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High throughput in vivo analysis of plant leaf chemical properties using hyperspectral imaging

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High throughput in vivo analysis of plant leaf chemical properties using hyperspectral imaging

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Abstract

The possibility of predicting plant leaf chemical properties using hyperspectral images was studied. Sixty maize and 60 soybean plants were used, and two experiments were conducted: one with water limitation and the second with nutrient limitation, with the purpose of creating wide ranges of these chemical properties in plant leaf tissues. A hyperspectral imaging system with a spectral range from 550 to 1700 nm was used to acquire plant images in a high throughput fashion (plants placed on an automated conveyor belt). Leaf chemical properties were measured in the laboratory. Partial least squares regression was implemented on spectral data to successfully model and predict water content, micronutrient, and macronutrient concentrations.

Introduction

High throughput plant phenotyping is important for the utilization of plant genomic data for gene discovery and crop trait improvement. Digital images of plants have been adopted as tools for rapid, non-destructive measurement of plant structural parameters. RGB imaging has been previously used to characterize biomass and growth of several agronomically important crops.

Hyperspectral imaging has been used to characterize the spectral variations of field crops under drought stress, but the literature is scant in the use of hyperspectral imaging to quantitatively measure chemical properties of plants at the single plant level (i.e., greenhouse phenotyping).

This study was based on the hypothesis that hyperspectral image data will be useful in the prediction of plant leaf chemical properties.

The objective was to investigate the usefulness of hyperspectral imaging to quantify chemical properties of maize and soybean plants in vivo. These properties included leaf water content, macronutrients: nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), and sulfur (S), and micronutrients: sodium (Na), iron (Fe), manganese (Mn), boron (B), copper (Cu), and zinc (Zn).

Methods

Figure 1. Plants with multiple treatments grown on benches

Figure 2. Ground-truth data collected using sensors

Figure 3. Plant images being obtained using the Lemnatec Scanalyzer3D system

Figure 4. Plants are destructively sampled and dried before laboratory chemical analysis

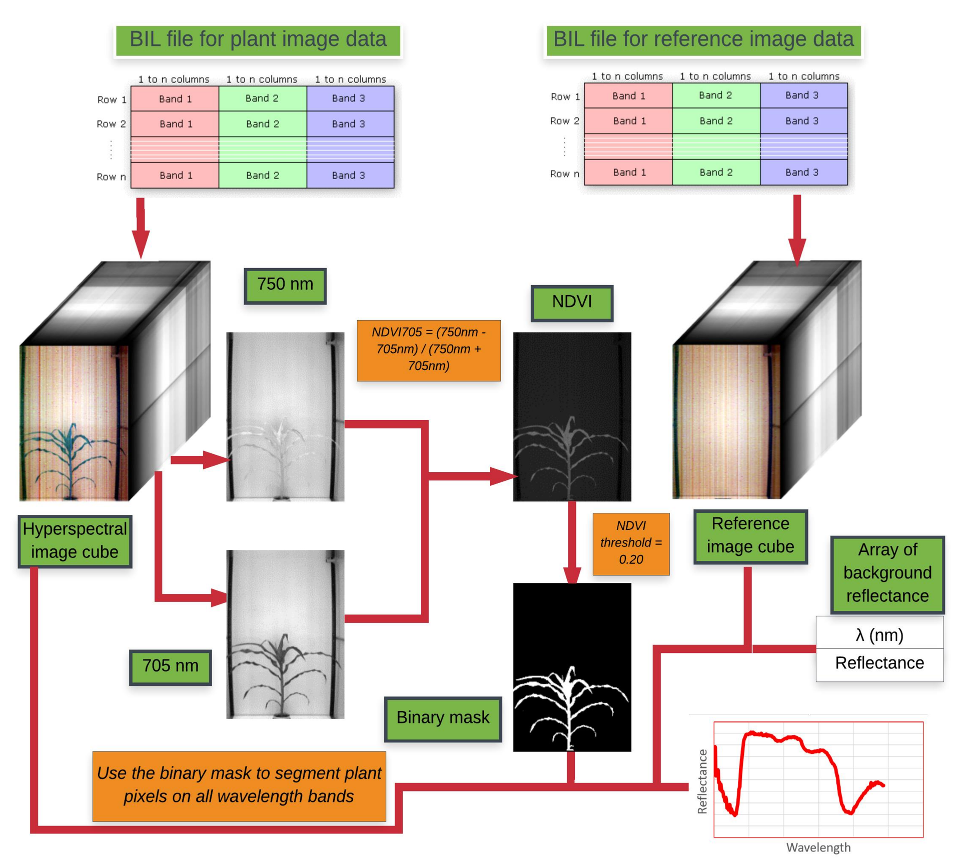

Figure 5. Steps in hyperspectral image processing

- Maize and soybean plants were grown at the Greenhouse Innovation Center at University of Nebraska-Lincoln.
- Two separate experiments were designed for drought stress (control and drought) and nutrient treatment (high, medium, and low nutrient).
- Data was collected between 27 and 64 days after sowing (DAS) to obtain greater variability in measurements.
- Hyperspectral images of plants were collected using the Lemnatec Scanalyzer3D system, along with a reference for every image.
- Leaf chlorophyll content, leaf temperature were measured for each plant. Fresh and dry shoot weight were obtained.
- Hyperspectral images were processed to get a spectrum per plant.
- Dried plant leaf material was sent to a commercial lab for chemical analysis for micro and macro nutrients.
- Principal component analysis was implemented on spectral data, followed by partial least squares regression to model and predict water content, micronutrient, and macronutrient concentrations.

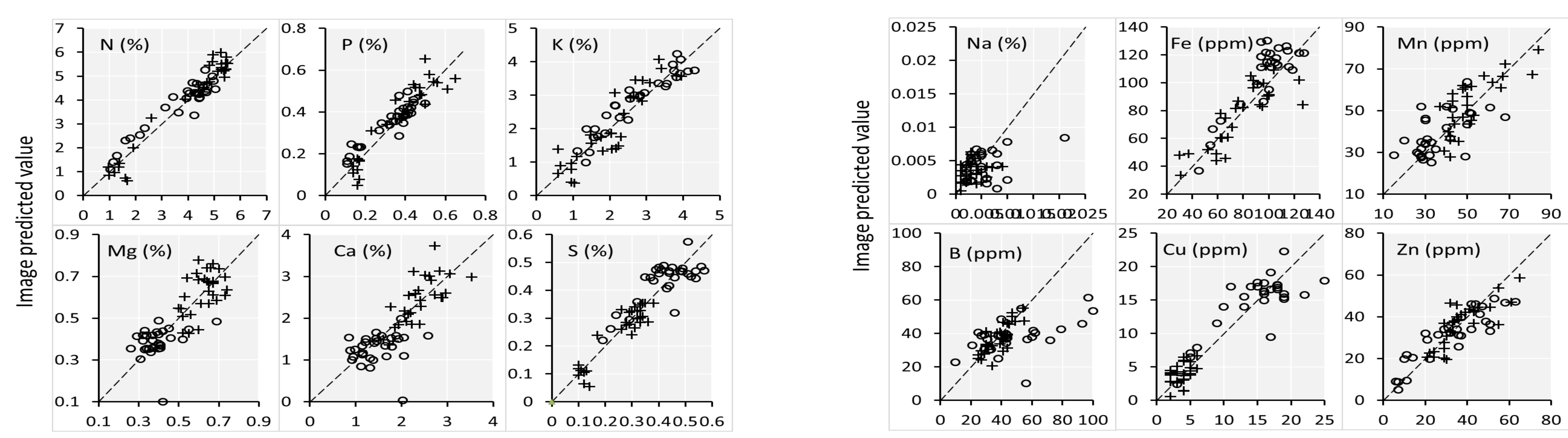

Figure 6. Scatterplot of the lab measured value versus the image predicted value of the concentrations of the macronutrients and micronutrients in plant leaves for the validation set (n = 60). Maize plants are denoted by circles and soybean plants are denoted by crosses.

Results

Table 1. Cross-validation and validation results of using hyperspectral images to predict plant leaf water content, macronutrient, and micronutrient concentrations with partial least squares regression.

	Cross-validation (n = 60)					Validation (n = 60)			
	R^2	$RMSE_{CV}$	RPD	MAPE (%)	Model Size	R^2	$RMSE_V$	RPD	MAPE (%)
WC (%)	0.97	1.18	5.64	1.1	12	0.93	1.62	3.80	1.6
N (%)	0.88	0.47	2.94	8.8	12	0.92	0.41	3.60	8.3
P (%)	0.71	0.075	1.86	13.8	10	0.83	0.056	2.43	12.3
K (%)	0.73	0.53	1.92	15.5	7	0.83	0.41	2.47	14.1
Mg (%)	0.69	0.088	1.81	13.1	5	0.69	0.078	1.79	12.2
Ca (%)	0.75	0.35	2.02	14.6	8	0.70	0.39	1.62	15.7
S (%)	0.71	0.068	1.88	13.0	11	0.83	0.052	2.46	12.2
Na (%)	0.19	0.003	1.13	46.2	7	0.18	0.003	1.09	49.5
Fe (ppm)	0.73	16.0	1.95	10.4	11	0.68	16.2	1.70	13.7
Mn (ppm)	0.51	11.1	1.45	21.2	7	0.64	9.56	1.62	17.3
B (ppm)	0.38	10.4	1.29	20.2	7	0.29	15.6	1.12	23.3
Cu (ppm)	0.80	3.01	2.25	24.9	12	0.86	2.52	2.69	20.8
Zn (ppm)	0.64	7.02	1.68	15.3	8	0.73	7.39	1.93	16.1

Conclusions

Statistics from the prediction models indicated that, among the 13 variables, leaf water content and N can be quantified very accurately. Predictions of P, K, Mg, Ca, S, Fe, Mn, Cu, and Zn were somewhat less accurate but still quite satisfactory. Na and B were the only two variables quantified poorly. To the best of our knowledge, this is the first study using hyperspectral imaging to probe the chemical properties of living plants. Apparently, hyperspectral imaging will be an essential imaging module for high throughput phenotyping for the chemical sensing of plants.

Future directions

Because of the positive results obtained in this study, it is conceivable that hyperspectral imaging would be used for phenotyping other plant chemical properties. For example, there is a wide interest to breed and improve energy crops for dedicated biofuel production (for example, biomass sorghum). It is quite possible that hyperspectral imaging can predict the chemical compositions of cell wall (such as lignin, cellulose, hemicellulose, and ash) from both plant leaves and stems.

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