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Research Article

Indirect Estimations of Lentil Leaf and Plant N by SPAD Chlorophyll Meter

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A Soil Plant Analysis Development (SPAD) chlorophyll meter can be used to screen for leaf nitrogen (N) concentration in breeding programs. Lentil (*Lens culinaris* L.) cultivars were grown under varied N regimes, SPAD chlorophyll meter readings (SCMR) were recorded from the cultivars leaves, and leaf N concentration was measured by combustion. Linear regression and the nonlinear Radial Basis Functions (RBF) neural networks models were employed to estimate leaf N concentration (LNC) based on the SCMR values. The closest estimates of LNC were obtained from the multivariate models in which the combination of plant age, leaf thickness, and SCMR was employed as the independent variable. In comparison, SCMR as the single independent variable in both models estimated less than 50% of LNC variations. The results showed significant effects of soil moisture and plant age on the association of LNC –SCMR as well as the relationship of LNC with plant N, grain yield, and days to maturity. However, the effect of cultivar on the measured variables was negligible. Although lentil N can be diagnosed by comparing SCMR values of the crop with those from a well-fertilized (N fixing) plot, the results did not support using SPAD chlorophyll meter for screening lentil LNC.

1. Introduction

The majority of leaf N is accumulated in the chloroplast, where photosynthesis takes place, resulting in a strong association between plant photosynthesis and leaf N status [1]. This association facilitates modeling plant growth and yield via leaf N assessment, because the latter can be rapidly estimated using SPAD chlorophyll meter. This widely used hand-held device measures the ratio of transmitted red (~650 nm) to infrared (~940 nm) electromagnetic radiation from the leaf surface and produces numeric outputs that are related to leaf chlorophyll (*chl*) content (Konica Sensing, Inc., Osaka, Japan). The SPAD Chlorophyll Meter Reading (SCMR) is correlated to leaf N concentration (LNC, e.g., leaf N mass per leaf mass) and can be used to evaluate soil and plant N status, estimate plant N requirement, predict grain yield, and forecast crop maturity [2, 3].

Despite its extensive application, association of SCMR-leaf *chl*/leaf N is often affected by soil and weather conditions, plant age, leaf thickness, leaf area, and leaf position in the canopy [2, 4]. Strategies such as data collection from the canopy apex at mid-day can eliminate daily variations of light and leaf starch concentration and improve the strength of SCMR-leaf N association models [5, 6]. Variations of leaf mass and leaf area can affect LNC (N is diluted in larger mass and area) and leaf spectral reflectance characteristics. Therefore, specific leaf weight (SLW: leaf mass per leaf area) and specific leaf N (SLN: N mass per leaf area) often have cofounding effects on SCMR-LNC models. In rice (*Oryza sativa* L.), LNC had stronger correlation with adjusted SCMR for leaf thickness (SCMR divided by SLW) than it had with SCMR [4]. In tobacco (*Nicotiana tabacum* L.), SCMR produced closer estimates for SLN than it did for LNC [7].

With an assumption of independency of input variables, SCMR (independent variable) is generally fitted against leaf *chl*/leaf N (dependent variable) in a standard linear regression model. When leaf thickness, plant age, soil, and weather affect the model, multivariate and polynomial linear models may result in stronger SCMR-LNC relationships compared to the standard linear model. In three different studies on rice and pigeon pea (*Cajanus cajan* L.), including SLW as the second independent variable improved the prediction power of the model for estimation of LNC based on SCMR values [3, 4, 6]. Similarly, a second-degree polynomial model allowed Castelli and Contillo [7] to interpolate data from two monocot and two dicot species and estimate leaf *chl* based on SCMR. However, combining several independent variables in multivariate linear models ($y = x_1, x_2, x_3, \ldots, x_n$) can violate the assumption of variables independency. Under such circumstances, nonlinear regression may provide more reliable fit for SCMR-LNC association than a linear model.

Nonlinear regression continually adjusts the estimated values of the parameters and improves the fit to a minimum satisfactory level [8]. From different nonlinear approaches, artificial neural networks models are widely used to develop association, classification, and prediction models in biology. These models consist of interconnected groups of artificial neurons that pass the information among a series of layers as the weights of observation are changed to achieve the best fit [9]. An advantage of these self-adaptive models is their capability of learning from an existing data set (training), as opposed to entirely relying on theoretical algorithms in linear approach. By employing back propagation neural networks and using plant and soil indexes, Liu et al. [10] estimated rice leaf *chl* concentration by SCMR values with 90% accuracy. In corn (*Zea mayz* L.), actual LNC values were strongly correlated ($r = 0.89$) to the estimated LNC from SCMR by a neural networks model [9]. Despite the complexity in calculation, most neural networks models, such as redial basis function (RBF), are available through statistical packages such as SAS and Matlab and can be employed directly or with minor modifications.

Lentil (*Lens culinaris* L.) is an annual legume plant that produces substantial amounts of leaf, enriched in N. In a field study, 60, 34, and 15% of the above ground biomass and 80, 45, and 13% of plant N content of lentil were accumulated in leaf at flowering, pod, and maturity stages, respectively [11]. We hypothesized that lentil leaf N directly links to plant performance and yield; therefore, plant N content and grain yield of lentil can be rapidly estimated via LNC measurement. To test the hypothesis, we (1) determined the associations of lentil LNC with plant biomass and N content at different growth stages, and with lentil grain yield at maturity, (2) estimated lentil LNC using a SPAD Chlorophyll Meter, and (3) developed linear and nonlinear models for computing lentil LNC via SCMR values.

2. Materials and Methods

2.1. Experiment Setup. Lentil cultivars were grown in Saskatoon (52.05° N and 106.43° W) and Indian Head (50.55° N and 103.65° W), Saskatchewan. In Saskatoon, three N fertility treatments of 50 kg N ha^{-1}, 5.6 kg ha^{-1} granular rhizobia (Nodulator, Becker Underwood, Saskatoon, SK), and a nontreated control were applied on eight lentil cultivars in 2006 and 2007 (N fertility trial). This trial was conducted in two different fields in 2006 and 2007. Compared to 2006, the 2007 field in the N fertility trial had low soil available N and no recorded history of legume crop cultivation and rhizobia inoculation. In the N fertility trial, CDC Greenland, CDC Plato and CDC Sedley (late-maturing group), CDC Milestone and CDC Viceroy (medium-maturing group), and CDC Blaze, CDC Red Rider, and CDC Rouleau (early-maturing group) were grown in both years [12]. The prefix "CDC" represents the Crop Development Centre at University of Saskatchewan, where the cultivars were developed. In both years, lentil was grown in a randomized complete block design (RCBD) with a split-plot arrangement in four replications. The main plots consisted of the three fertility treatments of control, inoculant, and N fertilizer and the subplots consisted of the eight lentil cultivars. In Indian Head, five lentil cultivars were subjected to two different no tillage (NT) durations, one 5 years (short-term NT) and the other 28 years (long-term NT). In this trial, average spring soil available N (NO_3-NH_4) over the years was 8.9 and 11.3 mg N kg^{-1} soil in the short- and long-term NT plots, respectively. In this trial, CDC Sedley, CDC Vantage and CDC Milestone (medium-maturing group), and CDC Robin and Redcap (early-maturing group) were grown in both years [12]. Here, five lentil cultivars were arranged in a CRBD with three replicates within each NT duration treatment. Varied rainfall and soil available N during the study resulted in four distinct situations: (1) N fertility trial in 2006, where a suitable growing season was terminated by a drought, (2) N fertility trial in 2007, where a severe mid-season drought and low soil N limited N_2 fixation, N uptake, and grain yield of lentil, (3) NT trial in 2006, where a suitable growing season was terminated by a mild drought, and (4) NT trial in 2007, where a substantial late season rainfall stimulated lentil biomass and N accumulations. More details about the weather, soil N, and lentil performance in these trials are found in [13, 14]. Overall, the N fertility trial, which had 8 lentil cultivars and 3 N fertility treatments, was conducted in Saskatoon in 2006 and 2007. The NT trial with 5 lentil cultivars and 2 no-till duration treatments was conducted in Indian Head in 2006 and 2007.

2.2. Data Collection. Leaf chlorophyll content was estimated using a SPAD Chlorophyll Meter (Model 502 Konica Minolta Sensing, Inc., Japan) at three growth stages of vegetative (up to node 12), first-pod (at least one pod per plant), and late-pod (when the canopy started turning yellow). To eliminate daily variations of light quality and leaf starch concentration, SCMR readings were limited to the uppermost leaves during 10:00 to 12:00 h of day. Three plants per plot were randomly selected and SCMR was recorded from the three fully expanded uppermost leaves of each plant. The average of nine SCMR values in each plot was considered as the plot SCMR value. The leaves were immediately detached and transferred on ice into a refrigerator for further measurements the next day. In the laboratory, leaf surface area was measured using

a leaf area meter (Model LI-3100C, LiCor, Lincoln, NE), and leaves were dried at 60°C for 24 hrs, weighed, and ground. Leaf N concentration was measurement by the combustion method, using a Leco carbon-nitrogen determinator (LECO CNS 2000, St. Joseph, MI, USA). Specific leaf weight (SLW), which represents leaf thickness, was the ratio of leaf dry weight (g) to leaf area (m^{-2}), SLN was the ratio of g leaf N (leaf weight × LNC) to leaf area (m^{-2}), and adjusted SCMR for leaf thickness was the ratio of SCMR to SLW. In addition, grain yield and N content of the entire plant biomass (from both trials) and average N content of leaf (referred to entire leaf biomass), stem, and pod from 5 plant $plot^{-1}$ at the three given growth stages (from the N fertility trial) were available.

2.3. Data Analysis. In each trial, the three leaf characteristics (LNC, SLW, and SLN) were analyzed for the effects of treatment and cultivar. In the analysis of variance, the main factor was N fertility treatment (in the N fertility trial) and no-till duration (in the NT trial), and the subfactor was cultivar. Data were analyzed as a year-combined RCBD for each trial-growth stage, with year, treatment, cultivar, and their interactions as fixed variables and block and interaction of block with the fixed factors as random variables [14]. Data were analyzed by the MIXED procedure in SAS, Version 9.2 (SAS Institute, Cary, NC), and differences amongst the means of the fixed effects were tested using Fisher's protected LSD at $P < 0.05$. Pearson's correlation coefficients for LNC with grain yield, harvest index, days to maturity, and biomass, N concentration, and N content (g N $plant^{-1}$) of leaf, stem, and pod at the three given growth stages were computed for the N fertility trial, using the CORR procedure of SAS.

Following the analysis of variance, the leaf characteristics data from two trials, three growth stages, and two years were pooled to compute the correlation of SCMR and adjusted SCMR with LNC, using the CORR procedure of SAS. This data set consisted of 740 data points for each of the leaf characteristics (LNC, SCMR, SLW, and SLN). The pooled data set was randomly divided into two groups, a training set of 630 data points (85% of the data) and a test set of 110 data points (15% of the data), to develop linear and nonlinear models by the GLM procedure in SAS and the Newrb function in Matlab, respectively. The training set was used in model development and the test data to validate the models. The three growth stages of vegetative, first-pod, and late-pod were arbitrary considered 1, 2, and 3, respectively.

For the nonlinear approach, we tested an RBF neural networks model. This model is linear combinations of radial basis that produces linear outputs based on nonlinear inputs. Using RBF requires specification of the number of hidden unit activation function, the number of processing units, a criterion for modeling a given task, and a training algorithm for finding the parameters of the network. Weight of the model is found through the training process, where network parameters are optimized to fit the network outputs to the given inputs [15]. Four groups of independent variables "SCMR," "SCMR + SLW," "SCMR + growth stage," and "SCMR + SLW + growth stage" were the input independent variables, and LNC was the only dependent variable. Hence, each of the linear and nonlinear approaches resulted in four equations differing in the independent variable (see Table 4 for the linear equations). The developed models were fed by the correspondence independent variable(s) from both the training and the test sets to estimate LNC. The estimated LNC from each set was correlated against the actual measured LNC from the same data set. Pearson's correlation coefficients of the estimated LNC and actual LNC from the training and test set were considered a measure of model accuracy and the model reliability, respectively.

3. Results

Averaged over the years, trials, treatments, and cultivars, lentil LNC decreased from 4.5% at vegetative to 3.8% at first-pod and 2.7% at late-pod growth stage (Table 1). Average SLN was similar at the vegetative and first-pod growth stages (2.0 g N m^{-2} leaf) and then decreased to 1.4 g N m^{-2} leaf at late-pod. In contrast to leaf N, leaf thickness (SLW) was increased from 42 g m^{-2} leaf at vegetative to 50 g m^{-2} leaf at first-pod and late-pod. Maximum variations of the three leaf properties occurred at the first-pod stage.

The leaf characteristics differed between 2006 and 2007 in the N fertility trial (Table 1), where soil N, soil indigenous rhizobia status, and rainfall distribution varied between the years. In this trial, LNC in 2006 was 0.7% (at vegetative) and 1% (at late-pod) greater than in 2007, but leaves were 8 (at first-pod) and 4 (at late-pod) g m^{-2} thicker in the dry year (2007). Variations of SLN between the years were not always similar to the LNC variations. For example, despite greater LNC at the vegetative growth stage in 2006 than 2007, SLN was similar in both years at this stage. Also, LNC was similar at first-pod growth stage in both years, but SLN in 2007 was 0.9 g N m^{-2} leaf greater than at the same stage in 2006. In the N fertility trial, lentil yielded more, fixed more atmospheric N_2, and accumulated more N and biomass in 2006 than in 2007 (Table 2, [13]).

In the NT trial, LNC was greater in 2006 than in 2007 at two final growth stages, SLW was similar during the entire seasons of both years, and SLN was greater in 2006 than in 2007 at vegetative, less in 2006 than in 2007 at first-pod, and similar in both years at late-pod. In this trial, lentil yield was similar between the years, but lentil accumulated more biomass and N due to more rainfall in 2007 than in the drier year of 2006 (Table 2, [14]).

3.1. Treatment and Cultivar Effects. Averaged over the treatments and years, LNC in the N fertility trial, where one third of the plots received 50 kg N fertilizer ha^{-1} at seeding, was 0.8% (at first-pod) and 1.5% (at late-pod) greater than in NT (Table 1). However, considering the year effect in the N fertility trial revealed that LNC variations were not solely due to the N fertility treatments. In 2006, LNC and SLW were independent of the fertility treatment and only SLN was greater in treated plots than in the control plots and only at first-pod stage. In 2007, LNC was 0.5% greater in the treated plots (averaged) than in the control at vegetative, 0.6% greater in the control and inoculated plots than in the fertilized plots at first-pod, and similar across the treatments

Table 1: Average leaf N concentration (LNC), specific leaf N (SLN), and specific leaf weight (SLW) of lentil at three growth stages of vegetative growth (VG), first-pod (FP), and late-pod (LP) under three N fertility treatments (top) and two NT duration treatments (bottom) in 2006 and 2007.

Year	Treatments	LNC (%)			SLW (g DW m^{-2} leaf)			SLN (g N m^{-2} leaf)		
		VG	FP	LP	VG	FP	LP	VG	FP	LP
					Fertility study					
2006	Control	4.5a	4.0c	3.8a	41b	50b	50bc	1.6b	1.6d	1.9a
	N fertilizer	4.6a	4.3b	4.1a	39b	47b	48c	1.6b	1.8c	1.9a
	Inoculant	4.5a	4.1c	3.9a	42b	49b	46c	1.6b	1.7c	1.8a
	Average	**4.5A**	**4.1B**	**3.9A**	**41A**	**49B**	**48B**	**1.6A**	**1.7B**	**1.9A**
2007	Control	3.6c	4.7a	3.1b	45a	56a	56a	1.6b	2.7a	1.7ab
	N fertilizer	4.0b	4.0c	2.8bc	41b	55a	52ab	1.6b	2.4b	1.4b
	Inoculant	4.2b	4.5a	2.9c	44a	56a	49b	1.8a	2.6a	1.4b
	Average	**3.9B**	**4.4A**	**3.0B**	**43A**	**57A**	**52A**	**1.7A**	**2.6A**	**1.5B**
					NT study					
2006	LT	4.7b	3.6ab	1.8b	41ab	46a	56a	1.9b	1.7b	1.1ab
	ST	4.9a	4.0a	2.3a	45a	48a	51ab	2.3a	1.8b	1.3a
	Average	**4.8A**	**3.8A**	**2.1A**	**43A**	**47A**	**53A**	**2.2A**	**1.7B**	**1.2A**
2007	LT	4.5b	3.4b	1.8b	39b	47a	49b	1.7c	2.1a	1.0a
	ST	4.4b	3.0c	1.9ab	38b	43b	54ab	1.7c	2.2a	1.1a
	Average	**4.4A**	**3.1B**	**1.8B**	**39A**	**46A**	**52A**	**1.7B**	**2.1A**	**1.0A**

Means followed by different small letters within columns within each study indicate significant effects of the fertility treatments (top) and the NT treatments (bottom) in the two years of the studies ($P < 0.05$).
Means followed by different capital letters within columns within each study indicate significant effects of the year in each study ($P < 0.05$).

Table 2: Correlation coefficients of LNC and N concentration of the entire leaf biomass with lentil performance in the N fertility trial in three stages of vegetative (VG), first pod (FP), and late-pod (LP).

			Yield	HI	DTM	%Ndfa	Above ground biomass			Leaf N	Stem		Pod	
	Year[†]	Stage	g m^{-2}	%	days	%	DM	N%	mg N $plant^{-1}$	mg N $plant^{-1}$	N%	mg N $plant^{-1}$	N%	mg N $plant^{-1}$
LNC (%)	2 years	VG	0.49	—	0.45	0.29	—	0.52	—	—	—	—	na[‡]	na
		FP	—	—	—	—	—	—	—	—	—	—	—	—
		LP	0.24	—	0.35	—	0.38	—	0.20	0.39	0.47	0.32	0.23	—
	2006	VG	—	—	0.43	—	0.29	—	0.33	0.37	—	—	na	na
		FP	—	—	—	—	—	0.37	—	—	—	—	—	—
		LP	0.25	−0.49	0.35	−0.30	0.61	0.58	0.25	0.45	0.49	0.39	—	—
	2007	VG	0.48	0.22	—	—	—	0.46	—	—	0.26	—	na	na
		FP	—	—	—	0.29	−0.39	0.32	—	—	0.30	—	—	—
		LP	—	0.25	0.29	0.28	−0.22	—	—	—	0.32	—	—	—
Total leaf biomass N%	2 years	VG	—	—	—	—	—	0.28	0.42	0.42	0.64	0.29	na	na
		FP	0.37	—	0.43	0.31	−0.23	0.37	—	0.30	0.78	0.21	0.65	—
		LP	—	—	0.22	—	—	—	—	0.47	0.53	0.27	0.30	—
	2006	VG	—	−0.36	—	—	—	—	0.63	0.63	0.37	0.40	na	na
		FP	0.30	−0.31	0.32	0.41	—	—	0.23	0.55	0.66	0.45	0.34	−0.40
		LP	—	−0.42	—	−0.44	0.41	0.55	—	0.54	0.67	0.49	0.38	—
	2007	VG	0.33	—	—	—	—	0.81	0.36	0.38	0.83	—	na	na
		FP	—	—	0.32	0.31	—	0.48	0.33	0.45	0.85	0.33	0.83	—
		LP	—	—	0.45	0.35	—	—	—	0.40	0.58	—	—	−0.27

[†]Correlation coefficients for the pooled data over the years (2 years) and for each year separately.
[‡]Only significant correlation coefficients are presented ($P < 0.05$).

at late-pod. In this year, SLW in the control and inoculated plots was greater than in the N fertilized plots at vegetative, similar across the treatments at first-pod, and again greater in the control than in the inoculated plots at late-pod. Also, SLN was the greatest in the inoculated plots at vegetative, the smallest in the fertilized plots at first-pod, and similar across the treatments at late-pod. In comparison to the leaf characteristics, the above ground biomass N concentration due to the control, N fertilizer, and inoculant treatments in 2007 was, respectively, 2.8, 3.5, and 3.1% at vegetative, 2.5, 2.1, and 2.7% at first-pod (all differences were significant), and 2.2% across all treatments at late-pod.

In the NT trial, where the spring soil N was greater in the long- than the short-term NT in both years, response of lentil yield to the NT duration treatment was limited to 2006 only (Table 1). In this year, LNC was greater in the short- than the long-term NT at both vegetative and late-pod; SLW was independent of the NT treatment; and SLN was greater in the short- than the long-term NT at vegetative. In the second year, NT treatment affected LNC and SLW at the first-pod only. Similarly, plant N concentration in the short- and long-term NT in 2006 was, respectively, 4.1 and 3.8% at vegetative, 3.6 and 3.2% at first-pod, and 2.7 and 2.4% at late-pod; the differences were all significant.

Variation of the cultivars for the three leaf characteristics appeared in the N fertility trial in 2007, only. In this case, cultivars CDC Red Rider and CDC Plato had the greatest and CDC Sedley had the smallest LNC at the vegetative, and CDC Red Rider and CDC Viceroy had greater LNC than the other cultivars at the first-pod (Figure 1). At the late-pod, cultivar CDC Viceroy had a greater LNC than both CDC Blaze and CDC Sedley, and the other cultivar had similar LNC. Compared to the other seven cultivars, CDC Sedley had small LNC at all three growth stages. Variations of cultivars for leaf thickness (SLW) in this year appeared at the vegetative and maximized at the first-pod. The early maturing small-seeded cultivars CDC Blaze and CDC Milestone and the large-seeded CDC Sedley had greater SLW than the other cultivars at all three growth stages. Cultivar CDC Rouleau had a smaller SLW than most cultivars at the vegetative, and the smallest SLW amongst the cultivars at the late-pod stage. In case of SLN variations in 2007, cultivar CDC Blaze had the greatest SLN amongst the cultivars at the vegetative stage, when the other cultivars did not differ for SLN. At the first-pod stage, CDC Greenland, CDC Plato, and CDC Rouleau had smallest SLN values than the other five cultivars. In the other cases (N fertility trial in 2006 and NT trial in both years), no difference existed among the five lentil cultivars for the leaf characteristics in either years.

3.2. Association of Leaf N and Lentil Performance. Association of leaf N with lentil performance was calculated for the N fertility trial only. In this experiment, correlation of LNC with N concentration of the entire leaf biomass was strongly positive ($r = 0.66$, $P < 0.05$) for the pooled data over the years, treatments, and growth stages and also for each separate year ($r = 0.69$, $P < 0.05$). Pearson's correlation coefficient of LNC and N concentration of entire leaf biomass at the vegetative, first-pod, and late-pod growth stages was, respectively, 0.27 ($P < 0.05$), 0.12 (ns), and 0.45 ($P < 0.05$) in 2006 and 0.42 ($P < 0.05$), 0.31 ($P < 0.05$), and 0.52 ($P < 0.05$) in 2007.

As a result of these variations, correlations of plant growth parameters and grain yield with N concentration of the top leaves (LNC) and N concentration of the entire leaf biomass were not consistent. For example, both LNC and entire leaf biomass N concentration were positively correlated to plant N concentration at late-pod stage in 2006 and at vegetative and first-pod stages in 2007 (Table 2). The entire leaf biomass N concentration and pod N concentration were strongly associated at first-pod of the drier year (2007), but this association was not observed in the other year or growth stages. Similarly, LNC in 2006 was strongly associated with biomass, plant N concentration, and plant N content at late-pod, but these associations were not seen in 2007. In this year, LNC at the vegetative growth stage was correlated with grain yield, but the entire leaf biomass did not have a strong association with grain yield in the pooled or separated data for each year. Both LNC and N concentration of the entire leaf biomass at late-pod were negatively correlated to harvest index in 2006. Days to maturity was not strongly linked to LNC and the entire leaf biomass N concentration. Plants with greater LNC (at first-pod and late-pod) produced more biomass in 2006, but this association reversed in 2007 when LNC was negatively associated with biomass at first-pod and late-pod.

3.3. Association of SCMR and Adjusted SCMR with LNC. Averaged over the treatments and cultivars, SCMR values were similar between the two trials (N fertility and NT trials) at the vegetative growth stage (30), greater in the N fertility trial (37) than the NT trial (26) at first-pod growth stage, and greater in the N fertility trial in 2006 (40) than in the other years-trials at the late-pod growth stage (24). In response to the N fertility treatments, inoculated lentil had greater SCMR than lentil in the control treatment at the late-pod stage in 2006 (Table 1). In the next year, SCMR was the smallest in the control plots at vegetative, greater in the control than in the fertilized plots at first-pod, and greater in the control than the inoculated plots at late-pod. In the NT trial, the SCMR was 6 units greater in short- than long-term NT plots at both first- and late-pod stages in 2006. In 2007, SCMR did not vary with the NT duration treatment.

Because of significant treatment and year effects in the N fertility trial compared to the NT trial, correlations of SCMR and adjusted SCMR with LNC, plant N, and SLN were conducted for the N fertility trial only (Table 3). In this case, averages of SCMR and adjusted SCMR (SCMR/SLW) over the treatments were strongly associated with LNC at late-pod in each year and also in the pooled data over years. Separate correlation analyses for each year showed that relationships between SCMR or adjusted SCMR with LNC were stronger in the drier year (2007) than in the year with better rainfall distribution (2006). Interestingly, relationships between SCMR or adjusted SCMR and plant biomass N concentration were moderately negative at late-pod stage. This might be related to the effect of severe drought in 2007, in which the leaves dried rapidly but the stem was still green.

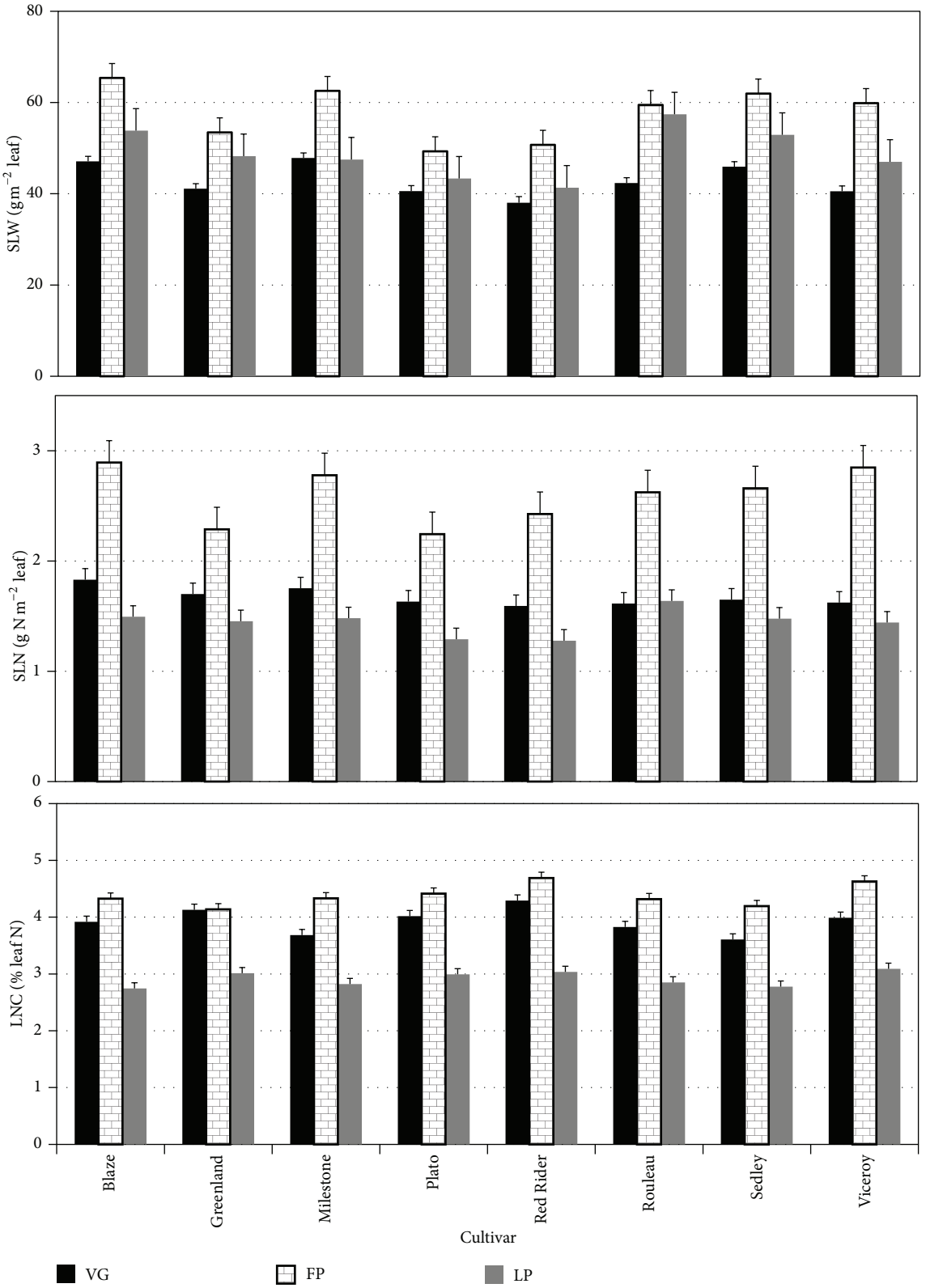

Figure 1: Variations of eight lentil cultivars for specific leaf weight (SLW), specific leaf N (SLN), and leaf N concentration (LNC) at three growth stages of vegetative (VG), first-pod (FP), and late-pod (LP) stages in the N fertility trial in 2007. Error bars are standard deviation.

Table 3: Correlation coefficients of SCMR and adjusted SCMR (SCMR/SLW) with leaf N concentration (LNC), plant N concentration (plant N), and specific leaf N (SLN) for each growth stage of vegetative, first-pod, and late-pod and for the pooled data over the growth stages.

Growth stage	Variable	SCMR	LNC	Plant N%	SLN
Vegetative	SCMR	1.00	ns[†]	ns	0.26
	Adjusted SCMR	0.58	0.35	0.36	−0.38
First-pod	SCMR	1.00	0.35	−0.19	0.37
	Adjusted SCMR	0.54	0.42	0.26	−0.37
Late-pod	SCMR	1.00	0.63	−0.43	0.65
	Adjusted SCMR	0.84	0.77	−0.40	0.40
Pooled	SCMR	1.00	0.50	ns	0.51
	Adjusted SCMR	0.70	0.75	0.40	ns

[†]All coefficients are significant ($P < 0.05$), unless otherwise indicated (ns).

Table 4: Equations are developed by linear regression to estimate leaf N concentration, using different combinations of SPAD chlorophyll meter reading (SCMR) values, growth stage (Stage), and specific leaf weight (SLW).

Linear model (independent variable)	Coefficient of determination (R^2)
$1.990 + (0.059 \times \text{SCMR})$	0.49
$3.162 + (0.048 \times \text{SCMR}) - (0.424 \times \text{Stage})^{\dagger}$	0.54
$3.737 + (0.053 \times \text{SCMR}) - (0.033 \times \text{SLW})$	0.64
$3.960 + (0.048 \times \text{SCMR}) - (0.023 \times \text{SLW}) - (0.026 \times \text{Stage})$	0.69

[†]Growth stages are 1, 2, and 3 for vegetative, first-pod, and maturity, respectively.

3.4. Estimation of LNC by SCMR. Four linear models were developed to estimate LNC based on various combinations of SCMR, growth stage, and leaf thickness as independent variables using the pooled data set from both trials (Table 4). These models were fed by the given independent variable(s) and their outputs (estimated LNC) were fitted against the actual measured LNC values to compute the accuracy of the models. Same procedure was followed for the nonlinear approach, and the accuracy and reliability of this approach was tested by comparing the models outcomes with the actual LNC observations in the test data set.

In both the linear and nonlinear models, SLW and, to some extent, growth stage had strong influence on the accuracy of LNC prediction models (Figure 2). When SCMR was the only independent variable, accuracy of the linear and nonlinear models was 53 and 38%, respectively. Including growth stage, SLW and the combination of SLW + growth stage with the SCMR improved the models accuracy by 12, 14, and 22% (linear) and 15, 43, and 48% (nonlinear), respectively. The linear approach was more accurate, when SCMR was the only independent variable, where the nonlinear approach provided more accurate outcomes with two or more independent variables.

For model validation (reliability), the models were fed by the test data set and then correlation coefficient of the models outcomes was calculated with actual LNC in the test data set. These correlation coefficients represent the models reliability for any new data. Results showed that reliability of the nonlinear models was always greater than the linear models. Correlation coefficients of the estimated LNC and actual LNC from the test set for the linear and nonlinear models were, respectively, 0.41 and 0.60 ($P < 0.05$), when SCMR was the only independent variable; 0.53 and 0.67 ($P < 0.05$), when SCMR + growth stage were independent variables; 0.61 and 0.81 ($P < 0.05$), when SCMR + SLW were independent variables; and 0.75 and 0.83 ($P < 0.05$), when SCMR + SLW + growth stage were independent variables. Accuracy as well as reliability of the linear and nonlinear models for SLN estimations was similar to the LNC estimation (data not presented).

4. Discussion

Leaf N concentration represented overall variations of plant N and grain yield of lentil due to the treatments and experimental conditions. In the fertility trial, varied plant N and grain yield between 2006 and 2007 and significant treatment effects in 2007 were all in agreement with the LNC variations in the given situations (Table 1). Likewise, yield advantage of lentil in the short-compared to the long-term NT in 2006 appeared in the LNC variations in this year. Similar findings on corn [16], rice [17], and cotton [18] suggest that LNC can be used to estimate shoot N concentration. In corn, LNC, SLN, and shoot N concentration all increased by increasing N fertilizer [19]. A strong association between LNC and plant N concentration at the vegetative stage allows using LNC to diagnose plant N deficiency, adjust soil N, and avoid yield loss later in the season. Compared to above ground biomass N concentration measurement, LNC measurement is fast and less expensive. In lentil, low LNC at vegetative stage due to the control treatment in the fertility trial in 2007 and due to the long-term NT in 2006 (Table 1) were in agreement with plant N status in the given treatments [13, 14].

Despite similar responses of LNC and lentil performance under the conditions of the experiment (Table 1), correlation of LNC-grain yield was not strong (Table 2). In comparison,

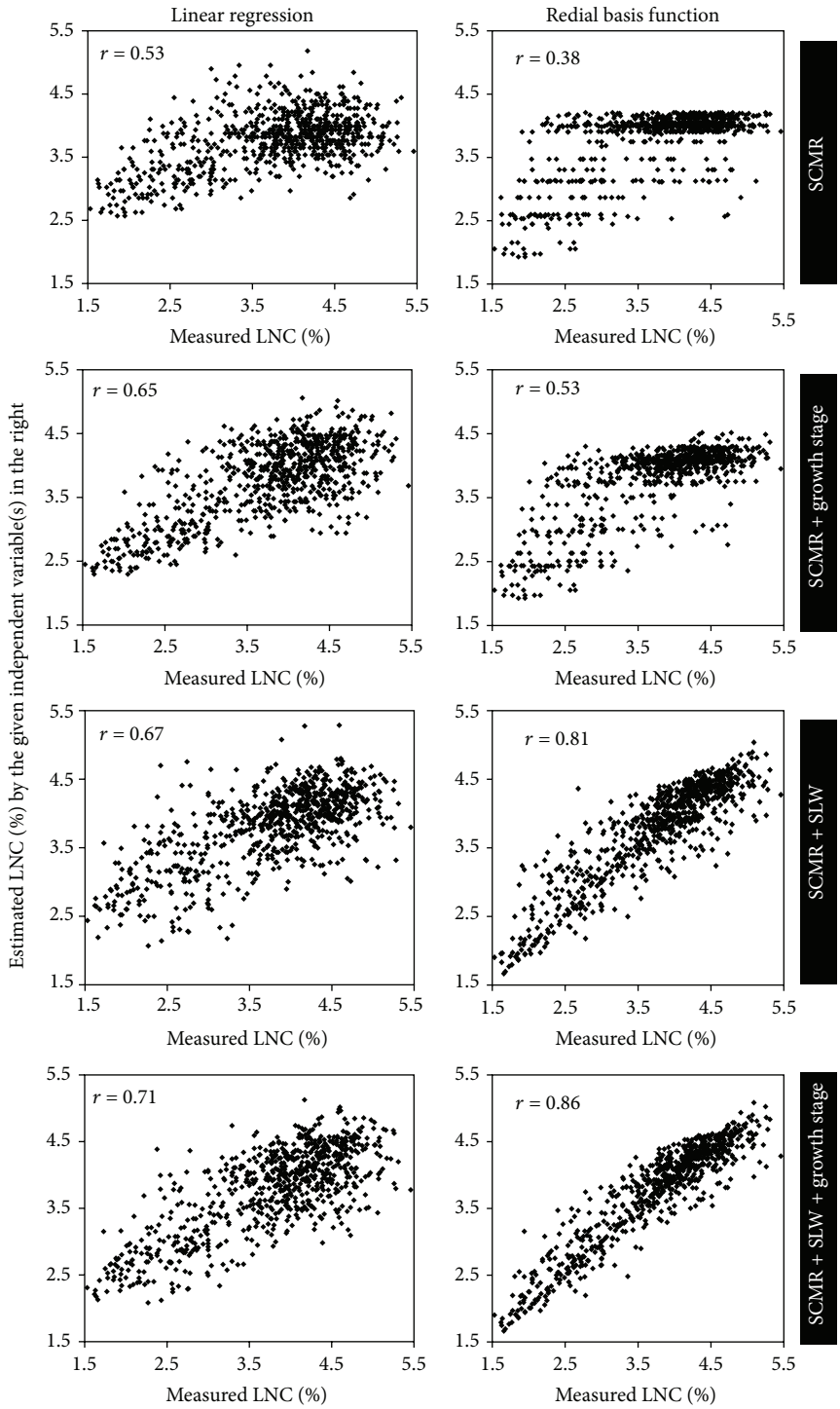

Figure 2: Association of estimated leaf N concentration (LNC) by linear regression (left) and nonlinear redial basis function (RBF) neural networks models (right) against actual LNC values. Data from two trials, two years, and three growth stages. Variables in the right side represent the combination of independent variables that generated each model.

corn LNC was strongly correlated to grain yield [20] and shoot N concentration [19]. These differences might relate to the ability of lentil for biological N fixation under normal conditions [21], which narrows down the range of LNC variation. As in Table 2, in a few occasions and only under marginal plant N status (at late-pod when plant N is low and at vegetative stage of 2007 in the N fertility trial that soil N was low), correlation coefficients of LNC with plant N concentration and days to maturity and with grain yield were moderately positive ($r > 0.40$). In addition, variation of cultivars phenology could result in different leaf N status in the sampling date. In soybean, correlation of leaf *chl*-SCMR was only strong in cultivars that had wide ranges of leaf *chl* concentration [22].

The results demonstrated that leaf position, plant growth stage, and number of sampled leaves (top leaves versus the entire leaf biomass) must be considered when LNC is used to estimate plant N status. Although plant age generally affects LNC amongst the field crops [23], the effect of leaf position is stronger in dicots whose canopy architecture limits light penetration into the canopy base [5]. Lack of adequate light inhibits leaf photosynthesis and stimulates N remobilizations from the lower leaves, resulting in LNC gradient along the canopy depth [5]. In addition, genotype [22] and number of sampled leaves [24] can alter the effect of leaf position on leaf *chl* and LNC.

The treatments affected SCMR in both trials, but the treatments effects on SCMR were not always similar to their effects on LNC (Table 2). As a result, SCMR-LNC association in the pooled data over the years ranged from very poor at vegetative to strong ($r = 0.67$, $P < 0.05$) at late-pod (Table 3). Association of SCMR-leaf N/leaf *chl* was strong at reproductive stage in soybean [2], it did not differ by growth stage in pigeonpea [3], and it was stronger in early rather than late-season sorghum [3], rice [25], and tobacco [7]. Lack of a strong association between SCMR and leaf *chl* in soybean was attributed to narrow range of leaf *chl* concentrations [22]. This can be the case in our study, because the range of LNC due to the treatments was narrower at lentil vegetative than first- and late-pod stages. Lack of a strong association between SCMR and LNC compared to previous works [4, 6, 24, 26] might also be associated with genotypic variations for LNC and leaf thickness. Fritschi and Ray [22] concluded that wide genotypic variations increased the range of *chl* concentration and improved the association of SCMR-leaf *chl*. However, varied responses of the cultivars to soil moisture in our study could alter the associations of LNC with leaf thickness and SCMR values. Most studies that have found strong SCMR-LNC associations have focused on one genotype within one environment, whereas varied flowering and maturity dates of lentil cultivars and different soil moisture of different environment could interfere with the LNC-SCMR correlation.

Adjusted SCMR (SCMR/SLW) showed a stronger correlation with LNC than SCMR (Table 3). These results are in agreement with findings in rice [4, 6], corn [24], sorghum, and pigeonpea [3]. To avoid the confounding effects of SLW (and other parameters) on the association of SCMR-LNC, farmers are suggested to grow a fully N-fertilized stripe and use it as N reference. SCMR values from the fertilized plots are compared with SCMR from the field for plant N diagnosis [26]. Alternatively, nonlinear models may handle the confounding effects of known and unknown variables on the LNC-SCMR association [9, 27]. Including SLW as the second independent variable increased the accuracy of both linear and nonlinear models by 14 and 53%, respectively (Figure 2). Similarly, Suen and Eheart [15] concluded that the back-propagation neural networks model, which we employed in our study, was more accurate than traditional regression analysis. By using this nonlinear model, the accurately estimated rice leaf *chl* concentration [10] and corn leaf N concentration [9] by SCMR values were improved compared to linear regression.

5. Conclusion

Lentil LNC and SCMR variations due to the experiment were in agreement with the plant N and grain yield changes. This similarity suggests that lentil SCMR from any field can be compared with a well fertilized plot in the same field to diagnose lentil N status. In two cases of treatment effects on plant N in the NT trial in 2006 and in the N fertility trial in 2007, plant N deficiency due to the treatments was detected from the SCMR values (Table 1). Although postflowering N deficiency in legume crops is less expected, performance of lentil in the long-term NT duration treatments in 2006 showed that rapid estimation of plant N by SPAD chlorophyll meter can lower the risk of yield loss due to inadequate N fixation. Variation of the lentil cultivars for the leaf characteristics and SCMR values in the drier year showed the confounding effects of drought and N on lentil leaf. In addition, variations of the leaf characteristics, especially SLW and to some extent SCMR, amongst the cultivars in the dry year of 2007 suggest the potential of screening for these traits under drought stress. We failed to demonstrate that SPAD chlorophyll meter is suitable for leaf and plant N concentration screenings amongst numerous lentil genotypes. This is explained by the effect of plant physiological age on leaf thickness, leaf area, and leaf N concentration. Including a complimentary device to SPAD Chlorophyll Meter for simultaneous measurements of leaf thickness and leaf *chl* content can substantially extend the applicability of this device in breeding programs.

Abbreviations

LNC: Leaf nitrogen concentration
CDC: Crop Development Centre, University of Saskatchewan
NT: No tillage
SPAD: Soil Plant Analysis Development
SCMR: SPAD Chlorophyll Meter reading
SLW: Specific leaf weight
SLN: Specific leaf nitrogen.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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