Monitoring cotton (*Gossypium hirsutum* L.) leaf ion content and leaf water content in saline soil with hyperspectral reflectance

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Abstract

The objectives of this study were to establish quantitative models for monitoring the leaf ion and water content under saline conditions. The best spectral indices for estimating leaf ion content and leaf water content were found to be normalized difference spectral indices (NDSI (R₁₃₄₀, R₂₃₀₆)), ratio spectral indices (RSI (R₂₃₀₆, R₁₃₄₇)) for K⁺, NDSI (R₁₃₄₆, R₂₂₇₆), RSI (R₂₂₇₆, R₁₃₄₃) for Na⁺, NDSI (R₁₃₈₀, R₂₂₇₇), RSI (R₂₂₇₇, R₁₃₅₀) for Ca²⁺; NDSI (R₁₂₀₀, R₂₂₁₁), RSI (R₂₂₁₁, R₁₃₆₁) for Mg²⁺; NDSI (R₁₁₅₄, R₂₃₁₇), RSI (R₂₃₁₇, R₁₁₅₄) for SO₄²⁻ and NDSI (R₁₂₂₂, R₂₂₆₄), RSI (R₂₂₆₄, R₁₃₂₃) for RWC, respectively. The regression models based on the above spectral indices were formulated with R² greater than 0.46. The high fit between the measured and estimated values indicate that the present models based on RSI could be used to estimate leaf ion and water content feasibly under saline conditions.

**Keywords:** cotton; saline soil; hyperspectral reflectance; ion content; leaf water content; monitoring model.

Introduction

Salinity is always an important threat to global agriculture. Currently, salinity stress is becoming even more prevalent as the intensity of land use increases in the world [Meloni et al., 2003]. The salt-induced water deficit is one of the major constraints for plant growth in saline soils. Nevertheless, it was recently reported that leaf area and fresh weight accumulation were significantly reduced by salinity [Heuer and Nadler, 1995]. In addition, ionic toxicity generated from salt contaminated soil has negative effects on plants growth and development [Munns et al., 2006]. Excess content of various ions associated with
salinity can cause enzyme inhibition, which alters metabolism or physiologic function [Flowers and Dalmond, 1992]. Therefore, determination of the physiological status can be used to detect and study plant stress and consequently has important potential implications on crop stress detection, agricultural field management and especially for precision agriculture practices. Analysis of the plants themselves can be used as indicator of the salinity effects on plants within the growth period; however, the destructive methods in used currently are time-consuming and cannot sufficiently reflect the spatial variability. Instead, remote sensing fulfills the demand of a rapid, accurate, and simple method for documentation of chemical composition in plant analysis [Pasquini, 2003]. Remotely sensing data have been widely used to develop vegetation indices as indicators of crop growth and yield development. Several studies have suggested that crop spectral reflectance can be used to detect abiotic and biotic environmental stress [Osborne et al., 2002], estimate plant growth and physiology [Serrano et al., 2000; Zhao et al., 2003], quantify the canopy characteristics of crops [Curran, 1989], and some researchers also found that leaf spectral reflectance increases in portions of the near-infrared range as a plant physiological stress experience [Carter, 1994; Hansen and Schjoerring, 2003]. Relative water content (RWC) is one measure of plant water status and is widely used [Pu et al., 2003]. Over the past decades, many studies have been used to directly estimate leaf parameters, like leaf relative water content (RWC) [Penuelas et al., 1993; Pu et al., 2003; Tian et al., 2001] using hyperspectral reflectance in non-saline soil. Similarily, Bowyer and Danson [2004] have showed that both NIR (near-infrared) and SWIR (short-wave infrared) ranges were necessary for retrieving leaf water status at the leaf level in non-saline soil. But, the ability of hyperpectral reflectance to estimate mineral contents and ratios has received less attention. Earlier works reporting near-infrared spectroscopy (NIRS) measurement of elements were mainly focused on analysis of hay samples [Clark et al., 1989; Clark et al., 1987; Saiga et al., 1989] and natural grass [Ruano-Ramos et al., 1999]. Cozzolino and Moron [2004] analyzed Na, S, Cu, Fe, Mn, Zn, and B in two types of legumes. Good results were also obtained in prediction of mineral in tobacco leaves [Wang et al., 2004]. However, most of the above studies were studied under non-salinity conditions, and less information is available in saline soil for estimating RWC and leaf ion content through remote sensing approach. So, it is necessary to develop new and accurate techniques for the remote estimation of RWC and leaf ion content under saline conditions.

In analysis of plant spectral data for extracting characteristic information on target objects many methods have been adopted, including vegetation parameters, and other derived functions, in attempt to minimize background noise and enhancing capacity of spectral information utilization and accuracy of estimating models [Claudio et al., 2006; Colombo et al., 2008; Jacquemoud and Baret, 1990; Wu et al., 2009]. However, among them, normalized difference vegetation indices (NDVI) and ratio vegetation indices (RVI) have been widely used for analyzing multispectral information in crop plants because they are constructed with simple form and for easy calculation [Claudio et al., 2006; Gao, 1996; Penuelas et al., 1997; Seelig et al., 2008; Yilmaz et al., 2008]. Therefore, further investigations are needed to explore consistent feature wavebands and to construct simpler and applicable vegetation indices (normalized difference spectral indices (NDSI), ratio spectral indices (RSI)) for more accurate monitoring models in RWC and leaf ion content estimation.

Thus, the study aims were (1) to define the wavelengths specifically sensitive to the presence of leaf ion content ($K^{+}$, $Na^{+}$, $Ca^{2+}$, $Mg^{2+}$, $Cl^{-}$, and $SO_{4}^{2-}$) and RWC in functional cotton leaves; (2) to quantify the relationship of leaf ion content ($K^{+}$, $Na^{+}$, $Ca^{2+}$, $Mg^{2+}$, $Cl^{-}$, and $SO_{4}^{2-}$) and RWC
with NDSI, RSI; and (3) to identify reliable regression models for leaf ion content and RWC estimation.

**Materials and Methods**

**Design of field experiments**

Pool (plot) experiments were conducted in the summer of 2007 and 2008 in a greenhouse at the Pailou experimental station of the Nanjing Agricultural University located at Nanjing (32°02' N and 118°50' E), Jiangsu Province, China. Cotton cultivars Sumian 12 (salt-sensitive) [Zhang et al., 2011] which is widely grown in the Yangtze River Valley in China was used. Cottonseeds were sown in a nursery bed on 25 April 2007 and 25 April 2008; seedlings with three true leaves were transplanted to pools (plot) at final populations of 37,500 plants/ha. The experimental area consists of 30 pools (plots) 4m×3m size, and 3 replications for each treatment. The randomized block design was used for the experimental layout with salinity treatments as the factor. The details of soil nutrient content, physical and chemical properties are listed in Table 1 and 2. Light supplementation, (Osram 36 W cool white lamps, 120 μmol m$^{-2}$ s$^{-1}$) was given from 7:00 to 10:00 and from 16:00 to 22:00. Maximum greenhouse air temperature ranged from 22 to 34°C, with a minimum night temperature of 18°C. Minimum relative humidity ranged from 50-60%. Soil water content was monitored daily by portable TDR soil moisture device (TRIME-EZ, imko, German) and watering the pools (plot) in order to maintain the soil relative water content ranged from 70 to 80% constantly.

**Table 1 - Nutrients contents and physical properties of the basic soil in the experiment in 2007 and 2008.**

| Year | Nutrient content (mg·kg$^{-1}$) | Physical properties |
|------|--------------------------------|---------------------|
|      | TNC$^A$ | ANC$^B$ | APC$^C$ | AKC$^D$ | pH | BD$^E$ (g·cm$^{-3}$) | FWC$^F$ (%) | EC$^G$ (ds·m$^{-1}$) |
| 2007 | 0.91×10$^4$ | 110.15 | 30.14 | 110.34 | 7.50 | 1.25 | 28.21 | 1.24 |
| 2008 | 1.11×10$^4$ | 88.92 | 27.83 | 132.73 | 6.62 | 1.23 | 28.55 | 1.22 |

$^A$ Total N content, $^B$ Available N content, $^C$ Available P content, $^D$ Available K content, $^E$ Bulk density, $^F$ Field water capacity, $^G$ Electrical conductivity.

**Table 2 - Chemical properties of the basic soil in the experiment in 2007 and 2008.**

| Year | Total salinity content (%) | HCO$_3^-$ (cmol·kg$^{-1}$) | SO$_4^{2-}$ (cmol·kg$^{-1}$) | Cl$^-$ (cmol·kg$^{-1}$) | Ca$^{2+}$ (cmol·kg$^{-1}$) | Mg$^{2+}$ (cmol·kg$^{-1}$) | Na$^+$ (cmol·kg$^{-1}$) | K$^+$ (cmol·kg$^{-1}$) |
|------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| 2007 | 0.04 | 0.18 | 4.43 | 0.53 | 2.03 | 0.39 | 5.58 | 0.32 |
| 2008 | 0.04 | 0.21 | 4.72 | 0.53 | 1.96 | 0.37 | 5.33 | 0.29 |

Seven kinds of salts (sodium carbonate (Na$_2$CO$_3$), sodium bicarbonate (NaHCO$_3$), sodium chloride (NaCl), calcium chloride (CaCl$_2$), magnesium chloride (MgCl$_2$), magnesium sulfate (Mg$_2$SO$_4$) and sodium sulfate (Na$_2$SO$_4$)) were mixed into naturally dried, sieved soils at an even molar ratio, forming soils with five levels of salinity (ECe, electrical conductivity of 1:5 soil/water extract), 0.20%(CK, control), 0.35% (S1), 0.60% (S2), 0.85% (S3) and 1.0% (S4), corresponding to electrical conductivity of 1.25 dS m$^{-1}$, 5.80 dS m$^{-1}$, 9.61 dS m$^{-1}$, 13.23 dS m$^{-1}$, and 14.65 dS m$^{-1}$, respectively, which similar to the local coastal saline soil.
Leaf collection and spectral measurements

Leaves at different positions in the stem may exhibit distinctive spectral characteristics. In order to minimize the confounding influence of location on spectral measurements, we chose the youngest fully expanded main stem leaf (fourth or fifth leaf from the top, functional leaves) and then randomly selected 10 plants of each of the 5 treatments for sampling. All leaves were collected in 10 June (seedling stage, SS), 3 July (budding stage, BS), 15 August (flowering and boll-forming stage, FBS), and 5 September (boll-opening stage, BOS) 2007 and 2008. They were immediately sealed in plastic bags, kept in an ice chest, and then transported to the laboratory for spectral measurements. Leaf reflectance was measured with a Field Spec Pro FR 2500 (Analytical Spectral Devices, Boulder, CO, USA). The measured procedure followed that employed by Pu et al. [2003]. The light source was a 100W halogen reflectorized lamp. All spectra were measured at the nadir direction of the radiometer with a 25° FOV. A standard whiteboard was employed as the white reference and measured every five minutes to convert leaf radiance to spectral reflectance. Reflectance spectra of leaves, picked randomly from the adaxial part of leaf, were collected by measuring spots of 10 mm diameter using a plant probe. The distance between the spectroradiometer and the leaf samples was about 5cm to allow within-leaf area radiance measurement. Each leaf sample consisted of an over-lapped piling of 2-3 leaves to eliminate the possible effect of background (black cloth) on the spectrum (based on our experiment, a spectrum of an overlapped piling of 2 cotton leaves becomes stable. The adaxial surfaces of a sample were measured five times, from which an average spectral reflectance was generated. Spectral reflectance was originally measured over the ranges of 350-1000nm at 1.4nm intervals and 1000-2500nm at 2.2nm intervals. The entire spectral range (350-2500nm) was automatically resampled to 1nm resolution.

Measurement of RWC and leaf ion content

For each leaf, the fresh weight was quickly measured after spectral measurement. Then they were hydrated until saturation (constant weight) for 24 h at 5°C in darkness (Turgid Weight, TW). Leaves were then dried in an oven at 80°C until a constant weight (DW) reached, and ground to determine the ion contents.

Leaf relative water content (RWC) was calculated by using equation [1] [Inoue et al., 1993].

\[
RWC = \frac{(FW - DW)}{(TW - FW)} \quad [1]
\]

where FW (g), DW (g) and TW (g) are the fresh, dry and turgid weight of cotton leaf, respectively.

About 0.5 g ground leaves were put into digesting tubes, 10 ml concentrated nitric acid and 3 ml perchlorate acid was added. All the samples were soaked for 12 h, and then burned at 300°C for 3 h, the residue was transferred to a 50 ml volumetric flask, and set the volume to 50 ml with distilled water. Finally, cation contents were measured using an atomic absorption spectrophotometer (TAS-986, Persee, China) [Zheng et al., 2008]. For the determination of Cl⁻ and SO₄²⁻ contents, leaf samples of 0.1 g were extracted in 10 ml of distilled water by heating at 80°C for 3 h [Ashraf and Orooj, 2006]. The Cl⁻ and SO₄²⁻ contents in the extracts were analyzed with ion chromatography (DX-300, Sunnyvale, CA, USA) [Liu et al., 2010].
Definition of normalized difference spectral index and ratio spectral index

In the present study, all possible two band combinations of spectral indices (NDSI and RSI) within the full spectral range of 350-2500 nm were constructed in the form of matrix linkage [Yao et al., 2010]. This concept and underlying content is beyond the traditional definition of normalized difference vegetation index, thus a more adaptable and descriptive spectral index was named, i.e. normalized difference spectral index (NDSI), or ratio spectral index (RSI). NDSI and RSI were calculated as follows:

\[
RSI = \frac{R_{\lambda_1}}{R_{\lambda_2}} \quad [2]
\]

\[
NDSI = \frac{R_{\lambda_1} - R_{\lambda_2}}{R_{\lambda_1} + R_{\lambda_2}} \quad [3]
\]

where \( R_{\lambda_1}, R_{\lambda_2} \) are the spectral reflectance of random wavebands (\( \lambda_1 \) and \( \lambda_2 \)).

Construction of reduced precise sampling method

With the hyperspectral data in the present study, a new method of reduced precise sampling was designed by integrating the reduced sampling and precise sampling to realize fast analysis and feature extraction from the observed data sets. Firstly, the reduced sampling method was adopted to construct NDSI and RSI. In this procedure, the spectral reflectance data from 2007 were read at the interval of 10 nm within the range of 350-2500 nm. With the duo matrix formulation, all possible NDSI and RSI based on any two individual bands at the interval of 10 nm in 350-2500 nm were regressed against leaf ion content (\( K^+, Na^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-} \)) and RWC. According to the changing coefficients of determination (\( R^2 \)), a contour plot of \( R^2 \) was made. From this map, the sensitive spectral range with relative greater \( R^2 \) was identified.

Then, the precise sampling was carried out to read the spectral reflectance at 1nm interval of the sensitive range identified by the above reduced sampling method, and all possible NDSI and RSI were formulated and regresses with leaf ion content (\( K^+, Na^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-} \)) and RWC. Based on \( R^2 \) and standard error (SE), the best feature bands and corresponding vegetation indices were determined. All these procedures for statistical analysis and contour mapping of \( R^2 \) and SE values were completed with self-programmed software based on MATLAB [MathWorks, 2000].

Statistical analysis

The data in 2008 [Zhang et al., 2012] were used to test the derived model equations, and the estimated results were compared with the measurements to evaluate reliability and accuracy of the equation output. The \( R^2 \), root mean square error (RMSE), and slope were used to evaluate the fitness between the predicted and observed values, along with the 1:1 plotting of the two sets of values. The RMSE is calculated with the following equation:
where $Y_j$ and $X_j$ are the predicted and observed values, respectively, and $M$ is the number of samples.

**Results**

**Effects of soil salinity on ions and relative water content of cotton leaves**

Soil salinity significantly affected the ion and water content of cotton leaves (Fig. 1). Contents of Na$^+$, Cl$^-$, and SO$_4^{2-}$ increased with increasing soil salinity, while K$^+$ and Ca$^{2+}$ contents decreased. RWC was highest at 1.25 dS m$^{-1}$ salinity, and decreased gradually as soil salinity increased. The highest RWC values were observed at the FBS growth stages, at various levels of soil salinity. However, except for differences in Ca$^{2+}$ content among the SS, and FBS, BOS growth stages, leaf ion content at the different growth stages did not differ significantly according to soil salinity.

![Figure 1 - Effect of soil salinity on the leaf ion content and leaf water content of functional cotton leaves in 2007. SS: Seedling stage, BS: Budding stage, FBS: Flowering and boll-forming stage, BOS: Boll-opening stage. Bars are ± one standard error (SE). When no bar is visible, the SE is smaller than the symbol. Values followed by the different letters within the same salinity rate are significantly different at $P=0.05$ probability level. Each data represents the mean of three replications.](image)
The correlation coefficients for ion content and RWC of cotton leaves are presented in Table 3. Clear and significant correlations were observed between ion contents and RWC; RWC was significantly negatively correlated with Na\(^+\), Mg\(^{2+}\), Cl\(^-\), and SO\(_4^{2-}\) content, with coefficients of –0.82, –0.78, –0.93, and –0.80, respectively. Significant positively correlations were observed between RWC and K\(^+\) and Ca\(^{2+}\) contents, with correlation coefficients of 0.79 and 0.72, respectively. Relationships between ions were also significantly correlated.

**Table 3 - Correlation between ion content and RWC of cotton leaves and between any two leaf parameters.**

| Leaf parameter | RWC | K\(^+\) | Na\(^+\) | Ca\(^{2+}\) | Mg\(^{2+}\) | Cl\(^-\) | SO\(_4^{2-}\) |
|----------------|-----|--------|--------|----------|---------|------|--------|
| RWC            | 1   |        |        |          |         |      |        |
| K\(^+\)        | 0.79*** | 1      | -0.87** | 0.92**   | -0.53** | -0.89** | -0.80** |
| Na\(^+\)       | -0.82** | -0.87** | 1      | -0.90**  | 0.76**  | 0.94** | 0.84** |
| Ca\(^{2+}\)    | 0.72** | 0.92** | -0.90** | 1        | -0.53** | -0.87** | -0.77** |
| Mg\(^{2+}\)    | -0.78** | -0.53** | 0.76** | -0.53**  | 1       | 0.75** | 0.66** |
| Cl\(^-\)       | -0.93** | -0.89** | 0.94** | -0.87**  | 0.75**  | 1     | 0.85** |
| SO\(_4^{2-}\)  | -0.80** | -0.80** | 0.84** | -0.77**  | 0.66**  | 0.85** | 1      |

RWC is the relative water content, all ion symbols, such as Na\(^+\), K\(^+\), in this study mean concentration of anion/cation related, respectively, **\(p<0.01\).**

**Changes in spectral reflectance of cotton leaves**

The spectral reflectance of cotton leaves changed dynamically with differing soil salinity levels at the four growth stages of cultivar Sumian 12 (Fig. 2). As soil salinity increased, leaf spectral reflectance did not change in the range of visible wavelengths but increased in the near-infrared (700–1300 nm) and middle-infrared (1300–2500 nm) ranges, with obvious differences among the five salinity levels. Previous experiments revealed that changes in reflectance in the near- and middle-infrared regions were attributable to absorption of water and organic molecules [Clark et al., 1987]; we therefore examined correlations in these regions to predict mineral content in relation to salinity.

Differences in reflectance of leaf samples among different salinity levels were considerably large in the 920–1120 nm, 1650–1850 nm, and 2000–2400 nm wavelength regions. In addition, reflectance in the 1650–1850 nm and 2000–2400 nm absorption bands increased with increasing salinity.

Similar spectral responses were observed at different growth stages; differences among spectra in relation to soil salinity were larger during the SS and BOS than during the BS and FBS. This is likely to have been a result of differences in leaf biochemical components among the different growth stages and salinity levels, which influenced spectral reflectance characteristics. The dynamic changes in leaf spectral reflectance at different salinity levels and growth stages provide a basis for analysing quantitative relationships between leaf ion content and RWC to characterise leaf reflectance in cotton.
Figure 2 - Spectral reflectance of functional cotton leaves under different soil salinity rate for cultivar Sumian 12 in 2007. —— 1.25 dS m$^{-1}$, —— 5.80 dS m$^{-1}$, —— 9.61 dS m$^{-1}$, 13.23 dS m$^{-1}$ and —— 14.65 dS m$^{-1}$, SS: Seedling stage, BS: Budding stage, FBS: Flowering and boll-forming stage, BOS: Boll-opening stage.
Correlations between leaf ion content, relative water content, and reflectance among wavebands

Correlations between spectral reflectance for all wavebands and leaf ion content and RWC from the pooled data are illustrated in Figure 3. For Na\(^{+}\), Mg\(^{2+}\), Cl\(^{-}\), and SO\(_4^{2-}\), two broad regions with relatively strong, positive correlations (>0.80) were observed in the near- and middle-infrared regions. Contents of K\(^{+}\) and Ca\(^{2+}\) and RWC were negatively correlated (<–0.80) in the same regions. Wavelengths with high R\(^2\) values can be useful for predicting ion contents. Bands with strong correlations were consistent with spectral regions associated with mineral and leaf water content reported by others [Aldana et al., 1995; Cozzolino and Moron, 2004; Huang et al., 2009; Tian et al., 2001]. The visible region from 350 to 700 nm showed a weak correlation with leaf ion contents and RWC. Other authors who analysed minerals in forage crops reported similar absorption regions [Cozzolino and Moron, 2004].

Figure 3 - Correlation coefficient of the leaf ion content (K\(^{+}\), Na\(^{+}\), Ca\(^{2+}\), Mg\(^{2+}\), Cl\(^{-}\), and SO\(_4^{2-}\)) and RWC of cotton leaves and the leaf spectral reflectance under different salinity rates.

Spectral index and construction of estimation models

To identify sensitive band ranges, we analysed the relationships between leaf ion content and RWC using NDSI and RSI at 10 nm intervals from 350 to 2500 nm, according to the reduced precise sampling method of Yao et al. [2010]. A number of “hot spots” with strong coefficients between leaf ion content, RWC, and NDSI/RSI were observed in near- and middle-infrared bands (Fig. omitted). R\(^2\) values >0.50 based on NDSI and RSI were observed for linear regressions of the ions and RWC as follows: K\(^{+}\), NDSI of 1240–1340 and 2280–2380 nm, RSI of 1150–1400 and 2250–2350 nm; Na\(^{+}\), NDSI of 1100–1350 and 2150–2400 nm, RSI of 1100–1400 and 2100–230 nm; Ca\(^{2+}\), NDSI of 1100–1400 and 2100–2400 nm, RSI of 1100–1400 and 2100–2400 nm; Mg\(^{2+}\), NDSI of 1100–1250 and 2200–2500 nm, RSI of 1200–1380 and 2100–2250 nm; Cl\(^{-}\), NDSI of 900–1300 and 2000–2400 nm, RSI of 800–1400 and 2000–2400 nm; SO\(_4^{2-}\), NDSI of 950–1250 and 2200–2500 nm, RSI of 1000–1350 and 2050–2400 nm; and RWC, NDSI of 850–1250 and 2050–2450 nm,
RSI of 700-1400 and 2000-2400 nm.

We conducted additional sampling at sensitive wavelengths to analyse leaf ion contents and RWC using NDSI and RSI for pairs of wavebands, at 1 nm intervals. Using strong \( R^2 \) values as an indicator, the following spectral indices were identified as optimal for estimation of leaf ion and water content in cotton: NDSI (\( R_{1340} \), \( R_{2306} \)) and RSI (\( R_{2306} \), \( R_{1347} \)) for \( K^+ \); NDSI (\( R_{1346} \), \( R_{2276} \)) and RSI (\( R_{2276} \), \( R_{1345} \)) for \( Na^+ \); NDSI (\( R_{1380} \), \( R_{2309} \)) and RSI (\( R_{2309} \), \( R_{1350} \)) for \( Ca^{2+} \); NDSI (\( R_{1200} \), \( R_{2311} \)) and RSI (\( R_{2302} \), \( R_{1365} \)) for \( Mg^{2+} \); NDSI (\( R_{1300} \), \( R_{2250} \)) and RSI (\( R_{2264} \), \( R_{1335} \)) for \( Cl^- \); NDSI (\( R_{1154} \), \( R_{2317} \)) and RSI (\( R_{2317} \), \( R_{1154} \)) for \( SO_4^{2-} \); and NDSI (\( R_{1222} \), \( R_{2264} \)) and RSI (\( R_{2264} \), \( R_{1335} \)) for RWC.

Linear, power, and exponential equations were used to construct models for monitoring leaf ion and water content based on the best NDSI and RSI derived from the sensitive wavelengths. These models demonstrated excellent predictive power (Tab. 4). The following parameters provided highly accurate linear equations: \( K^+ \), NDSI (\( R_{1340} \), \( R_{2306} \), \( R^2 = 0.5329 \), \( SE = 0.23 \)), RSI (\( R_{2306} \), \( R_{1347} \), \( R^2 = 0.5421 \), \( SE = 0.23 \)); \( Na^+ \), NDSI (\( R_{1340} \), \( R_{2276} \), \( R^2 = 0.6392 \), \( SE = 0.41 \)), RSI (\( R_{2276} \), \( R_{1345} \), \( R^2 = 0.6421 \), \( SE = 0.40 \)); \( Ca^{2+} \), NDSI (\( R_{1380} \), \( R_{2309} \), \( R^2 = 0.4868 \), \( SE = 0.45 \)), RSI (\( R_{2309} \), \( R_{1350} \), \( R^2 = 0.4898 \), \( SE = 0.23 \)); \( Mg^{2+} \), NDSI (\( R_{1200} \), \( R_{2251} \), \( R^2 = 0.5154 \), \( SE = 0.15 \)); \( SO_4^{2-} \), NDSI (\( R_{1154} \), \( R_{2317} \), \( R^2 = 0.9315 \), \( SE = 0.48 \)); and RWC, RSI (\( R_{2264} \), \( R_{1335} \), \( R^2 = 0.6607 \), \( SE = 0.0026 \)). The following power equations provided strong correlations: \( Mg^{2+} \), RSI (\( R_{2202} \), \( R_{1361} \), \( R^2 = 0.5276 \), \( SE = 0.16 \)); \( Cl^- \), NDSI (\( R_{1305} \), \( R_{2250} \), \( R^2 = 0.6631 \), \( SE = 0.83 \)); \( SO_4^{2-} \), RSI (\( R_{2317} \), \( R_{1154} \), \( R^2 = 0.9388 \), \( SE = 0.20 \)); and RWC, NDSI (\( R_{1222} \), \( R_{2264} \), \( R^2 = 0.6588 \), \( SE = 0.0028 \). The exponential equation provided a strong correlation: \( Cl^- \), RSI (\( R_{2264} \), \( R_{1335} \), \( R^2 = 0.6890 \), \( SE = 0.24 \).

**Model testing for estimation of leaf ion and water content**

An independent dataset from 2008 [Zhang et al., 2012] was used to test the reliability of performance of the regression models. Three statistics: \( R^2 \), RMSE, and slope between observed and estimated values were used for the evaluation (Tab. 5).

Compared with NDSI, monitoring models derived using RSI indices of (\( R_{2306} \), \( R_{1347} \)), (\( R_{2276} \), \( R_{1345} \)), (\( R_{2309} \), \( R_{1350} \)), (\( R_{2202} \), \( R_{1361} \)), (\( R_{2264} \), \( R_{1335} \)), (\( R_{2317} \), \( R_{1154} \)), and (\( R_{2264} \), \( R_{1335} \)) had showed the best performance, with \( R^2 \) of 0.78, 0.80, 0.69, 0.68, 0.85, 0.93, and 0.81, respectively; RMSE of 0.57 g kg\(^{-1} \), 1.48 g kg\(^{-1} \), 2.49 g kg\(^{-1} \), 1.13 g kg\(^{-1} \), 3.42 g kg\(^{-1} \), 1.86 g kg\(^{-1} \), and 0.01 %, respectively; and slope of 0.7594, 0.9492, 1.0564, 1.3924, 0.8975, 1.3284, and 0.8649, respectively. These results suggest that the RSI indices provide a better indicator for leaf ion content and RWC than the NDSI indices. One-to-one plots of observed and predicted values well reflected the reliability and accuracy of the derived models (Fig. 4). The more accurate the predictive equations, the more closely the points clustered near the theoretical 1:1 line.

Over all, validation of the monitoring models indicated good agreement between observed and estimated values for different salinity levels. Thus, the selected RSI indices could be reliably used for accurate estimation of leaf ion and water content in cotton at all growth stages.
Table 4 - Quantitative relationships between leaf ion content, RWC of cotton leaves and NDSI, RSI under different salinity rates.

| Leaf parameters | Spectrum parameters | Equations                                                                 | R²  | SE  |
|----------------|---------------------|--------------------------------------------------------------------------|-----|-----|
| K⁺             | NDSI(R_{1446} - R_{2306}) | y = 49.085x - 12.811                                                    | 0.5329 | 0.23 |
|                | y = 1.6893e^{3.834x} | y = 49.349x^{2.1059}                                                   | 0.5049 | 0.25 |
|                | y = -57.772x + 31.413 | y = 53.549e^{-4.5188x}                                                  | 0.5176 | 0.25 |
|                | RSI(R_{2306} - R_{1447}) | y = 2.7782x^{-1.3223}                                                   | 0.5421 | 0.23 |
| Na⁺            | NDSI(R_{1446} - R_{2276}) | y = -91.239x + 56.282                                                   | 0.6329 | 0.41 |
|                | y = 671.65e^{-4.4731x} | y = 0.552x^{4.1183}                                                    | 0.6366 | 0.45 |
|                | y = 102.22x - 23.175 | y = 335.89x^{3.1874}                                                   | 0.6412 | 0.41 |
|                | RSI(R_{2276} - R_{1441}) | y = 53.549e^{-4.5188x}                                                  | 0.6329 | 0.41 |
|                | y = 2.7782x^{-1.3223} | y = 0.552x^{4.1183}                                                    | 0.6366 | 0.45 |
| Ca²⁺           | NDSI(R_{1340} - R_{2307}) | y = 26.723x^{1.6168x}                                                  | 0.4628 | 0.45 |
|                | y = 100.93x^{0.7489} | y = 35.015x^{1.1057}                                                   | 0.4628 | 0.45 |
|                | y = -112.91x + 91.572 | y = 27.619x^{0.6039}                                                   | 0.4628 | 0.45 |
| Mg²⁺           | RSI(R_{2306} - R_{1350}) | y = 105.52e^{-2.0283x}                                                  | 0.4628 | 0.45 |
|                | y = 27.619x^{0.6039} | y = 35.015x^{1.1057}                                                   | 0.4628 | 0.45 |
| Cl⁻            | NDSI(R_{1200} - R_{2211}) | y = 48.062x^{2.6923x}                                                   | 0.5135 | 0.16 |
|                | y = 5.1894x^{1.2663} | y = 35.015x^{1.1057}                                                   | 0.5135 | 0.16 |
|                | y = 34.342x - 0.9211 | y = 4.4862e^{2.5967x}                                                  | 0.5201 | 0.16 |
|                | RSI(R_{2202} - R_{1361}) | y = 27.619x^{0.6039}                                                   | 0.4628 | 0.45 |
|                | y = 35.015x^{1.1057} | y = 4.4862e^{2.5967x}                                                  | 0.5201 | 0.16 |
|                | y = -189.96x + 122.15 | y = 27.619x^{0.6039}                                                   | 0.4628 | 0.45 |
|                | RSI(R_{2264} - R_{1321}) | y = 3.3616e^{7.0804x}                                                  | 0.9315 | 0.49 |
|                | y = 138.37x - 14.253 | y = 427.33x^{2.6574}                                                   | 0.6744 | 0.80 |
|                | RSI(R_{2317} - R_{1154}) | y = 3.3616e^{7.0804x}                                                  | 0.9315 | 0.49 |
|                | y = 235.35x^{1.7771} | y = 108.35x + 85.466                                                   | 0.9315 | 0.49 |
|                | NDSI(R_{1200} - R_{2211}) | y = 1.694e^{7.7856x}                                                  | 0.689  | 0.24 |
|                | y = 427.33x^{2.6574} | y = 108.35x + 85.466                                                   | 0.9315 | 0.49 |
|                | RSI(R_{2264} - R_{1321}) | y = 235.35x^{1.7771}                                                  | 0.9315 | 0.49 |
|                | y = 427.33x^{2.6574} | y = 108.35x + 85.466                                                   | 0.9315 | 0.49 |

n = 80, R²_{0.01} = 0.0820.
Figure 4 - The 1:1 relationship between the observed and predicted values of leaf ion content and RWC in cotton leaves based on the better performance estimating models (n=40).
Table 5 - Testing results of the better performance estimating models based on RSI and NDSI to leaf ion content and RWC of cotton, the independent data set from 2008 were used to test the performance of these models.

| Leaf parameters | Spectral index | Regression equation | $R^2$ | RMSE | Slope |
|-----------------|----------------|---------------------|-------|------|-------|
| K$^+$           | RSI($R_{2306}$, $R_{1347}$) | $y = 0.7594x + 2.9359$ | 0.78  | 0.57 | 0.7594 |
|                 | NDSI($R_{1340}$, $R_{2306}$) | $y = 0.6855x + 4.3317$ | 0.70  | 1.00 | 0.6855 |
| Na$^+$          | RSI($R_{2270}$, $R_{1437}$) | $y = 0.9492x - 0.0503$ | 0.80  | 1.48 | 0.9492 |
|                 | NDSI($R_{1340}$, $R_{2276}$) | $y = 0.7326x + 3.0947$ | 0.65  | 2.01 | 0.7326 |
| Ca$^{2+}$       | RSI($R_{2306}$, $R_{1350}$) | $y = 1.0564x - 1.5824$ | 0.69  | 2.49 | 1.0564 |
|                 | NDSI($R_{1380}$, $R_{2307}$) | $y = 0.9052x + 6.6975$ | 0.51  | 2.87 | 0.9052 |
| Mg$^{2+}$       | RSI($R_{2202}$, $R_{1361}$) | $y = 1.3924x - 5.9025$ | 0.68  | 1.13 | 1.3924 |
|                 | NDSI($R_{1200}$, $R_{2211}$) | $y = 1.0754x - 1.1772$ | 0.51  | 1.27 | 1.0754 |
| Cl$^-$          | RSI($R_{2264}$, $R_{1335}$) | $y = 0.8975x + 2.7172$ | 0.85  | 3.42 | 0.8975 |
|                 | NDSI($R_{1200}$, $R_{2251}$) | $y = 0.8702x + 2.52$ | 0.80  | 4.32 | 0.8702 |
| SO$_4^{2-}$     | RSI($R_{2137}$, $R_{1154}$) | $y = 1.3248x - 8.3754$ | 0.93  | 1.86 | 1.3284 |
|                 | NDSI($R_{1154}$, $R_{2317}$) | $y = 1.4237x - 10.781$ | 0.93  | 2.10 | 1.4237 |
| RWC             | RSI($R_{2264}$, $R_{1335}$) | $y = 0.8649x + 0.1083$ | 0.81  | 0.01 | 0.8649 |
|                 | NDSI($R_{1222}$, $R_{2264}$) | $y = 1.0289x - 0.02$ | 0.74  | 0.01 | 1.0289 |

Discussion and conclusions

In saline soils, uptake of water by plants is driven by the difference in water potential between the soil and plant roots. Decreased soil water potential under high salinity restricts water flow into plant roots, which reduces pressure-driven xylem transport of water to aboveground tissues [Lee et al., 2005]. Increased salt content is expected to negatively affect plant water relations and water content, with RWC decreasing as salt increases. In the present study, the effect of soil salinity on RWC in cotton showed similar results among different growth stages.

It is reported that NIR and SWIR are sensitive to the presence of various trace elements, depending on the forage type and crop species [Ruano-Ramos et al., 1999]. Some minerals (e.g. Ca and P) do not exhibit absorption in the NIR region but could be indirectly detected through their connections to organic complexes and chelates [Moron and Cozzolino, 2002], which agrees well with our preliminary conclusions under different salinity conditions. RWC is a synthetic reflection of leaf water status in crops, and thus is considered a key indicator for diagnosing plant water status and for estimating stress levels [Ghulam et al., 2008]. Here, we showed that the regions of intermediate absorption by leaf water in middle-infrared wavelengths near 1650 and 2200 nm and the weak absorption bands in the near-infrared region near 970 and 1200 nm were suitable for estimating plant water status. Our observations of the reflectance of bands from 700 to 1400 nm and 2000 to 2400 nm in relation to leaf water content were similar to findings reported in previous studies [Bowman, 1989; Gao, 1996].

A number of new two-band combinations designed to be optimally related to a specific crop variable were tested previously [Blackburn, 1998; Gitelson et al., 1996]. Therefore, we adopted a new approach using reduced, precise sampling derived from all two-band combinations at 1 nm intervals, based on these sensitive spectral ranges, to estimate leaf water and ion content in cotton grown in saline soil.
Using this method, we identified a series of novel wavelengths for monitoring leaf water and ion content. However, the wavelengths selected for monitoring $K^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$, $Cl^−$, and $SO_4^{2−}$ content in cotton grown under saline conditions are generally not consistent with other published findings [Aldana et al., 1995; Cozzolino and Moron, 2004]. This may have been a result of the elements existing in different complexes, which would contribute to the differences in wavelengths used for the equations [Clark et al., 1989].

The 1222 and 2264 nm bands near the middle-infrared wavelengths and weak absorption bands in the near-infrared region have been shown to be reliable for remote sensing of plant water status [Gao, 1996; Penuelas et al., 1993; Penuelas et al., 1996; Tucker, 1980]. The 1321 nm band in the near-infrared range is believed to be determined by tissue and cell structural properties, which are influenced by salinity [Munns and Termaat, 1986], this band can be considered as representative of turgor pressure in bulk tissue [Grant, 1987]. This indicates that the best bands for determining leaf water content of cotton in saline soils are those in the near-infrared and short-wavelength infrared range, consistent with Ceccato et al. [2001] and Bowyer and Danson [2004] who showed that these ranges were necessary for assessing leaf water content under non-saline conditions.

Selection and exploration of key wavebands is an important technology in vegetation remote sensing and has been performed in a number of studies [Hansen and Schjoerring, 2003]. As expected, the results obtained for predicting mineral elements were not as reliable as those for organic compounds due to the lack of a direct relationship between the elements and spectra [Shenk and Westerhaus, 1985; Smith et al., 1991]. The coefficients of determination for predicting ion contents were smaller in our study, similar to the findings of Clark et al. [1989] and Ruano-Ramos et al. [1999], who estimated the mineral contents of forage and semi-arid grassland samples. This is probably a result of the narrow range of concentrations of minerals our leaf samples and the low concentration of associated organic compounds detected by hyperspectral sampling. Although prediction of ion contents by hyperspectral sampling has limitations, the speed of analysis and minimal sample preparation provide advantages over other methods (e.g. atomic absorption spectroscopy).

In this paper, we constructed new, simple spectral NDSI and RSI indices for $K^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$, $Cl^−$, $SO_4^{2−}$, and RWC. The vegetation indices (NDSI and RSI) composed of near-infrared and short-wavelength infrared bands should be useful for effective monitoring of leaf water and ion contents in cotton plants, and for reflecting stress caused by soil salinity. The models derived from this study are simple and applicable, with reasonable explanatory power using the principles of remote sensing.

In summary, a series of novel wavelengths were identified using a reduced, precise sampling method, and seven sets of new sensitive spectral indices were determined with corresponding regression models for reliable detection of leaf ion and water content: $K^+ = −57.772 \text{RSI (} R_{2300−1345} \text{)} + 31.413$; $Na^+ = 102.22 \text{RSI (} R_{2276−1345} \text{)} − 23.175$; $Ca^{2+} = −112.91 \text{RSI (} R_{2300−1350} \text{)} + 91.572$; $Mg^{2+} = 35.015 \text{RSI (} R_{2202−1361} \text{)}^{0.1057}$; $Cl^− = 1.694e^{7.7856\text{RSI (} R_{2264−1335} \text{)}}$; $SO_4^{2−} = 235.35 \text{RSI (} R_{2317−1154} \text{)}^{1.7771}$, and $\text{RWC} = −0.7137 \text{RSI (} R_{2264−1321} \text{)} + 1.0143$. These findings help to create a technical foundation for rapid and accurate monitoring of plant salinity status in cotton using hyperspectral remote sensing data, and will be useful for developing non-destructive monitoring techniques for leaf ion and water content in cotton using portable equipment for ground-based spectral reflectance under saline conditions. This study was conducted under different salinity conditions in the pool environment.
in four growth stages of cotton; the models based on hyperspectral reflectance were established and tested under an identical set of ecological conditions. Thus, these key indices and models require further verification in other conditions, with different cultivars and salinity treatments. Such additional studies will enable these tools to be refined for accurate estimation and application to field management.

Acknowledgements
This work was supported by the National High Technology Research and Development Program of China (863 Program) (Grant no. 2007AA10Z206) and the National Natural Science Foundation of China (31301262).

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