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Detection of calcium, magnesium, and chlorophyll variations of wheat genotypes on sodic soils using hyperspectral red edge parameters

Malini Roy Choudhury [a,*], Jack Christopher [b], Sumanta Das [a], Armando Apan [c,d], Neal W. Menzies [a,b], Scott Chapman [a,b], Vincent Mellor [a], Yash P. Dang [a]

[a] *The University of Queensland, School of Agriculture and Food Sciences, St Lucia, Queensland 4072, Australia*
[b] *The University of Queensland, Queensland Alliance for Agricultural and Food Innovation, Leslie Research Facility, Toowoomba, Queensland 4350, Australia*
[c] *University of Southern Queensland, School of Civil Engineering and Surveying, Toowoomba, Queensland 4350, Australia*
[d] *University of the Philippines Diliman, Institute of Environmental Science and Meteorology, Quezon City 1101, Philippines*

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ABSTRACT

Plants grown on sodic soils can suffer from macronutrient deficiencies, such as calcium (Ca), magnesium (Mg), and potassium (K), reducing health and growth. Nutrient concentrations in plant tissue could potentially provide a signal to identify cultivars tolerant to sodic conditions. However, conventional approaches to diagnosing crop nutrient and chlorophyll status involve determining total elemental content in plant tissues. These methods are time-consuming, tedious, and expensive, requiring destructive sampling of plant parts and complex laboratory analyses. Here, we propose a novel approach using hyperspectral sensing to determine macronutrient and chlorophyll variations/deficiencies of 18 different wheat genotypes grown in moderately sodic (MS) and highly sodic (HS) soil conditions in north-eastern Australia. Canopy reflectance was measured using a handheld spectroradiometer close to flowering to compute red edge spectral indices, such as normalized difference red edge index (NDRE), red edge inflection point (REIP), and red edge chlorophyll index ($CI_{rededge}$). Plant Ca, Mg, and K concentrations were also measured by destructive sampling of young mature leaves followed by laboratory analysis. The maximum first derivative of reflectance spectra for 18 wheat genotypes were observed at 722–728 nm and 719–725 nm for the MS and HS site, respectively and was used to determine REIP for the genotypes using a four-point linear interpolation method. Ca and Mg had a significant positive association with both REIP and NDRE, with Ca more closely correlated than either Mg or K. REIP was more closely associated with Ca ($R^2 = 0.72$; RMSE=0.02 for the MS site and $R^2 = 0.57$; RMSE=0.02 for the HS site) than NDRE. This suggests that REIP has a great potential to detect structural variations of wheat genotypes in sodic soil environment. Furthermore, Ca was also significantly (p<0.0001) and positively correlated with $CI_{rededge}$ at both sites with $R^2 = 0.53$ and 0.51 for the MS and HS site. This suggests that plant structural variations in sodic soil can regulate leaf chlorophyll concentration and, in turn, photosynthetic activities. Overall, results demonstrate that hyperspectral sensing can be efficiently used to detect plant Ca, Mg, and chlorophyll concentrations. The study improves understanding of genotypic nutrient variation for tolerance to different levels

* Corresponding author.
E-mail address: m.roychoudhury@uq.net.au (M. Roy Choudhury).

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of sodic soil conditions using optical properties of plant structure and can be beneficial to the plant science community for developing new approaches to study plant physiology.

1. Introduction

Sodic soils adversely affect 581 million ha of land worldwide, and Australia has the most extensive coverage of sodic soils in the world (340 million ha) (Rengasamy, 2016). In Australia, soils are classified as sodic when exchangeable sodium percentage (ESP) in soil is >6%, and highly sodic when ESP is >15% (Northcote and Skene, 1972). Sodicity causes serious soil structural degradation, aeration problems, and restricted water movement that can reduce root growth, plant water, and nutrient uptake, and seedling emergence (Anzooman et al., 2018; Rengasamy, 2002). The co-occurrence of salinity in sodic soils, particularly sodic subsoils, can also hinder plant nutrient uptake due to its osmotic effects and the presence of toxic concentrations of ions, such as chloride (Cl), that reduce crop growth and yield (Dang et al., 2008).

Calcium (Ca), magnesium (Mg), and potassium (K) are important cationic elements responsible for structural development, photosynthetic activity, and abiotic stress resistance of crops (Hasanuzzaman et al., 2018; Karley and White, 2009; Tränkner and Jamali Jaghdani, 2019). Plants grown in sodic soil can suffer due to macronutrient deficiencies including Ca, Mg, and K (Naidu and Rengasamy, 1993; Sugar Research Australia Ltd, 2020). Severe Ca deficiency (<1 mM) adversely impacts plant development, and especially cell wall structure, resulting in reduced plant growth with necrosis (spotted leaves) (Dang et al., 2016; Reuter and Robinson, 1997), which may affect photosynthesis by reducing leaf chlorophyll concentration. Mg plays a vital role in plant metabolism including chlorophyll synthesis; therefore, deficiency of Mg could reduce leaf chlorophyll concentration (Karley and White, 2009; Tränkner and Jamali Jaghdani, 2019). A K deficit plant is more susceptible to abiotic stress (Cakmak, 2005; Hasanuzzaman et al., 2018). Thus, the adequacy of these macronutrients in plant tissue is essential for crop healthy growth and productivity. Nutrient deficiency is one of the major constraints to the growth and development of wheat in the sodic soil-dominated northern grains growing regions of Australia (Dang et al., 2016).

Evaluation of plant nutrient concentrations before flowering and/or grain filling can provide useful insights into the susceptibility and tolerance to sodic soil constraints (Dang et al., 2016). However, conventional approaches to diagnosing crop nutrient and chlorophyll status involve determining total elemental content in plant tissues. These methods are time-consuming, tedious, and expensive, requiring destructive sampling of plant parts and laboratory analyses (Li et al., 2005). In contrast, high resolution remote sensing techniques may provide a promising approach for diagnosing plant nutrient status using optical properties of plant leaves, which is non-destructive, as well as time and labour efficient. A Study reported that red edge wavelengths (680–750 nm) can be useful to detect plant Ca deficiencies on non-sodic soils (Li et al., 2005). In addition, studies reported that healthier and well-structured plants have greater contrast in reflectance in red edge wavelengths, creating an abrupt ascending slope between 680 and 750 nm and that can be used to detect plant Ca concentration in non-sodic soils (Clevers et al., 2002; Tian et al., 2011). While a few studies have been reported the utility of red edge wavelengths to understand crop health, structural variations, etc., this has increased the imperative that how these techniques can be used to detect genotypic macronutrient (Ca, Mg, and K) and chlorophyll variations and that too in a sodic soil environment where soil constraints may have variable genotypic impacts on health. Thus, there is potential to use hyperspectral remote sensing to determine genotypic macronutrient and chlorophyll variations on sodic soil.

A number of red edge parameters can be computed to critically examine crop condition, health, and structural development (Ju et al., 2010). A study used a red edge inflection point (REIP) as the maximum first derivative of reflectance spectra to identify optical features from leaf reflectance that provides potential insights into leaf structural changes and chlorophyll status for the detection of Ca deficiency on non-sodic soils (Li et al., 2005). The REIP describes the maximum slope in reflectance of the red edge region (680–750 nm). When the REIP is shifted towards longer wavelengths, this usually indicates healthy plants (Mutanga and Skidmore, 2007; Velichkova and Krezhova, 2019). Thus, REIP is considered a useful red edge parameter (Boochs et al., 2007; Li et al., 2005; Tian et al., 2011) that may be used to evaluate genotypic macronutrient variations, in turn, variations in health, stress tolerance, photosynthetic activity, and structural development on sodic soils. In addition, studies report that a normalized red edge vegetation index (NDRE) and the red edge chlorophyll index ($Cl_{red\ edge}$) can also be useful indicators to quantify vegetative health, physiological changes, and chlorophyll concentration on non-sodic soils (Gitelson et al., 2005; Li et al., 2005; Micasense, 2014). Hence, these red edge parameters can be comprehensively used to study genotypic macronutrients and chlorophyll variations and/or deficiencies in constrained sodic soil environments.

The present study aimed to test whether hyperspectral remote sensing can be used to quantify macronutrient (Ca, Mg, and K) and chlorophyll variations of different wheat genotypes grown on different levels of sodic soil constraints and improve our understanding of genotypic tolerance to sodic soil conditions by reducing the need for expensive, labour-intensive, and tedious manual plant sampling to determine crop nutrients and chlorophyll. We experimented with eighteen wheat genotypes on a moderately sodic (MS) and a highly sodic (HS) soil site in north-eastern Australia and used high resolution narrow-band hyperspectral sensing to examine genotypic nutrient and chlorophyll variations on sodic soil. We hypothesized that sodic soil constraints can affect crop nutrients uptake, photosynthetic activity, and structural development and if hyperspectral sensing could quantify the smallest differences in genotypic nutritional and chlorophyll concentrations to the different levels of sodic soil conditions that may improve our understanding to study plant growth, physiological properties, and tolerance to sodic soil using a non-invasive and non-destructive way. The specific objectives of this study were: (1) to quantify the impact of sodic soil on crop nutrient status with relevance to structural development, (2) to evaluate the performance of hyperspectral red edge spectral parameters in remote detection of crop macronutrient (Ca, Mg, and K) and chlorophyll concentration on sodic soils, and (3) to differentiate wheat genotypes performance to different levels of sodic soil constraints to help identify tolerant genotypes to soil constraints.

2. Materials and methods

2.1. Site selection and experimental setup

Two experiment sites were located on MS (28.15°S and 150.22°E) and HS (28.08°S and 150.15°E) soils near Goondiwindi in north-eastern Australia (Das et al., 2021). The sites were located at an average elevation of 268 m from mean sea level and have well-structured Gground samplesrey Vertisol soils with high clay content and good water holding capacity. Both sites have adequate and mostly similar concentrations of soil nutrients from 0–150 cm depth (Das et al., 2021), which is favourable for crop production. However, studies reported that the availability of these nutrients for crops can be strongly restricted by sodic soil constraints, particularly in the presence of chloride (Cl) toxicity in the subsoil in this region (Dang et al., 2008, 2019). In this region of Australia, the winter wheat cropping season is between May and October. Seasonal (May to October 2018) air temperature varied between ∼5° and ∼35 °C for both the experiment sites with a similar seasonal mean air temperature of 14.7 °C and 14.9 °C and in-season rainfall of 86.2 and 85.6 mm for the MS and HS site, respectively (Das et al., 2021).

We used a randomized complete block design (RCBD) at both experiment sites, each with eight replications. Four replications (i.e., 72 plots) were designed for destructive sampling and plant biophysical measurements close to flowering, 110–112 days after sowing (DAS), and another four for grain yield (72 plots) measurements at maturity (152 DAS) (yield data not reported in this study). At each experiment site, 18 different wheat genotypes were tested (Supplementary Table 1) (Das et al., 2021), making a total of 144 plots (4 columns and 36 rows) (Supplementary Figure 1). Each plot was 5 × 2 m with five planting rows and 30 cm spacing between each other. Crops were sown on 24th May 2018 and harvested on 2nd November 2018.

2.2. Soil sampling

A relatively uniform area for soil constraints was selected for the experiments at both MS and HS sites based on acquired apparent electrical conductivity (ECa) readings. Soil samples were collected prior to sowing from a minimum of eight locations within each selected area to determine soil moisture availability (Das et al., 2021). At each sampling point, volumetric water content was determined from a 50-mm diameter soil sample collected to 150 cm using a hydraulic soil sampling rig (Dang et al., 2019; Das et al., 2021). The soil samples were dried at 40 °C and ground to pass through a 2 mm sieve. In a 1:5 soil water suspension, pH, electrical conductivity (EC), and Cl were measured using standard methodology (ISO, 2005). The EC of saturated extract (EC_{se}) was computed from EC 1:5 and clay content ratio (Shaw, 1997). Exchangeable Na^+ and cation exchange capacity (CEC) were measured using a 1 M NH_4Cl (pH 8.5) extraction solution (Tucker, 1985) and ESP was calculated from exchangeable Na^+ relative to CEC. The volumetric moisture content in percentage was calculated by multiplication of gravimetric soil moisture content with bulk density (BD) of the soil (Das et al., 2021). The soil physico-chemical characteristics for the MS and HS sites are illustrated in our earlier studies (Das et al., 2021; Roy Choudhury et al., 2021).

2.3. Plant biophysical measurements

Plant Ca, Mg, Na, and K concentrations were determined from destructively sampled youngest mature leaf (YML) at close to flowering (110–112 DAS). Earlier studies reported that a close to the flowering is the most crucial time to measure plant biophysical, spectral, and nutrient concentrations in sodic soil environments that can be used to differentiate crop/cultivars relative performance (Dang et al., 2019, 2016; Das et al., 2021; Roy Choudhury et al., 2019). A minimum of 50 young leaves was sampled from the middle three rows (3 × 0.5 m area) of each destructive sampling plot (72 plots) (Supplementary Figure 2). These leaves were washed with deionized water before oven drying at 70 °C and grinding to <0.5 mm. The ground samples were digested in a mixture of nitric (HNO_3 and perchloric ($HClO_4$) acids before measuring element concentrations using an inductively coupled plasma spectroscopy (ICP) (Munns et al., 2010; Svensson, 2017). A SPAD chlorophyll metre (SPAD-502, Minolta Co. Ltd., Osaka, Japan) was also used to measure plot-wise relative leaf chlorophyll concentrations close to flowering (110–112 DAS) on the destructive sampling plots. The readings were taken from the young mature leaves, with a minimum of 10 measurements taken in each plot to derive a plot average.

2.4. Canopy reflectance measurements and data processing

Canopy reflectance was measured using a hyperspectral spectroradiometer (ASD FieldSpec® HandHeld 2, Malvern Panalytical Ltd, USA) under the cloud-free, low wind, and sunny conditions between 9:00 and 15:00 hrs. Weekly in-situ monitoring of crop development was used to identify a date near flowering (110–112 DAS). A date near flowering was chosen since this is the most crucial time before grain filling when the canopy is most fully developed and likely to modify the reflectance most efficiently. The instrument was calibrated using a standard white spectralon calibration panel at each time of point shoot to reduce the effects of background reflectance on canopy spectral measurements. The continuous spectra were recorded in VIS to NIR from 325 nm to 1075 nm with ±1 nm accuracy and a resolution of <3 nm for each spectrum. In each plot, five spectral measurements of 0.4 m diameter area of the canopy were recorded from the middle three rows by locating the sensor in a horizontal position 0.5 m above the canopy cover. The measured canopy reflectance data was then exported into an ASCII file using ViewSpec Pro, an integrated package with RS^3 spectral acquisition software (Analytical Spectral Devices Inc., Colorado, USA) (Suarez et al., 2017). Subsequently, the raw data was exported into the R Studio statistical software platform and converted into hyperspectral data by using the hyperSpec package (Beleites, 2015). The pre-processing ('cleaning') was carried out using an excel spreadsheet program. Based on this, the bands introducing excessive variation into the data (901–1050 nm) and very short wavelength bands (325–399 nm) were excluded. Further, we performed a Savitzky–Golay derivative filter with the spectroscopic data at 400–900 nm in MATLAB R2020a (The Mathworks® Inc., USA) software platform for smoothening and removing noise from the data (King et al., 1999). Finally, the various spectral indices were derived from hyperspectral data for further analyses as described below.

2.4.1. Red edge inflection point (REIP)

The present study used a four-point linear interpolation technique (Guyot et al., 1992) to extract REIP from hyperspectral red edge wavelengths (680–750 nm) for 18 genotypes over two experimental sites. Previous studies have considered the four-point linear interpolation using four spectral bands (670, 700, 740, and 780 nm) as the most promising and simple approach to compute REIP (Baret et al., 1987; Cho and Skidmore, 2006; Velichkova and Krezhova, 2019). To determine the REIP in this study, the first derivative of reflectance spectra was determined first using Eq. (1) (Dawson and Curran, 1998). The maximum first derivative of reflectance spectrum in red edge region is used to determine REIP (Li et al., 2005). Therefore, using the maximum first derivative of reflectance for all the genotypes at both sites, we realized the REIP 1 using a four-point linear interpolation (Eq. (2)) might be more useful than REIP 2 or 3 for the current study. The REIP 1 using a range of hyperspectral wavebands (700, 740, and 780 nm) was also suggested to be a strong indicator of nitrogen status of winter wheat in non-sodic soil environments (Prey and Schmidhalter, 2019).

$$D_{\lambda_{(i)}} = \left(R_{\lambda(j+1)} - R_{\lambda_{(j)}}\right) / \Delta\lambda \tag{1}$$

$$REIP = (700\ \text{nm} + 40) \times \left[\frac{\left(\frac{670\ \text{nm}\ +\ 780\ \text{nm}}{2}\right) - 700\ \text{nm}}{(740\ \text{nm} - 700\ \text{nm})} \right] \tag{2}$$

where $D_{\lambda_{(i)}}$ represents the first-difference transformation at wavelength i midpoint between j and $(j+1)$. $R_{\lambda_{(j)}}$ and $R_{\lambda(j+1)}$ are the reflectance at wavelength j and $(j+1)$, respectively, and $\Delta\lambda$ is the difference in wavelengths between j and $(j+1)$.

2.4.2. Normalized difference red edge index (NDRE)

The NDRE was derived using near-infrared and red edge wavelengths from hyperspectral data using Eq. (3) (Fitzgerald et al., 2006). The range of NDRE values for vegetation canopies varies between 0 and +1, with a higher value indicating favourable greenness, chlorophyll concentration, and structural development of the plants.

$$NDRE = \frac{(R_{790} - R_{720})}{(R_{790} + R_{720})} \tag{3}$$

where R_{790} and R_{720} are the reflectances of wheat genotypes for the respective wavelengths of 790 nm (NIR) and 720 nm (red edge).

2.4.3. Red edge chlorophyll index ($Cl_{\text{red edge}}$)

$Cl_{\text{red edge}}$ was calculated using Eq. (4) from hyperspectral data (Gitelson et al., 2005) and correlated with ground-measured SPAD chlorophyll data to check the association between them.

$$Cl_{red\ edge} = (R_{750}/R_{710}) - 1 \tag{4}$$

where R_{750} and R_{710} are the reflectances of wheat genotypes for the respective wavelengths of 750 and 710 nm.

2.5. Statistical analyses

Various statistical analyses including t-test, analysis of variance (ANOVA), principal component analysis, and stepwise regression were performed in RStudio 3.6.2 (RStudio, PBC) and MATLAB R2020a (The Mathworks® Inc., USA) software platforms.

A paired sample t-test was used to understand whether any significant differences exist between the soil constraints and the site means of Na, Ca, Mg, and K concentrations at the MS and HS sites. A two-way ANOVA was performed to determine if there are any significant interactions between the genotypes at MS and HS sites based on REIP, NDRE, and nutrient concentrations. The testing of significant differences between levels within each factor was performed using Fisher's protected least significant difference (LSD) test at the 5% significance level. The variables were treated as nested effects, while replicate was fitted as a random effect.

We performed principal component analysis (PCA) to reduce data redundancy between response (plant nutrients) and predictor (red edge spectral parameters) variables before performing stepwise regression analysis. A stepwise regression analysis was performed using 'n' standardization with a 5% significance level to test for correlations between red edge spectral parameters and plant nutrient concentrations in the leaves. A 10-fold cross-validation method was used to validate the results in the regression analysis. A test of significance at $p < 0.05$ from '0' was used to compare the results between variables. The accuracy of the regression models was assessed using the coefficient of determination (R^2) and root mean square error (RMSE) in the cross-validation.

3. Results and discussion

3.1. Impacts of sodic soil constraints on plant nutrient concentration

The depth-wise distribution of soil physico-chemical constraints suggested that both sites have higher ESP in the subsoil (18%–20% for the MS and 23%–27% for the HS site at 100–150 cm) than the surface soil (ESP of 2.7% for the MS and 14% for the HS site at 0–10 cm). The profile ESP of the HS site was also significantly higher ($p < 0.05$) than the MS site. In addition, both sites have a high Cl concentration in the subsoil at 100–150 cm depth, with concentrations substantially higher ($p < 0.001$) at the HS site (>2300 mg/kg) compared to the MS site (700–750 mg/kg). High ESP and Cl concentrations can restrict nutrient uptake by roots, by reducing the rooting depth (Dang et al., 2008, 2019). It would be anticipated that the higher subsoil ESP and Cl at the HS site would have a greater impact on nutrient uptake by crops than at the MS site. However, crops at both sites might have grown with restricted nutrient availability.

The greater impact of the conditions at the HS site on plant growth was supported by a paired sample t-test, which showed that leaf Na, Ca, Mg, and K concentration (%) were significantly different ($p < 0.0001$) between the MS and HS sites (Fig. 1). The mean leaf Na concentration for the HS site (∼0.05%) was significantly higher than the MS site (∼0.02%) (Fig. 1a). The previous study has shown the accumulation of Na concentration in the leaf tissue of Australian wheat genotypes is <0.1% (Liu et al., 2000), with Na > 0.1% adversely impacting plant physiology (Dang et al., 2019; Munns et al., 1988). Therefore, our results indicate that Na toxicity was unlikely to be limiting wheat growth at either site. The mean Ca concentration in the leaf tissue at the MS site was ∼0.35% and at the HS site was ∼0.27% (Fig. 1b). Most of the genotypes at the HS site had <0.25% Ca, which is below the critical limit (0.25%) of leaf Ca concentration (Dang et al., 2019, 2016), suggesting inadequate structural development of crops was likely to have developed on the HS site due to Ca deficiency.

The mean Mg concentration in the leaf tissue at both sites was adequate and higher than the critical limit (>0.15%) (Snowball and Robson, 1991). We observed that most of the wheat genotypes at the MS site had >0.22% Mg in their leaf tissue (Fig. 1c), which was significantly higher ($p < 0.0001$) than the HS site (∼0.17%). Considering the role of Mg in plant metabolism including chlorophyll synthesis (Karley and White, 2009; Tränkner and Jamali Jaghdani, 2019), the results suggest that wheat genotypes at the MS site might have grown with greater chlorophyll concentrations and thus, photosynthetically more active compared to the HS site. The mean K concentrations in the leaf tissue were ∼2.5% and ∼1.7% for the MS and HS sites, respectively (Fig. 1d), with the genotypes at the MS site having a significantly higher K uptake than the HS site. Most of the genotypes at the HS site had K concentrations in the leaf tissue of <1.5%, which is below the critical limit (Snowball and Robson, 1991). This suggests that wheat genotypes grown at the HS site might be more susceptible to abiotic stress compared to the MS site due to poor K uptake (Hasanuzzaman et al., 2018). Coskun et al. (2017) suggested K deficiency also interrupts other nutrients, including Ca and Mg translocation and source–sink relationship. A recent study also demonstrated that high sodic soil constraints can significantly reduce wheat K uptake than a moderately sodic soil that increased plant water stress (Das et al., 2021). Overall, this study demonstrates that high levels of sodic soil constraints can significantly reduce the availability of essential plant nutrients by reducing uptake.

3.2. Spectral signature curve analysis of wheat genotypes on sodic soils

Spectral reflectance curves of the wheat genotypes at the MS and HS sites using the far red to NIR wavelengths (650–900 nm) (Fig. 2a and b) showed variations among the genotypes. A steep ascending reflectance slope was clearly observed in the red edge region (680–750 nm). Well-structured genotypes reflect more to create a maximum slope and shift towards

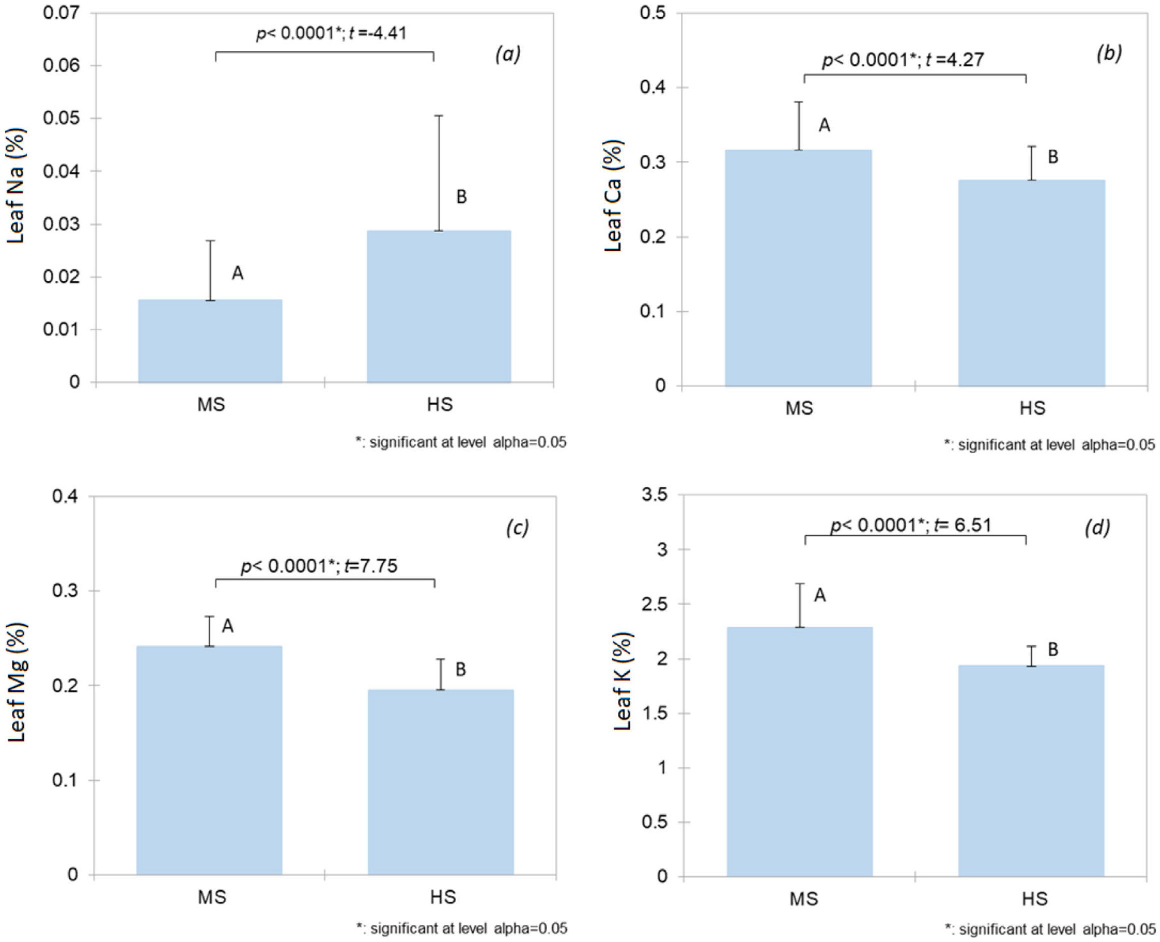

Fig. 1. Site interactions of the mean leaf nutrient concentrations of 18 wheat genotypes between a moderately sodic and highly sodic soil for leaf nutrients; Na (a), Ca (b), Mg (c), and K (d) in (%). The error bars represent the standard deviation of the mean of 72 plots (18 genotypes with 4 replications each). A significant difference between the site means is shown using different letters (^{A,B}).

longer wavelengths (>750 to 900 nm). Although by visual observation, the mean spectra of some of the wheat genotypes showed some overlaps, especially between 690 and 720 nm, furthermore, ANOVA results showed significant differences ($p < 0.05$) in spectral reflectance of all the genotypes between 710 to 900 nm at the MS and 723 to 900 nm at the HS site. Overall, we identified that *Lancer, Mitch, Janz*, and *Gregory* strongly reflected in red edge and NIR regions, and *Flanker, Wallup*, and *Emu Rock* had a comparatively weaker reflectance.

Significant differences in reflectance spectra of rice genotypes and grapevine species from red edge to NIR region have also been reported on non-sodic soils (Das et al., 2018; Maimaitiyiming et al., 2016). Our results showed a relatively less steep reflectance slope created by the genotypes at the HS site than at the MS site. Thus, significant differences occurred between the genotypes at the HS site at longer wavelengths (>723 nm) compared to the MS site (710 nm). Overall, genotypic spectral observation supports the suggestion that wheat genotypes grown at the HS had comparatively weaker reflectance than those at the MS site, likely due to the adverse impacts of high soil constraints. Hence, to quantify the impacts of variable sodic soil constraints on genotypic nutrient concentration and structural development, we further analysed genotypic response in red edge spectral indices at both sites (below in Section 3.3).

3.3. Genotypic response in red edge spectral indices on sodic soils

The REIP of the 18 wheat genotypes varied between 722–728 nm at the MS site and 719–725 nm at the HS site (Fig. 3a). Significant differences ($p < 0.05$) were observed between fifteen wheat genotypes based on REIP at site interactions between MS and HS sites. However, wheat genotypes *Flanker, Trojan,* and *Wallup* were not significantly different ($p > 0.05$). The first derivative of reflectance spectra for 18 genotypes showed a first prominent and maximum peak at ~729 nm for the MS site and ~718 nm for the HS site (Supplementary Figure 3). The maximum first derivative spectra, indicating REIP, divides the entire red edge area by far red (670 nm) and NIR (760 nm) lines (Supplementary Figure 3), suggesting

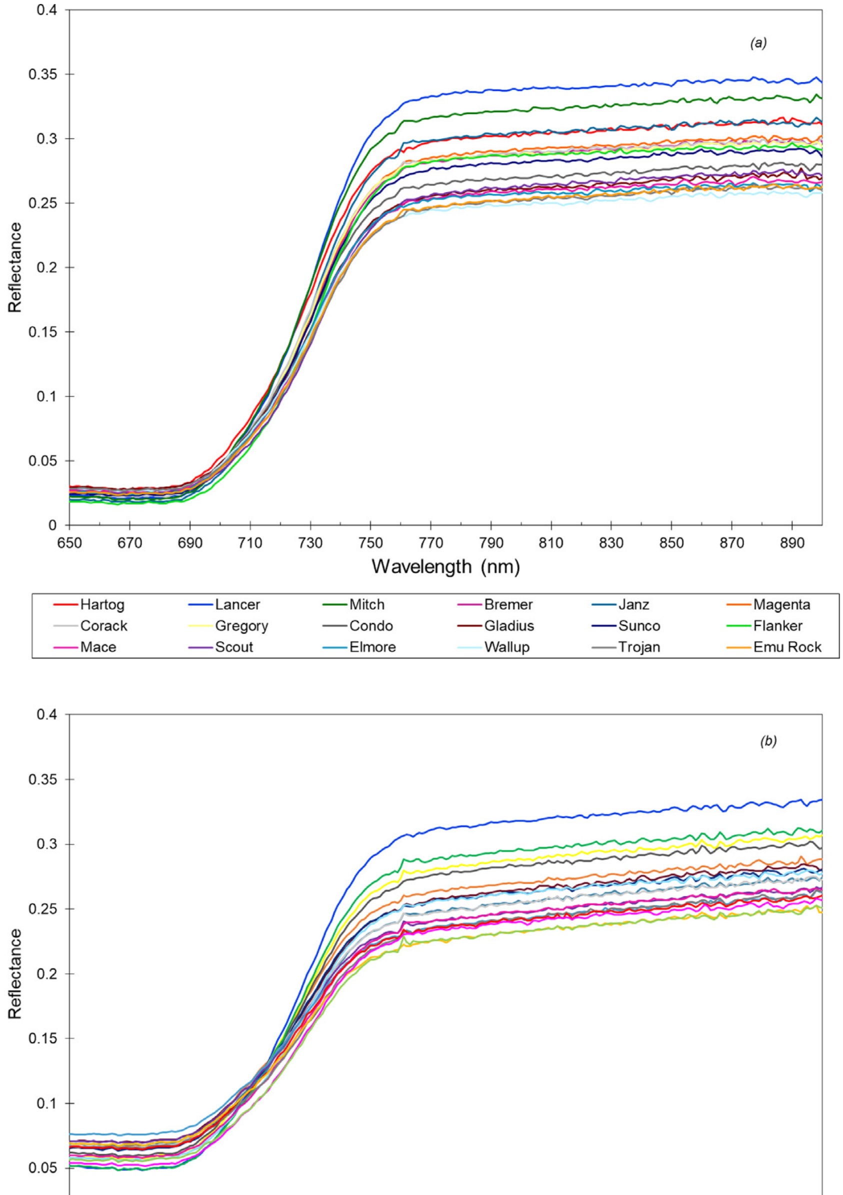

Fig. 2. The spectral signature curve for 18 wheat genotypes at moderately sodic (a) and highly sodic (b) sites. The data shows the mean reflectance of each genotype with four replications for each of 670–900 nm, including red edge and NIR wavebands.

a significant shift of healthier genotypes towards longer wavelengths. A first derivative reflectance peak at a relatively higher wavelength at the MS site than the HS site, clearly suggests that more shifting of healthier genotypes towards longer wavelengths. A greater REIP position at the MS site than the HS site may also indicate a greater accumulation of leaf chlorophyll concentrations and photosynthetic activities by the genotypes.

Previous studies have found the effectiveness of red edge position to determine heavy metal contamination in river flood plains, leaf chlorophyll, and/or chlorophyll fluorescence (Clevers et al., 2010; Ju et al., 2010; Zarco-Tejada et al., 2003). Studies also found REIP as a useful indicator of leaf chlorophyll concentration on non-sodic soils (Filella and Penuelas,

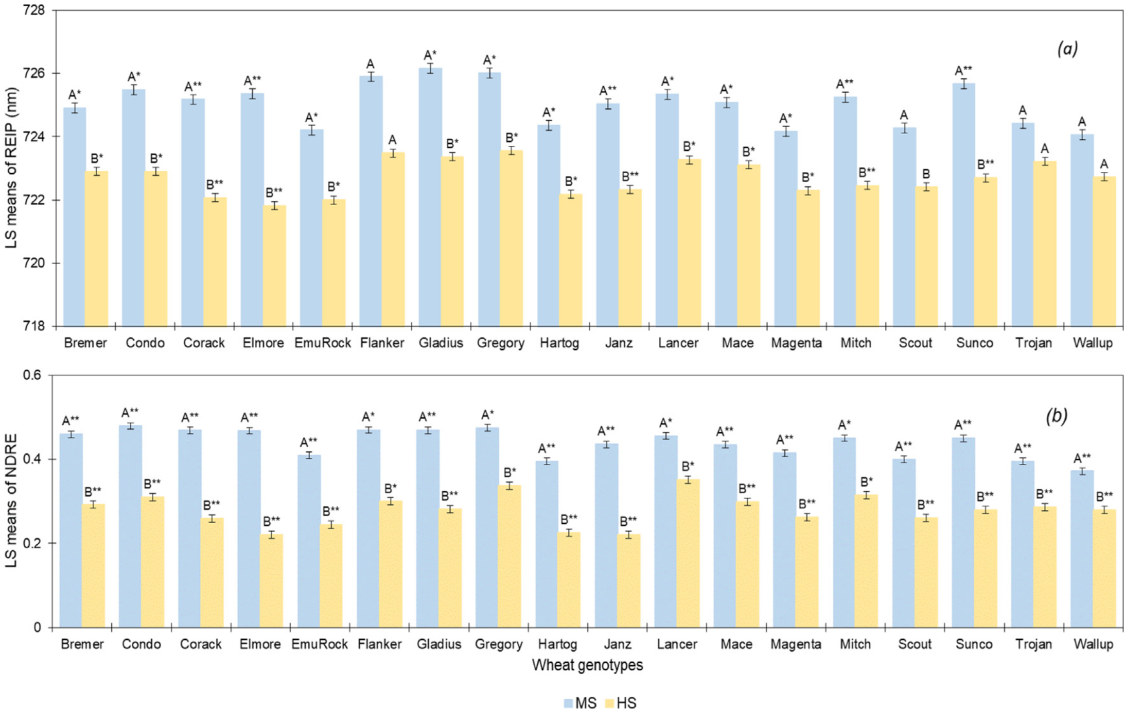

Fig. 3. Least significant (LS) means of REIP (a) and NDRE (b) for 18 wheat genotypes for a moderately sodic and highly sodic site measured close to flowering. The error bars show the standard error of the mean for genotype with four replications in each. A significant difference between the sites for each genotype is shown using different letters (^{A,B}) and the probability of their interactions was indicated using asterisks at $p < 0.01^{**}$, $< 0.05^{*}$

2007; Li et al., 2005). Our study demonstrates that REIP can be used to differentiate genotypic responses in sodic soil environments.

The NDRE also indicated that the wheat genotypes at the MS site were healthier (mean NDRE = ∼0.46) and significantly better ($p < 0.05$) than those at the HS site (mean NDRE = ∼0.27) (Fig. 3b). Researchers suggested that NDRE can be more useful indicator than a normalized difference vegetation index (NDVI) to determine leaf chlorophyll status since red edge can penetrate deeper into the leaves than visible 'red' wavelength, therefore pigments detection sensitivity is higher than NDVI (Fitzgerald et al., 2006; Li et al., 2014). NDRE also overcomes the saturation problems of NDVI and is less sensitive to background reflectance (Li et al., 2014; Nguy-Robertson et al., 2012). Eitel et al. (2010) found the usefulness of red edge spectral variables to estimate leaf chlorophyll content ($R^2 > 0.73$) on non-sodic soils. Overall, results indicate that wheat genotypes; *Flanker, Gladius, Gregory, Lancer, Mace, Trojan,* and *Sunco* had a greater response in both REIP and NDRE compared to the others and suggest that the REIP and NDRE may further be used to determine crop structural, nutritional, and chlorophyll variations on sodic soils.

3.4. Determination of crop nutrient concentrations using hyperspectral red edge parameters

PCA results showed a positive association between REIP and NDRE with Ca, Mg, and K concentrations for both the MS and HS sites (Fig. 4a and b). A PCA was tested against five components (PC1 to PC5) at each experimental site. At the MS site, the first two principal components PC1 and PC2 contributed with 70.7% and 15.9%, respectively, which together explained 86.6% of the data variability in the model. The eigenvalues were 3.53 and 0.79 for PC1 and PC2, respectively. Whereas, at the HS site, PC1 and PC2 contributed with 59.9% and 17.1%, respectively, which together explained 77.0% of the data variability in the model. The eigenvalues were 2.99 and 0.85 for PC1 and PC2, respectively. The high proportion of data variability explained in PC1 and PC2 (>75%) at both sites suggests that the variability of crop nutrients data can further be effectively explained as a function of red edge spectral parameters using a linear and/or stepwise regression model.

The REIP showed a relatively closer association with leaf nutrients compared to NDRE for both sites with $R^2 = 0.72$ and 0.57 for Ca; $R^2 = 0.50$ and 0.34 for Mg; and $R^2 = 0.15$ and 0.10 for K at the MS and HS sites, respectively (Fig. 5). Moreover, stepwise regression results (Table 1) showed that leaf Ca was closely and significantly ($p < 0.01$) correlated with both REIP and NDRE for both sites. Mg was also significantly correlated with REIP at both sites, however, the correlation with NDRE was only significant at the MS site. Further, leaf K concentration was not significantly associated ($p > 0.05$) with

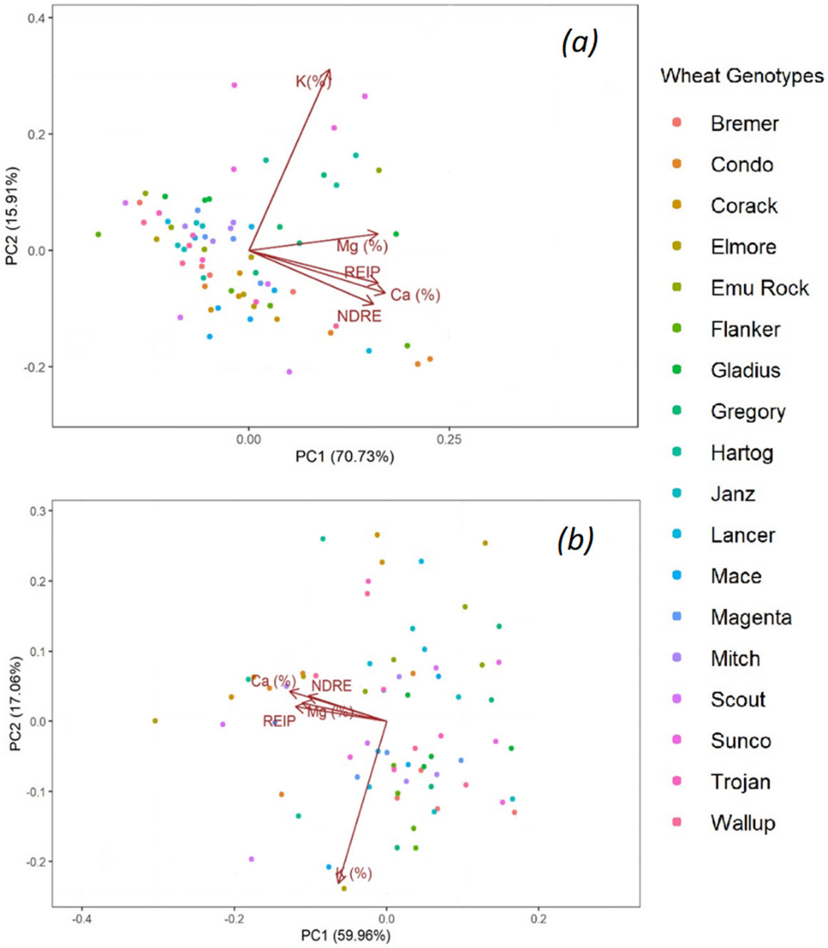

Fig. 4. PCA biplot with active observations (genotypes) and variables (NDRE, REIP, Ca, Mg, and K) showing the association between the variables and observations for the first two principal components (PC1 and PC2); n = 72 (four replicates of 18 wheat genotypes); (a) moderately sodic site and (b) highly sodic site.

Table 1
Stepwise regression variables and cross-validation between red edge parameters and leaf nutrient concentrations at the moderately sodic and highly sodic sites.

Sites	Variables	Ca (%)			Mg (%)			K (%)		
		RMSE (%)	*F*	*p*-value	*RMSE (%)*	*F*	*p*-value	*RMSE (%)*	*F*	*p*-value
MS	REIP	0.02	52.7	<0.0001	0.02	16.0	**0.001**	0.3	4.1	0.06
	NDRE	0.03	28.6	<0.0001	0.02	8.8	**0.004**	0.4	0.29	0.58
HS	REIP	0.02	42.4	<0.0001	0.02	20.6	<0.0001	0.1	3.5	0.07
	NDRE	0.03	9.6	**0.003**	0.03	0.34	0.56	0.1	0.36	0.55

Values in **bold** are different from '0' with a significance level alpha <0.05; n = 72.

NDRE or REIP at either site. The results suggest that of the parameters tested, REIP is the superior indicator of variations in crop nutrient concentration, particularly Ca and Mg, with closer association with Ca in a sodic soil environment. As Ca is an important driving factor of the structural development of crops, REIP, thus, has a strong potential to detect structural variations of genotypes on sodic soil.

Estimates of leaf Ca concentration were derived from a linear function of REIP data and were compared between sites. Furthermore, the ANOVA results (Fig. 6), indicate that leaf Ca concentration of most of the wheat genotypes at the MS site was significantly higher ($p < 0.05$) than those at the HS site. These results confirm the adverse impacts of high sodic soil

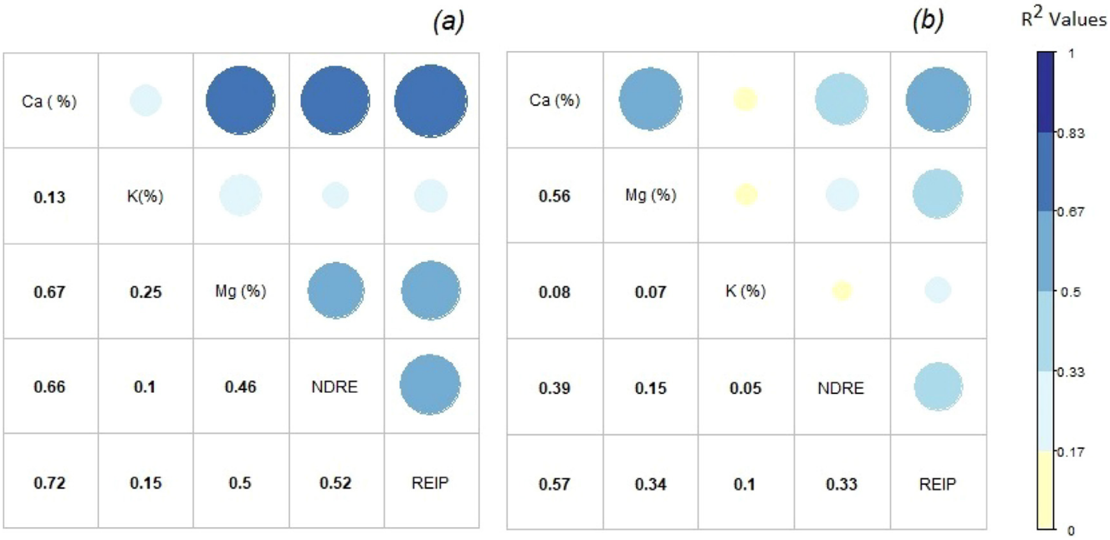

Fig. 5. Relationship between red edge parameters with leaf nutrient concentrations at moderately sodic (a) and highly sodic (b) sites; n = 72. The matrix shows the coefficient of determination (R^2) values between the variables below the diagonal as well as indicated by colour scale (above the diagonal). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

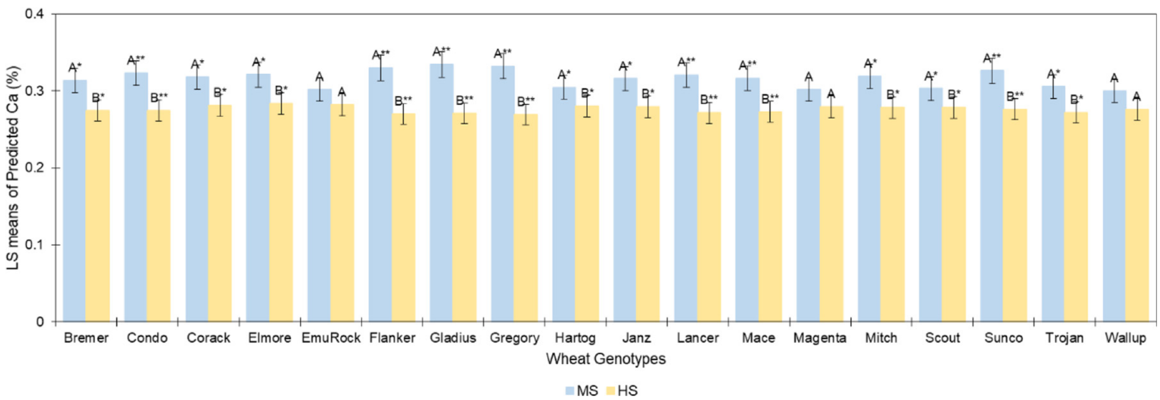

Fig. 6. Least significant (LS) means of estimates of leaf Ca concentration predicted using a linear function of REIP at a moderately sodic and highly sodic site during close to flowering. The error bars show the standard error of the mean for four replications of each of 18 genotypes. A significant difference between the sites for each genotype is shown using different letters (^{A,B}) and the probability of their interactions was indicated using asterisks at p < *0.01***, < *0.05**

constraints on genotypic macronutrient concentrations in plant tissue, especially Ca availability and/or deficiency and also demonstrate the potential of red edge position or REIP to detect the smallest differences of genotypic structural variations due to the changes in leaf Ca to different levels of soil sodicity.

3.5. Relationship between crop structural variation and chlorophyll concentrations on sodic soil

We derived $Cl_{red\ edge}$ from hyperspectral red edge wavelengths and then related these with leaf Ca concentrations. The correlations between $Cl_{red\ edge}$ and REIP, and $Cl_{red\ edge}$ and Ca both showed significant ($p < 0.05$) positive associations for both sites (Fig. 7). For REIP, R^2 was 0.43 for the MS site, and 0.38 for the HS site (Fig. 7a and b), while for Ca, $R^2$was 0.53 for the MS site and 0.51 for the HS site (Fig. 7c and d). In addition, hyperspectral derived $Cl_{red\ edge}$ index was closely correlated ($p < 0.001$) with ground measured SPAD chlorophyll data for both sites with R^2 of 0.54 for the MS site and 0.49 for the HS site. A previous study also identified a good association between $Cl_{red\ edge}$ and canopy chlorophyll concentrations for a wheat crop ($R^2 = 0.85$; n = 24) on non-constrained soil (Wu et al., 2009). Further, Zhang et al. (2011) found a reasonable positive correlation between SPAD values and a hyperspectral red edge reflectance index ($R^2 \sim 0.5$) for rice crop on non-sodic soils. In this study, although the correlation between $Cl_{red\ edge}$ index and SPAD values was reasonable and statistically significant for both the sites, we could not achieve a R^2 of >0.55. This might be site-specific and/or

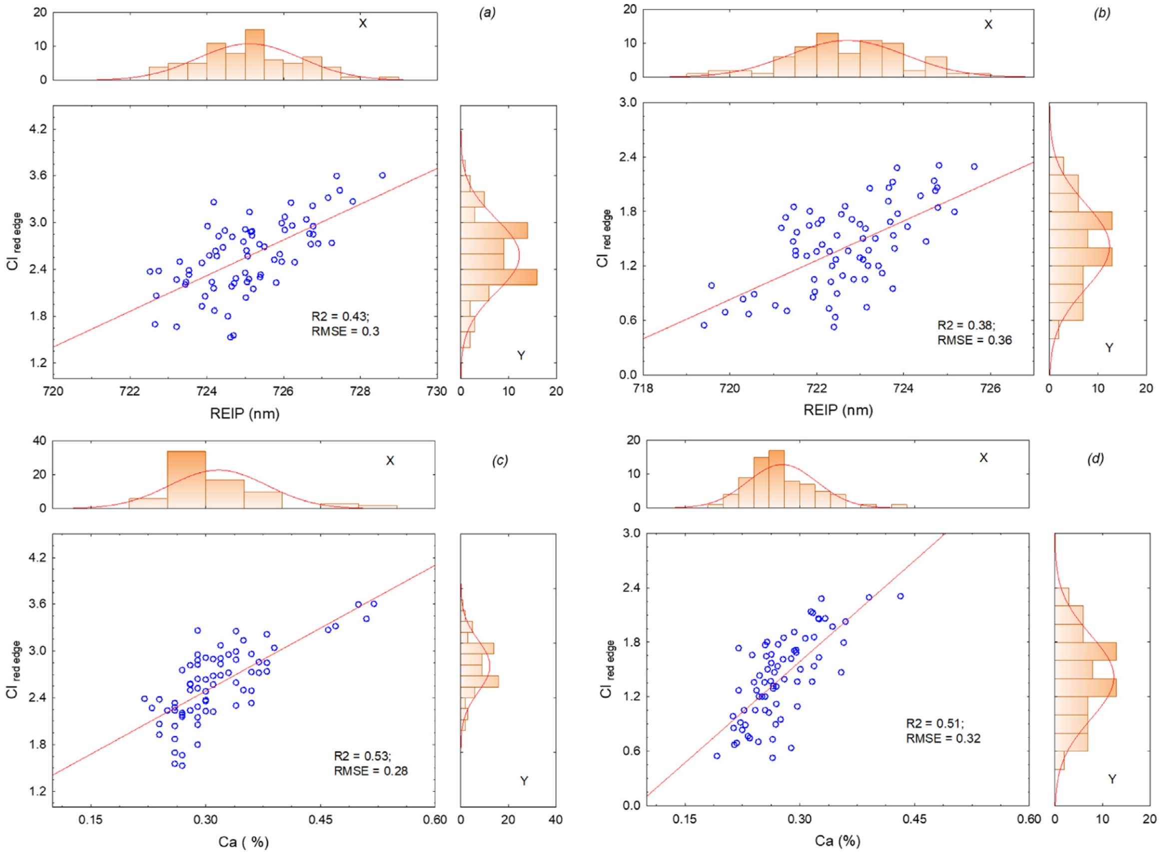

Fig. 7. Relationship between $Cl_{red\ edge}$ index with REIP and leaf Ca concentrations at a moderately sodic site (a) and (c); and a highly sodic site (b) and (d). Histograms indicate the distribution of data of X and Y variables in the graph; n = 72.

environment-specific or could be due to the variations in data points used for the correlation between earlier studies and our current study. It was also observed that the hyperspectral derived $Cl_{red\ edge}$ index was slightly better correlated with leaf Ca concentrations (R^2 = 0.53 and 0.51 for the MS and HS site, respectively) (Fig. 7c and d) than ground measured SPAD chlorophyll data (R^2 = 0.38 and 0.33 for the MS and HS site, respectively) (Supplementary Figure 4).

Previous studies report that the variable positions of REIP and shifting of leaf chlorophyll concentration with the changes of REIP, i.e. ~700 nm (low chlorophyll content), ~720 nm, ~725 nm, and ~735 nm (high chlorophyll content) on non-sodic soils (Boochs et al., 2007; Cho and Skidmore, 2006; Clevers et al., 2010; Tian et al., 2011). Likewise, we observed that a higher chlorophyll concentration was associated with the shifting of REIP to longer wavelengths on constrained sodic soils. A reasonable to good inter-relationship between plant Mg and chlorophyll concentrations were acknowledged by previous studies for crops, such as wheat, sunflower, and strawberry in non-sodic soil environments (Choi and Latigui, 2008; Tränkner and Jamali Jaghdani, 2019). Here, we also observed a significant positive ($p < 0.05$) correlation between Mg and chlorophyll concentrations of wheat on sodic soils (R^2 = 0.32 and 0.30 for the MS and HS site, respectively) (Supplementary Figure 5). This study, further indicated that plant Ca availability and variations can also have a significant and even slightly greater influence on leaf chlorophyll concentrations than Mg in sodic soil environments. As Ca plays a key role in the structural development of crops, it is, thus, determined that leaf chlorophyll concentrations and/or photosynthetic activities on sodic soil can be heavily reliant on crop structural growth. This also suggests likely differences in potential photosynthetic activities between different levels of sodic soil constraints, although this was not directly measured.

Overall, results suggest that a higher level of sodic soil constraints can significantly reduce plant nutrient and chlorophyll concentrations and restricted structural development and photosynthetic activities, more than a moderately sodic soil. As photosynthesis is related to primary productivity, it is thus clear that the primary productivity of crops in a sodic soil environment can be highly restricted by soil constraints. The results also indicated that hyperspectral red edge wavelengths have the potential to detect plant nutrients, especially Ca and Mg, and chlorophyll concentrations, suggesting there might be a reduced need for ground-measured and laboratory-based expensive, labour-intensive, and tedious process of determining plant nutrients and chlorophyll concentrations. The approach used here was sensitive to the effect of high levels of sodic soil constraints, which significantly reduce crop nutrient uptake from the soil, relative to moderately sodic soil, which leads to reduced crop development. Although the study successfully demonstrated the potential of narrow-band hyperspectral sensing and red edge wavelengths to provide insights into wheat genotypic chlorophyll and nutritional variations/deficiencies and their inter-relationships in sodic soil environments, using a greater diversity of crops and/or cultivars with multiple environments including non-sodic soils may provide more comprehensive outcomes while determining plant chlorophyll and nutritional properties.

4. Conclusions

This study aimed to test whether hyperspectral remote sensing can be used to determine macronutrient and chlorophyll variations of wheat genotypes grown on sodic soils and thus, improves our understanding of genotypic differences and tolerance to different levels of sodic soil constraints by reducing the need for expensive, labour-intensive, and tedious manual plant sampling to determine crop nutrients, chlorophyll, and health.

The study considered the elemental composition and chlorophyll concentrations of wheat genotypes grown on sodic soils, and demonstrated that hyperspectral sensing can be used to accurately detect and quantify variation in leaf nutrient, particularly Ca, Mg, and chlorophyll concentrations. Results support the proposition that observing red edge shift position towards longer wavelengths and quantifying genotypic responses to that shifting is useful to differentiate plant structural, nutritional, and chlorophyll concentration using plant optical properties. In addition, REIP, as a red edge variable was identified to be a strong indicator of leaf Ca and chlorophyll in sodic soil environments. Follow-up studies should focus on implementing these techniques with a greater diversity of genotypes and a greater number of sodic sites to extend these results.

Overall, this study offers a promising, remote sensing-based approach to improve understanding of genotypic variations in sodic soil environments due to the variations in nutrient and chlorophyll concentrations in plant tissue, and structural development using optical properties of plant structure in a non-destructive way. The study can be beneficial to the researchers, working on the development of new approaches to advance the science of plant physiology using remote sensing techniques and may assist farmers and breeders in selecting cultivars tolerant to sodic soil constraints.

The key findings of this research are the following:

- Hyperspectral sensing could detect the reduction in wheat Ca, Mg, and chlorophyll concentrations resulting from sodic soil constraints.
- The REIP, as a red edge parameter, was more closely associated with leaf nutrients and chlorophyll concentrations than the NDRE on sodic soils.
- Leaf Ca concentration was more closely associated with red edge parameters than either Mg and K.
- Reduced structural development and leaf Ca correlation were both associated with reduced chlorophyll absorption. This suggests a possible reduction in potential photosynthetic activity and the primary productivity of the plants on highly sodic soil.

CRediT authorship contribution statement

Malini Roy Choudhury: Conceptualization, Data curation and processing, Methodology, Software, Validation, Visualization, Investigation, Writing – original draft. **Jack Christopher:** Data curation, Supervision, Reviewing and editing. **Sumanta Das:** Conceptualization, Methodology, Software, Validation, Reviewing and editing. **Armando Apan:** Data curation and processing, Supervision, Reviewing and editing. **Neal W. Menzies:** Conceptualization, Supervision, Reviewing and editing. **Scott Chapman:** Supervision. **Vincent Mellor:** Software, Validation. **Yash P. Dang:** Conceptualization, Supervision, Project administration, Resources, Reviewing and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.eti.2022.102469.

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