Application of visible and near-infrared spectrophotometry for detecting salinity effects on wheat leaves (Triticum aestivum L.)

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Abstract: Soil and water salinity is the most limiting factor for plant growth and productivity. Due to a high rate of evaporation, agricultural lands become saline in arid regions after a while. This leads to a decline in plant production. The present study investigated the capability of visible and near infrared (VNIR) spectrophotometry as a non-destructive method in detecting salinity effect on wheat leaves. A completely randomized design was worked out with four salinity levels and three replicates. Wheat seeds were planted in plastic pots and irrigated with four levels of saline water [0 (control), 4, 8 and 12 dS/m] Leaf spectrophotometry at VNIR (190-1100 nm) wavelength was performed on wheat leaves at the nodule-formation growth stage. The results indicated that treatments are discriminated mostly by reflectance and absorption spectra of 530-660 nm although a difference existed between the control treatment and the other treatments at 700-1100 nm. The difference between the treatments of T0, T4 and T12 was found to be significant ($P<0.01$) in the reflectance with an absorption value of 530-660 nm. Although all the treatments were discriminated at 700-1100nm visually, the difference between them was statistically insignificant at this wave range.

Keywords: Saline water, leaf elemental concentration, spectral curve, reflectance, absorbance, completely randomized design

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1 Introduction

In arid and semi-arid regions, relatively high temperature causes considerable amount of daily evaporation and increases soil salinity by accumulating water-soluble salts in the soil (Chen et al., 2010; Singh and Abrol, 1985). Therefore, soil and water salinity is known as a major limiting factor affecting plant growth and productivity in these areas. Although soil salinity can be reduced by soil soluble-salt leaching from its root zone by either increasing the quantity or frequency of irrigation water (Ali, 2011), water with sufficient quality and quantity is unavailable to satisfy the requirements of crops grown in arid and semi-arid regions. In these conditions, farmers have to keep the crop production at a reasonable level on one hand and to minimize the water amount for irrigation on the other hand. In order to optimize this balance, it is necessary to have information about crop water requirement, to measure water and soil salinity, and to determine crop salinity tolerance threshold at different crop growth stages. This requires appropriate monitoring of crop growth and determination of the minimum threshold for salt tolerance at different growth stages (Ragab, 1995). Using information on these activities, the effects can be alleviated by management practices such as soil drainage improvement and appropriate irrigation scheduling (Abrol et al., 1988).

Currently, laboratory tests are conducted to measure soil and water salinity and plant nutrient status. Nevertheless, soil sampling and plant analysis are limited due to financial and time constraints (Foley et al., 1998).

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However, crop salinity tolerance threshold and salinity effects on crops can be determined in a controlled environment that is normally varied from natural growth surroundings, and a lot of assumptions about uncertain matters need to be considered. In addition, salinity affects the crop yield before visible signs appear on crop organs. The complex interaction among soil elements does not allow relating the laboratory test results directly to the plant health and its nutrient status (Yerokun and Christenson, 1990) and, as a result, plant material analysis is inevitable (Jeffrey, 1987). In recent years, non-destructive, cost-effective and rapid analytical techniques such as spectrophotometry have become increasingly common in the field of agricultural studies, which makes it possible to remotely sense the effects. This method has proved to be able to identify plant nutrient status (Cayuela, 2008; Menesatti et al., 2010), soil properties (Chang et al., 2001; Minasny et al., 2009), wheat and wheat grain hardness assessment (Windham et al., 1991; Zayas et al., 1992), crop disease (Delwiche and Kim, 2000; Delwiche et al., 2009), wheat pre-harvest sprouting (Wu et al., 2012), and safflower oil content (Elfadl et al., 2010). The above-mentioned studies have mainly used the visible and near-infrared (VIS/NIR) part of the reflected spectrum for plant tissues analysis.

The present study evaluates the potential of VIS/NIR spectrophotometry in detecting soil salinity effects on wheat crops befor visible toxicity signs appear. Although salinity effects can occur in any plant organ, to do the analysis, a selection was made of leaves because they are known as the most physiologically active, available plant tissues and mineral stowing place (Embleton et al., 1973). Leaf growth is reported to be more sensitive to salinity than the other plant organs (Rawson, 1986).

2 Materials and methods

2.1. Data collection

A variety of autumn wheat seeds were planted in a greenhouse with a controlled temperature. The wheat seeds germinated and grew in small plastic pots (15 cm in diameter and 20 cm in height) filled with sandy soil containing humus (30%, v/v). Prior to seeding, all the pots were irrigated once with distilled water. Ten seeds were planted in each pot and, after germination, six strongest plants were maintained. In order to evaluate the salt effects, a completely randomized design was worked out with four salinity levels and three replicates. Four levels of saline water [0 (control), 4, 8 and 12 dS/m] were provided by dissolving pure NaCl salt in distilled water used as a treatment at each irrigation time. These levels were selected since wheat plants are reported to be moderately tolerant to soil salinity (4 dS/m), and the yield decreases at salinity of 6 dS/m (Hussain et al., 2003). The levels from this point forward are called T0, T4, T8 and T12 respectively. Salt treatment was initiated at the 5^{th} leaf stage of the plants. Nitrogen fertilization as ammonium nitrate (1 mmol/L NH_4NO_3) was added once to the pots at the stem elongation stage. Leaf sampling was performed for spectral and chemical analyses at the wheat nodule formation growth stage. All the treatments were made with no visible toxicity sign on the wheat organs at the sampling time.

In order to determine the irrigation frequency, gravimetric soil water content was measured (GSWC) by subtracting the weight of the dry soil from the weight of the flooded and then drained wet soil. This measurement was used to approximate the field capacity moisture, the upper limit soil moisture content for plant growth. Based on the GSWC and the field capacity measurement, the irrigation frequency was once three days during the experimental period. The constancy of salinity level at each treatment was checked by the Electrical Conductivity (EC) measurement of the irrigation water drained out of the pots using Jenway Model 4071 Laboratory conductivity meter. At the end of the experiment, the leaves harvested from each pot were used for both chemical and spectrophotometric analyses.

2.2. Leaf water content

It is reported that the amount of leaf water content (LWC) is affected by increasing salinity (Fricke and Peters, 2002). In addition, LWC is known as an appropriate indicator for soil water content, plant water status and drought stress assessment (Yan et al., 2012; Zhang et al., 2014). Water content in a plant organ is defined as dry weight or fresh weight. In this study, LWC was measured as the moisture percentage using the follow equation (Sande-Bakhuyze, 1928):

$$LWC = \frac{Fresh.weight - Dry.weight}{Fresh.weight}$$

2.3. Chemical analysis

Chemical analyses were conducted on the wheat leaves under treatment, and the results were statistically compared at the four salinity levels. The concentrations of certain elements in the leaves, including Na, Ca, K, Mg, P, Cl, Cu, Zn and Fe, were determined. The other elements (Se, Co, Si, Rb, Sr, I), which are contained in plant tissues, were excluded from the analysis because they are normally not needed for plant growth and development. Prior to the mineral analysis, the leaves contained in porcelain crucibles were oven-dried at 78 ℃ for 48 h. Then, 0.3 g of the oven-dried material was used to determine the concentration of N using the Micro-Kjeldahl method (Behr Distillation units S4, Behr-Labor-Technik). The concentrations of the other elements were determined after incinerating the remaining oven-dried materials in an oven at 550 ℃ for 4 h and extracting them with hydrochloric acid (wet ash digestion method 6% v/v). The concentrations of Na+ and K+ contents in the leaves were measured by a flame photometer using standard solution samples, according to the method used by Jackson (1973). Ca and Mg contents of the samples were determined by a titration method using the chelating agent, EDTA (ethylenediamine tetra acetic acid) as practiced by Jackson (1973). Chloride and Phosphorous were determined using Mohr's methods as in Jackson (1973) and Olsen method as in the study by Olsen et al. (1954). Finally, the concentrations of Fe, Mn, Zn, Cu in the solutions were determined using a flame atomic absorption spectrophotometer (Model novAA 350, Analytik Jena, Germany). In addition, for each laboratory measuring test, three replications were performed, and the results were statistically analyzed. The nutrient concentrations were expressed as a percentage of leaf dry matter.

2.4. VIS/NIR leaf spectroscopy

VIS/NIR reflectance spectroscopy was done to evaluate any change in the spectra reflectance in relation to different salinity treatments. For this, a spectrophotometer instrument (Analytik Jena AG model Spec 210) with the capability of radiating 190 to 1100 nm electromagnetic wavelengths and reflectance measurement was used. The samples were illuminated by both deuterium and halogen lamps to cover the above-mentioned wavelengths. The middle part of the leaves, which had a sufficient surface for spectroscopy, was selected for the analysis. The instrument was equipped with a computer and specific software (WinASPECTver: 2.3.1.0) to acquire, calibrate and elaborate spectral data. Before sample measurement, a reference correction method was employed as recommended in the user manual of the instrument. The second option (i.e. standard correction) provided in the software was less accurate than the reference correction because of the possible difference between the measurement parameters used for the correction data measurement and the sample measurement. The Standard correction data were measured with device-internally defined measurement parameters and stored permanently. As the instrument was a double-beam spectrophotometer, a blank sample holder was placed in the reference beam unit for each measurement. The spectra were recorded with a 0.1-second integration time per 1 nm increment. For

each spectrum measurement, three replications were performed, and the results were averaged.

3 Results

The chemical analysis showed that NaCl salinity could affect the concentration of wheat leaf elements. The statistical results of the chemical analysis of the wheat leaves gained under different salinity treatments are shown in Table 1. Based on the results presented in this table, there are five groups of elements to recognize. The concentration of the first group, containing Na and Cl, is increased in the leaf tissues by an increase in the salinity level from T0 to T12. Therefore, salt stress increases the uptake of NaCl ions in wheat plants. In contrast, the concentrations of K and Ca, as the second-group elements, are decreased by an increase of salinity. Also, a different behavior is observed for the third (N and Zn) and the fourth (P, Cu and Mg) elemental groups as compared to the first and the second groups. In the third group, the concentration is elevated by an increase of salinity from T4 to T12, but the maximum value is related to T0. The concentration of P, Cu and Mg, as the forth-group elements, is increased by an increase of salinity form T4 level to T12; nevertheless, the value at T0 level is located between T4 to T12 values. Finally, the concentration of Fe, as the fifth-group element, is decreased by an increase of salinity from T4 to T12 although the value for T0 level is located around the value of T8 level. The variance of elements concentration was analyzed statistically and shown in Table 1. In this table, the means marked with the same letter are not significantly different according to Duncan's multiple range test at 1% level.

In order to evaluate the status of the plant leaves, their water content (LWC) was measured. The results confirmed an insignificant difference of LWC in different treatments. In addition to the chemical analysis, spectrophotometry of the leaves was conducted. In this study, measurements were made of both reflected and absorbed electromagnetic waves of the leaves ranging from 190 to 1100 nm. They are reported as percentages. Figures 1 and 2 show the obtained reflectance and absorbance spectral curves respectively. The values of each point shown in these figures are the average of three-leaf sample reflectance and absorbance measurements in the same treatment. The deviation of treatments from 530 to 660 and 700 to 1100 nm is noticeable in the graphs. Although the absorbance values of all the treatments diverge at the wavelengths of 700-1100 nm, the reflectance values are similar for T4, T8 and T12 at this range. Nevertheless, as Figure 2 suggests, there is no similarity between the reflectance values of T0 in the control and the other treatments. Since there was no specific pattern of spectral curve in 110-530 nm and no similarity was observed between the treatments in 660-700 nm, these ranges were excluded from further statistical analysis.

Table 1 Concentration of elements in wheat leaves for each treatment expressed as a percentage on dry matter

Salinity levels	Concentration, %									
	Group 1		Group 2		Group 3			Group 4		Group 5
	Na	Cl	K	Ca	P	Cu	Mg	Zn	N	Fe
T0	0.98^{c}	2.08^{b}	28.80^{a}	1.44^{a}	1.26^{c}	0.0052^{b}	0.94^{a}	0.0159^{a}	2.80^{a}	0.029^{ab}
T4	1.33^{c}	2.23^{b}	23.30^{b}	1.40^{a}	1.12^{d}	0.0049^{b}	0.56^{b}	0.0144^{a}	2.41^{b}	0.032^{a}
T8	2.88^{b}	2.42^{b}	21.42^{b}	1.37^{a}	1.51^{b}	0.0052^{b}	0.67^{b}	0.0152^{a}	2.49^{ab}	0.030^{ab}
T12	3.30^{a}	2.71^{a}	20.52^{b}	0.92^{b}	1.58^{a}	0.0066^{a}	1.07^{a}	0.0158^{a}	2.57^{ab}	0.027^{b}

Note. Any two means marked by the same letter are not significantly different.

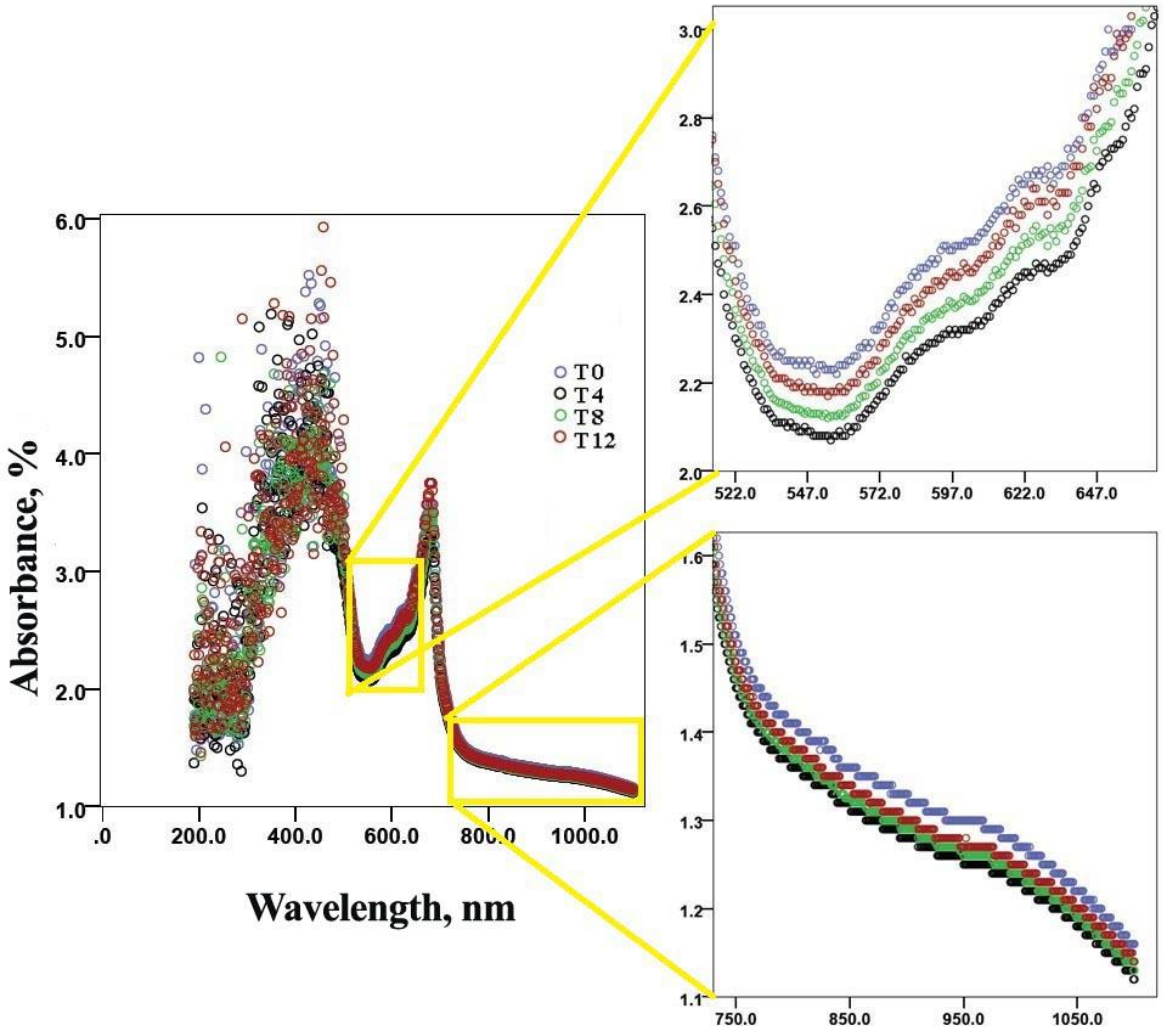

Figure 1 Spectral absorbance graph of wheat leaves at four levels of salinity

In addition to visual inspection of the spectral curves, the observed spectral values were statistically investigated. A comparison of the results presented in Table 2 indicates that the difference between the treatments is significant ($P<0.01$) for both spectral reflectance and absorbance measurement values at a wavelength ranging from 530-660 and 700-1100 nm. In addition, Duncan's multiple range test results presented in Table 2 suggests that, at the wavelength of 700-1100 nm, the mean differences between T0 and other treatment groups are significant, but the differences between T4, T8 and T12 prove to be insignificant. The results further reveal that all the treatments are better discriminated at wavelengths of 530-660 nm than 700-1100 nm for both reflectance and absorbance spectra. In this table, the means marked with the same letter are not significantly different according to Duncan's multiple range test at 1 % level. In this wave range, although the minimum mean value of reflectance belongs to T0 (control), the mean value is decreased as the salinity level increases from T4 to T12 (Table 2). A reverse pattern is observed for absorbance values in this table.

4 Discussion and conclusions

Since the variation of the spectral graphs is mostly correspondent to the variation of N and Zn in the chemical analysis results, the conclusion will be based on the relationship between the salinity treatments, the spectral results and the chemical results. This study demonstrates the capability of visible and near-infrared

Table 2 Comparison of means of wheat reflectance and absorbance values at four levels of salinity

Salinity levels	530-660 nm		700-1100 nm	
	Reflectance, %	Absorbance, %	Reflectance, %	Absorbance, %
T0	6.49[c]	2.41[a]	22.12[b]	1.328[a]
T4	7.19[a]	2.31[c]	22.72[a]	1.303[b]
T8	7.07[ab]	2.33[bc]	22.70[a]	1.305[b]
T12	6.96[b]	2.35[b]	22.67[a]	1.306[b]

Note. Any two means marked by the same letter are not significantly different.

spectrophotometry in detecting salinity effects on wheat leaves. From the investigation, it is understood that 530-660 nm of the visible range of spectrum forms the most effective waves for detecting salinity effects on wheat leaves. The decrease in the reflectance value from T4 to T12 at this wave range can be attributed to the decrease of leaf chlorophyll. This is in line with the report of Sims and Gamon (2002) in that the visible part of an electromagnetic spectrum (400-700 nm) provides information about leaf pigmentation, in which chlorophylls absorb red and blue light whereas green light is highly reflected at this wave range. In other word, leaf spectral reflectance patterns in the visible range are determined mainly by chlorophyll concentration (Vogelmann, 1993).

The difference between the treatments was insignificant in term of LWC. This can be due to plant osmotic adjustment under stress. However, since middle infrared wavelength is responsible for water content change, this result cannot be related to the graphs shown in Figures 1 and 2. In addition, this study suggests the use of leaf relative water content index instead of LWC measurement in future research. With regard to the results of the chemical analysis, the concentration of N has increased from T4 to T12. However, the difference among T8, T12 and T0 is insignificant. Table 1 shows that the difference is only significant between T4 and T0, but the concentration of this element in T0 (treatment without salinity stress) is higher than that in the other treatments. A decrease in N accumulation is reasonable as N and Cl have antagonist effects on absorption of each other. Nevertheless, the concentration of this element in plant tissues can also be increased due to an elevated salinity stress as reported by such researchers as Higbie et al. (2010) and Pessarakli and Tucker (1985). Leaf greenness is normally related to the chlorophyll content which is, in turn, directly related to the plant N content. Despite an increase in the leaf N content as a function of salinity, the deficiency of other elements (e.g. Fe) can cause a reduction in the chlorophyll concentration. This also can be due to plant nutrition imbalance as a consequence of salinity stress (Álvarez et al., 2012). However, salinity treatment with no effects on N absorption has also been reported in previous studies (Langdale and Thomas, 1971). The same conclusion can be drawn for the variation of Zn elements (Higbie et al., 2010). The concentration of Zn in plant tissues depends on many factors such as plant genotype and soil PH (Khoshgoftarmanesh et al., 2006). However, due to the lower concentration of Zn in saline stress treatments as compared to T0, it can be concluded that NaCl reduces the total concentration of Zn in wheat leaves.

In the near-infrared spectral region between 700 and 1100 nm, where the leaf reflectance is normally high (except water-related absorption bands of 960-1100 nm) and leaf structure is characterized by optical properties. Leaf pigments and cellulose are transparent to this wave range and, therefore, leaf absorbance is very small (10% maximally) while reflectance and transmittance can reach

50% (Blackmore et al., 2002). Nevertheless, the present study showed that all treatments are better discriminated by absorbance values than by reflectance values. Despite the similarity between the patterns of change of N and Zn to the variation of absorbance value at 700-1100 nm, the relationships cannot be easily drawn because this wave range is reported to provide more information than elemental concentration does about plant cell structure, number of cell layers, number of intercellular spaces and cell size.

This experiment was conducted in a controlled environment, and differences between the treatments were evaluated comparatively. Although a controlled environment is necessary to provide reproducible treatments, there are many factors in the field that could cause effects similar to those under salinity stress. Consequently, the main factors limiting plant stress must be known or identified when the presented methodology is applied in the natural plant growth environment. The combined effects of salinity and conditions of high evaporative demand, whether caused by high temperature, low humidity or increased wind, were reported to be more stressful than salinity stress alone. Sensitivity of plants to salinity is increased by elevating temperature whereas sensitivity to salinity is decreased by increasing humidity (Matthew, 2007). Therefore, the suggestion of this study for any future research is the evaluation of spectral curves of wheat leaves under salinity stress at different air humidity and temperature conditions.

Although elemental concentrations are not provided by the spectral analysis of leaves as affected by salinity stress directly, the ability to rapidly predict the salinity effect is an advantage over standard wet chemical analyses. Moreover, a chemical analysis only measures the total concentration of nutritional elements, while a visible and near-infrared spectral analysis properly discriminates among the diverse chemical compounds in which nutrients are held. Although this study made use of a lab-based spectrophotometer and a destructive method for leaf sampling, the use of field-based spectrophotometer for on-site measurement of live leaf spectrum is suggested. This method, however, does not provide a universal spectral curve for salinity stress and assessment of its effects on wheat leaves. As a result, comparison with the normal leaf spectral curve is inevitable. The presented methodology is rapid and low-cost as compared to chemical analysis. This study only used plant leaves as a target of chemical and spectral analyses. To gain a better understanding of how salinity stress affects plant elemental concentration, there seems to be a need for further research on the elemental concentrations in other plant organs such as shoots and stems.

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