making identification of unique spectral profiles an efficient non-destructive means of phenotypic characterization (Araus

and Cairns et al., 2014). Spectral imaging is divided into two overarching categories: multispectral and hyperspectral imaging, with the former's extent of measurements spans a handful of specific, narrow wavebands, and the latter doing so over multiple distinct wavelengths and wavebands across an entire spectral region. (Cozzolino et al., 2016). While both approaches are without a doubt powerful techniques, the broad scope of hyperspectral spectroscopy is arguably more fitting for this context as it covers a wider range, presumably capturing key signals reflectance signals that may be otherwise overlooked.

Taxonomic scaling

Predictive models have proven capable of estimating a wide range of plant functional traits at both broad and narrow taxonomic scales with remarkable accuracy. At the broad end of the scale spectrum lie undertakings to design models intended to estimate leaf biochemical and morphophysiological traits (e.g., carbon concentration, nitrogen concentration, leaf mass per area (LMA), cellulose and lignin content, etc.), utilizing hyperspectral leaf reflectance data from collections of species within moderately diverse communities of temperate forest trees (Serbin et al., 2014). At the opposing end of the spectrum lie single species, typically agronomically-oriented studies where models are built to predict characteristics pertinent to yield increase such as photosynthetic traits (e.g., xanthophyll, chlorophyll, and water content) (Silva-Perez et al., 2018; Yendrek et al., 2017). While the consistency in predictive power across these vastly different scales would seem to strongly validate this methodology, intermediate scales remain understudied, raising important questions – the most obvious of which concerns the validity of

the methodology when applied to mid-range scales, for instance diverse species within a genus for studies in plant evolutionary ecology.

This study was aimed at addressing this mid-scale range. Our scale consisted of a sufficiently diverse collection of leaves from hundreds of individuals of several dozen species of plants, representative of the following three genera of woody plants: *Cornus*, *Rhododendron*, and *Salix*. Leaves of these diverse species were sampled as part of a larger study on leaf trait evolution from plants grown under common conditions in the Arnold Arboretum of Harvard University. While biochemical, morphological, and physiological traits can indeed vary greatly within and across these three genera, there are no immediately conspicuous differences between this and other validated scales that could reasonably alter the expected degree of predictability.

PLSR vs SVM

Traditional predictive modeling hinges on a lattice of linear regressions and multivariate input (Des Marais et al., 2013). Partial least squares regression (PLSR) accomplishes exactly that in a familiar fashion; predictor data is regressed against the expected predictions under the assumption that response variables result from estimations based on undetectable latent variables (Rosipal & Krämer et al., 2006) The ability of PLSR models to account for collinearity and overall excellent performance have rendered it a common default approach to predictive modeling. Additionally, PLSR produces visual results that facilitate interpretation of findings as the latent factors of one variable can describe the latent structure that best explains the most variation of another responsible variable, making it remarkably useful for analyses involving spectral datasets (Hanse et al., 2013).

While PLSR is likely the most widespread and traditional method, other linear contenders (e.g., random forest regression) increasingly display outstanding predictive power, even outperforming PLSR in some cases (Zhai et al., 2013). The rise in popularity of non-linear, machine-learning-based modeling approaches have also introduced new formidable contenders such as support vector machine (SVM) regressions to the race for maximal predictability. While repurposed SVM regressions are something of a black box given their machine-learning nature, their ability to map data points onto discriminatory hyperplanes, thereby capturing and facilitating the interpretation of nonlinear relationships via kernel functions, represents an impressive advantage that may outweigh the lack of transparency.

For rigor's sake, this study includes the both PLSR and SVM as popular representatives of both linear and non-linear modeling approaches.

Methods

Sample preparation

The samples used in this study were collected from the common growing environment of Arnold Arboretum at Harvard University in Boston, Massachusetts. These living collections contain large swaths of species diversity for each of the focal genera across the Northern hemisphere, including Asia, Europe, and North America. Leaves were sampled in summer 2016 approximately four weeks after leaf-out. Fresh traits including leaf area and chlorophyll content were assessed, and leaves were dried at 60 °C in a forced-air drying oven. Leaf dry mass was obtained, and used to calculate leaf mass per area. Leaf samples were proportionately pooled across individuals within each species, and analyzed by the Louisiana State University's Soil Test and Plant Analysis Laboratory for nutrient analysis by ICP-MS. Remaining portions of leaf samples were ground into a fine homogenized powder with a Wiley Mill and/or mortar and pestle in advance of spectroscopy.

Spectral measurements

Hyperspectral reflectance measurements were taken using an Ocean Optics NIRQuest12-2.5 spectrometer (900-2500 nm), DH-2000 halogen/deuterium UV-VIS-NIR light source (~200-2500 nm), and mixed UV-visible & visible-NIR bifurcated optical fibers, with data collection made using Ocean View interfacing software (Ocean Insight, Largo, FL). The spectrometer was calibrated for well lit, indoor conditions according to manufacturer recommendations. The fiber optic probe was secured inside an anodized aluminum reflectance probe holder at 45°. Samples

were individually spread across a black, non-reflective, acrylic matte board (which was thoroughly cleaned between samples) and shuffled with a steel rod to avoid clumping, then condensed to a single pile, and ultimately compressed by a flat -surfaced coffee tamper to create a smooth, scannable surface. upon which measurements were taken by overlaying the probe holder. Thus far, we have conducted two studies used two tissue types – dried whole leaf tissue and homogenized ground tissue.

Model design

For analysis, we employed the approach of Serbin et al. (2014).We divided data into 80% training and 20% validation subsets per trait, such that the proportion of quantiles represented in each subset was preserve (i.e., we ensured training and validation datasets shared identical low (0-25th percentile) to high value (75-100th percentile) ratios, etc.). In order to calibrate the number of parameters used in PLSR modeling, we extracted a random 70% subset of the training data 100 times (each time with a different random 70% subset) and fit PLSR models to each these subsets. We then recorded the minimum predicted residual error sum of squares score (PRESS score) for each model throughout each iteration and used the number of components with the median minimum PRESS score for all subsequent PLSR models. We then fit PLSR and SVM models to the training data to in order to predict the validation data, and quantified comparisons in accuracy between validation and actual data as R^2, root mean squared error (RMSE), residual product deviation (RPD), and mean absolute percentage error (MAPE).

Next we performed 1000 jackknife iterations as follows: for each iteration, a random 70% subset of the training data was used to fit PLSR and SVM models and predict the validation data.

That is to say 1000 different subsets of 56% of the data (70% subset of training data which is itself an 80% subset of the data = 70% * 80% = 56%) were used to predict the remaining 20% (i.e., the validation data). For PLSR and SVM, we used the median of the predicted trait values of the validation data for each iteration and compared these predictions against actual trait data.

The inclusion of "regular" PLSR, SVM, and jackknife models is a central step to this exploratory analysis as such comparisons are imperative in determining which (if any) modeling approach yields stronger and more consistent predictive power.

Results

Overview

The table below outlines the predictive capabilities of each model, comparing results across taxa (i.e., predictions made across all genera as well as within each genus) and by modeling approach, (PLSR vs SVM regression) including the results of Jackknife resampling analyses for both PLSR and SVM regression as well an average of coefficient analysis exclusively for PLSR. For the sake of discussion, we designated an R^2 value of 0.4 as the cutoff for sufficient model predictive power, with performance above or below this threshold considered adequate or inadequate respectively. In general, no modeling approach was uniformly superior, and predictive power varied across traits and taxonomic levels. These distinctions in performance are subtle but important and are described throughout the remainder of this section, further elaborated upon in the discussion.

Table 1. Comparison of predictive power across Partial Least Square Regression and Support Vector Machine Models (including variants of each: Jackknife, Average of Coefficients) for plant functional traits across the genera: *Cornus* (n = 14), *Rhododendron* (n = 18), and *Salix* (n = 10) (All Genera n = 41), where n = the number of observations in validation data. All models with high predictive power ($R^2 > 0.40$) are listed, and models with substantially higher predictive power ($R^2 > 0.55$) are shown in bold.

		PLSR		SVM	
		Trait	R^2	Trait	R^2
Regular	All Genera	Nitrogen Content	0.473	**Aluminum Content**	**0.563**
				Calcium Content	**0.681**
				Manganese Content	0.507
				Nitrogen Content	0.511
				Potassium Content	**0.687**
				Zinc Content	**0.575**
	Cornus	Magnesium Content	0.499	Iron Content	0.504
		Manganese Content	**0.616**		
		Zinc Content	0.487		
	Salix	Sulfur Content	0.430	None	
	Rhododendron	Manganese Content	0.472	Phosphorus Content	0.463
Jackknife	All Genera	Nitrogen Content	0.467	**Aluminum Content**	**0.556**
				Calcium Content	**0.673**
				Manganese Content	0.486
				Nitrogen Content	**0.581**
				Potassium Content	**0.662**
				Zinc Content	**0.572**
	Cornus	Copper Content	0.422	None	
		Magnesium Content	**0.633**		
		Manganese Content	0.524		
		Zinc Content	0.526		
	Salix	Aluminum Content	0.420	None	
	Rhododendron	Manganese Content	0.445	**Manganese Content**	**0.584**
				Chlorophyll Content	0.502
Average of Coefficients	All Genera	Nitrogen Content	0.467	None	
	Cornus	**Manganese Content**	**0.619**	None	
		Zinc Content	0.478		
	Salix	None		None	
	Rhododendron	Manganese Content	0.485	None	
		Lipid Content	0.402		

PLSR vs SVM

PLSR models proved adept at estimating traits *within* genera, particularly leaf elemental concentrations at the time of sampling. These included magnesium, manganese, and nitrogen, all foliar elements closely associated with photosynthetic capacity and related processes (Silva-Perez et al., 2018; Yendrek et al., 2017). Predictive power for manganese content was considerably higher than that of magnesium and nitrogen estimations. Interestingly, PLSR predictive power was consistently high for a housekeeping trait (omitted in table) – number of leaves contained in the ground sample. This housekeeping trait persisted with an R^2 value well above the cutoff threshold in all PLS regressions, and could indicate that leaf size (which determined the number of leaves sampled) is correlated with a chemical trait detectable in ground powder. In contrast to PLSR, SVM regression demonstrated superior predictive power across genera (the unpartitioned dataset). As in PLSR models, models for elemental concentrations demonstrated highest predictive power. However unlike PLS regressions, SVM regressions appeared more sensitive to the spectral signatures of aluminum, calcium, and zinc, all metals most pertinent to root-shoot development, plant growth, and nutrition (Taiz & Zeiger, 2006).

Differences Among Genera

PLSR models notably favored *Cornus* leaves in its predictive capacity, with a mere maximum of two traits well-predicted in *Rhododendron* as opposed to a maximum of four in *Cornus*. This difference did not hold in SVM regression models, where lower predictive power was observed for *Cornus* but roughly the same for *Rhododendron*. Unlike these two genera,

predictive power for *Salix* leaves remained much lower, likely a consequence of its lower validation sample size (n = 10).

Regular, Jackknife, and Average of Coefficients Models

For both PLSR and SVM regression, jackknifing appears to have marginally increased models' predictive power, typically increasing the number of predictive traits at nearly all taxonomic levels of analysis, while also raising R^2 values for several traits, irrespective of modeling approach. The average of coefficients analysis for PLSR greatly reduced predictability in *Cornus*, characterizing it as the most poorly performing model.

Discussion

Scale-Sensitive Predictive Power

Our study makes a compelling case for sensitivity to taxonomic scale in hyperspectral reflectance spectroscopy – an otherwise reliable, developing methodology for estimating plant functional traits at maximum efficiency. A brief comparison of results obtained to that of contemporaries in this vein will quickly inform readers of this previously unexplored problem. Singh et al (2015), for instance, reported R2 values no lower than consistent values approximating $R2 > 0.70$ when fitting spectral data fitted to foliar traits scaled from leaf to canopy via PLS regression modeling for traits similar to those examined in this study (Singh et al., 2015). Equally consistent predictability is observed in studies making estimations at the crop level with little to no disturbance (Silva-Perez et al., 2018; Yendrek et al., 2017).

Given that scaling from individual leaves to a canopy made up of a single species has been demonstrated, it may be surprising to find scaling issues based on plant taxonomy. However, while scaling from an individual leaf to a canopy involves the incorporation of larger and larger amounts of variation in traits of interest, many traits remain consistent, or consistently correlated within a species. This intraspecific variation is often dwarfed by that encountered at the interspecific and intergeneric level. Because spectral signatures are composites of many underlying traits, different degrees of variation in multiple traits may cause traits with less variation to be obscured. At an inter-family community level (e.g. Serbin et al., 2014), variation in most traits will be maximized, which may reduce the ability of one highly variable trait to obscure variation in other traits within a dataset. These dynamics may make intermediate scales most susceptible to interference from non-target traits and other sources of noise variation. One

solution may be to explicitly examine the degree of trait variation being modeled. Another may be to include key interfering morphophysiological traits as covariates in models, to reduce their ability to obscure variation in focal traits. Yet another may be the incorporation of phylogenetic structure into modeling, as correcting for the hierarchical relatedness of species may serve to remove interfering trait-driven covariation. Beyond this, considerable phenotypic plasticity and interdependence of plant traits, convergent evolution of focal traits in different trait backgrounds, and the absence of established best practices for spectral data collection or modeling are all factors that need to be explored to improve the utility of hyperspectral reflectance at scales relevant to evolutionary ecology.

Phenotypic Plasticity and Trait Interdependence

Let us first consider the extent of plant trait phenotypic plasticity and what it might represent for hyperspectral spectroscopy. Leaves, in particular, are immensely variable structures, even at the individual level, further morphing in response to environmental pressures – a remarkable evolutionary advantage that can unfortunately become a hurdle, hindering spectroscopic analyses. Because leaf traits can acclimate both within the lifespan of a plant and within the lifespan of a single leaf (Mason et al., 2020), hyperspectral data collection and leaf harvesting and trait assessment must be as tightly paired as possible in space and time. Pairing spectra from one leaf with traits assessed on another may work well enough at some scales but will introduce substantial noise at others. As leaf traits shift plastically within individuals over space and time, they do not do so independently. Traits such as leaf mass per area and leaf nutrient contents are deeply interdependent, influencing each other in an inversely proportional

fashion, thereby altering rates of photosynthesis, leaf thickness, and internal physiological machinery across a broad range that is often driven by resource availability and ambient climatic conditions (Wright et al., 2004; Mason et al., 2015; Mason et al., 2020). Given this known interdependence, it may be possible to improve the predictive power of hyperspectral reflectance models by explicitly including easy-to-assess traits as covariates in modeling.

Convergent Evolution

Much like trait interdependence, convergent evolution may be a factor hindering hyperspectral reflectance models in diverse genera. As lineages diversify, individual lineages may evolve trait values in an atypical phenotypic background. For example, a genus may contain a clade with high leaf nitrogen and another clade with low leaf nitrogen. Within the low leaf nitrogen clade, a single species may experience selection for high leaf nitrogen content. This outlier species may have other leaf traits (and an overall leaf reflectance spectrum) more similar to the other members of its clade with low nitrogen. Without a sufficient number of intermediates spanning variation in many traits to untangle leaf nitrogen content from other traits that often covary, it may not be possible to build a model based on hyperspectral reflectance alone. It is not difficult to imagine how such a scenario could cause confusion and poor performance at the level of diverse genera grown under common conditions, as commonly used in studies in evolutionary ecology. One solution to this may be to incorporate phylogenetic structure explicitly into modeling, an area of research currently in development.

Best Practices for Spectral Data Collection and Modeling

The results of this study indicate a strong need for evidence-based best practices for spectral data collection and modeling steps for leaf hyperspectral reflectance. At present, there are no comprehensive simulation studies or other computational examinations of the consequences of investigator choices in the collection, processing, and analysis of leaf reflectance data for the prediction of leaf functional traits. Though certainly not the only factor muddling predictability, understanding the consequences of these choices, including the differences between modeling approaches is imperative and not merely a matter of researcher preference. Our results indicate that both the PLSR and SVM approaches are quite capable of predicting a wide range of traits, but with inconsistent performance across datasets. PLSR models outclassed SVM models in predicting traits within Cornus, but largely failed to make predictions within other genera, and across all three genera. PLSR models boast impressive features that have crowned it the go-to method for analyzing spectral data for decades (Ollinger et al., 2002). Among these are ability to check collinearity, computational effectiveness, statistical versatility (capable of univariate or multivariate modeling), ease of interpretability (often eliminating the need for extensive a priori knowledge of the relationship between wavelengths and plant functional traits) (Wold et al., 1984), and perhaps most importantly, its capacity to make use of a vast set of predictors across a given spectral region (Ollinger et al., 2011; Dahlin et al., 2013; Xing et al., 2018). On the other hand, PLSR suffers from a destabilizing sensitivity to variation – the inability to adapt to the addition of dissimilar data points. At the expense of many of the aforementioned merits of PLSR, SVM models offer non-linear methods that succeed where PLSR fails. By virtue of the self-informing design of machine

learning algorithms, SVM models can withstand a wider degree of variation in datasets, handled by dimensionality reduction. In our datasets, SVM regression was superior for the largest dataset, but clearly requires either a larger sample size or broader degree of variation to be effective. Though there is a risk of decreasing the potential of estimating certain traits when reducing data dimensionality to the extent machine learning algorithms do, the tradeoff is stronger predictive power across all genera. More exhaustive examination of the relative strengths and weaknesses of these methods will increase the utility of these approaches for plant science researchers.

Conclusion

Solving the scale problems requires a nuanced, multifaceted set solutions that exceed the scope of this study. We do advocate, however, looking beyond the daunting elements of nonlinear modeling (namely SVM models) as its promise increases in tandem with the number of investigative studies published on the matter, all of which appear to arrive at the consensus that is our conclusion. A priori assessment of the situation our results depict would seem to suggest it isn't unreasonable to assume any gaps in SVM models' predictive power can be mended by expanding calibration datasets to whatever extent necessary such that they become optimal representations of trait diversity within a given genus (or multiple genera), and thereby optimal training datasets capable of calibrating SVM models to possess the predictive power the intermediate taxa scale demands.

References

Araus JL, Cairns JE. 2014. Field high-throughput phenotyping: the new crop breeding frontier. *Trends in Plant Science* 19:52–61

Cozzolino D, Roberts J. 2016. Applications and developments on the use of vibrational spectroscopy imaging for the analysis, monitoring and characterisation of crops and plants. *Molecules* 21: 1–9.

Dahlin KM, Asner GP, Field CB. 2013. Environmental and community controls on plant canopy chemistry in a Mediterranean-type ecosystem. *Proceedings of the National Academy of Sciences* 110:6895–6900

Des Marais D, Hernandez K, Juenger T. 2013. Genotype-by-environment interaction and plasticity: exploring genomic responses of plants to the abiotic environment. *Annual Review of Ecology Evolution and Systematics* 44:5–29

Gholami R, Fakhari N. 2017. *Support Vector Machine: Principles, Parameters, and Applications*. *Handbook of Neural Computation* (1st ed.). Elsevier Inc.

Hansen MC, Potapov PV, Moore R, Hancher M, Turubanova SA, Tyukavina A, Thau D, Stehman SV, Goetz SJ, Loveland TR, Kommareddy A, Egorov A, Chini L, Justice CO, Townshend JRG. 2013. High-resolution global maps maps of 21st century forest cover change. *Science* 850:2011–2014

Mason CM, Donovan LA. 2015. Evolution of the leaf economics spectrum in herbs: Evidence from environmental divergences in leaf physiology across *Helianthus* (Asteraceae). *Evolution* 69: 2705-2720.

Mason CM, LaScaleia MC, De La Pascua DR, Monroe JG, Goolsby EW. 2020. Learning from dynamic traits: seasonal shifts yield insights into ecophysiological tradeoffs across scales from macroevolutionary to intra-individual. *International Journal of Plant Science* 181, 88-102

Ollinger SV, Smith ML, Martin ME, Hallett RA, Goodale CL, Aber JD. 2002. Regional variation in foliar chemistry and N cycling among forests of diverse history and composition. *Ecology* 83:339–355

Ollinger SV. 2011. Sources of variability in canopy reflectance and the convergent properties of plants. *New Phytologist* 189:375–394

Rosipal R, Krämer N. 2006. Overview and recent advances in partial least squares. Lecture Notes in Computer Science (Including Subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics), 3940 LNCS, 34–51.

Serbin SP, Singh A, Mcneil BE, Kingdon CC, Townsend PA. 2014. Spectroscopic determination of leaf morphological and biochemical traits for northern temperate and boreal tree species. *Ecological Applications* 24:1651–1669

Singh A, Serbin SP, McNeil BE, Kingdon CC, Townsend PA. 2015. Imaging spectroscopy algorithms for mapping canopy foliar chemical and morphological traits and their uncertainties. *Ecological Applications* 25:2180–2197

Silva-Perez V, Molero G, Serbin SP, Condon AG, Reynolds MP, Furbank RT, Evans JR. 2018. Hyperspectral reflectance as a tool to measure biochemical and physiological traits in wheat. *Journal of Experimental Botany* 69:483–496.

Taiz L, Zeiger E. 2006. Plant Physiology. 4th Edition. Sinauer Associates. Sunderland, MA.

Wang R, Gamon, JA. 2019. Remote sensing of terrestrial plant biodiversity. *Remote Sensing of Environment* 231: 111218.

Wold S, Martens H, Wold H. 1983. The multivariate calibration problem in chemistry solved by the PLS method. In: Ruhe A, Kagstrom B, eds. Matrix pencils, lecture notes in mathematics. Springer, Heidelberg. 286–293.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, et al. 2004. The worldwide leaf economics spectrum. *Nature* 428: 821-827.

Xing L, Pittman JJ, Inostroza L, Butler TJ, Munoz P. 2018. Improving predictability of multisensor data with nonlinear statistical methodologies. *Crop Science* 58: 972–981.

Zhai Y, Cui L, Zhou X, Gao Y, Fei T, Gao W. 2013. Estimation of nitrogen, phosphorus, and potassium contents in the leaves of different plants using laboratory-based visible and near-infrared reflectance spectroscopy: Comparison of partial least-square regression and support vector machine regression met. *International Journal of Remote Sensing* 34: 2502–2518.