Application of hyperspectral image to identify the salinity effects on lettuce leaves


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Abstract

Soil and irrigation salinity are key factors in the growth of most of vegetables, causing problems in agriculture, mainly in arid or semi-arid regions. Industrial development and population growth have caused contamination and salinization of surface and underground water and, thereby of agricultural soil. Saline soils represent a great area in the southeast of Spain, where are the main region of horticultural crops in the country. Lettuce (Lactuca sativa L.) is one of the most important crops in this region, being Murcia the main producer region in the European Union. This production is employed mainly for fresh consumption and ready-to-eat product.

In general, lettuce presents a moderate sensitivity to salinity. In moderated levels of salinity lettuce crops do not show visible damages in the leaves and seem to be normal, although their growth and yield decrease. They could have deep green leaves, with more density and thickness, and more succulent tissues. When salinity induces imbalances in concentrations of certain mineral elements, necrosis, chlorosis and tipburn can appear in the leaves. However a moderate saline stress in lettuce crop can have positive effects in post-harvest conservation for fresh and ready-to-eat products because of less polyphenoloxidase and peroxidase activity after cutting. Therefore, lower enzymatic and microbiological degradation occur along the shelf-life.

Hyperspectral image has been widely employed to detect salinity levels in soils and canopy in remote sensing, developing many indexes to estimate saline concentration in relation with reflectance at different wavelengths. However, there are no studies at laboratory level to observe changes produced in the structure and composition of the leaf tissues when saline concentration is increasing. The aim of the present work is to apply hyperspectral imaging techniques as a non destructive procedure to identify the influence of saline stress in just harvested “baby” lettuce.

Four salt treatments were applied under a hydroponic growth system: a control treatment without NaCl (Ct) and three levels of salt through the addition of different concentrations of NaCl to the nutrient solution: S1 (50 mM NaCl), S2 (100 mM NaCl), S3 (150 mM NaCl). The application of salt treatments was proportionally established in three days and conducted for a total of 10 days. At the end of experiments, leaves were harvested and a sample of 10 leaves per treatment, with similar size, was selected for the acquisition of hyperspectral images.

Hyperspectral images from the 40 selected leaves were taken with a hyperspectral vision system consisting in a CCD camera and a VIS-NIR spectrometer (Headwall Photonics HyperspecTM) working in the range of 400 – 1000 nm. 189 wavelengths were considered along the range, obtaining a spectral resolution of 3.2 nm.
For segregating leaves at different salinity levels a model based on principal component analysis and a non linear index (Lettuce Salinity Index, LSI) combining three relevant wavelengths were proposed. Both methods were able to sense the salinity effects on the leaves. LSI showed slight better performance, with the advantage of using only three wavelengths (675, 710 and 745 nm).

Keywords: non destructive assessment, multivariate analysis, ready-to-eat product, salinization, Lactuca sativa L.

1 Introduction

Soil and irrigation salinity are key factors in the growth of most of vegetables, causing many problems in agriculture, mainly in arid or semi-arid regions (Lamsal et al., 1999; Shannon et al., 1994). Industrial development and population growth have caused contamination and salinization of surface and underground water and, thereby of agricultural soil. Saline soils represent a great area in the southeast of Spain, where there is the main region of horticultural crops in the country. Lettuce (Lactuca sativa L.) is one of the most important crops in this region, being Murcia the main producer region in the European Union. This production is employed mainly for fresh consumption and ready-to-eat product.

Lettuce presents a moderate sensitivity to salinity; when the electrical conductivity (EC) is greater than 1.3 dS/m, the growth is affected, decreasing its yield by 13% for each unit of EC above such level (Ünlükara et al., 2008).

Salinity affects crop growth mainly in two ways: a) increasing the osmotic potential of the soil, which causes less availability of the water to the plants, and b) by specific effects of some elements in excessive concentration. Herbaceous crops affected by salinity in moderated levels do not show visible damages in the leaves and seem to be normal, although their growth and yield decrease. They could have deep green and more succulent leaves, with more density and thickness. However, when salinity induces imbalances in concentrations of certain mineral elements, like Cl, Na, B or Ca, necrosis, chlorosis and tipburn can appear in the leaves (Carassay et al., 2012).

Hyperspectral imaging has been widely employed to detect salinity levels in soils and canopy in remote sensing, developing many indexes to estimate saline concentration in relation with reflectance at different wavelengths (Poss et al., 2006; Hamzeh et al., 2013). This technique has been applied in remote sensing on many different crops and plants: sugarcane (Hamzeh et al., 2013), cotton, corn, cogon grass, reed, saltcedar, suaeda or aeluropus (Zhang et al., 2011). There are also many studies in remote sensing and at laboratory level to observe the effect of water stress (Harris et al., 2006; Clevers et al., 2010) or nutrient deficiencies (Masoni et al., 1996; Pacumbaba & Beyl, 2011). However, there are no studies at laboratory level to observe changes produced in the structure and composition of the leaf tissues when saline concentration is increasing. The aim of the present work is to apply hyperspectral imaging techniques as a non destructive procedure to identify the influence of saline stress in just harvested “baby” lettuce, detecting the distribution of the effects in the surface of the leaves.

2 Materials and methods

2.1 Materials and analytical measurements

Plants of lettuce (Lactuca sativa L. var capitata) were obtained from seeds. The seeds were placed into polystyrene cylinders with Hoagland’s nutrient solution. The composition of the nutrient solution was: 7 mM K⁺, 4 mM Ca²⁺, 14 mM NO₃⁻, 1 mM Mg²⁺, 1 mM PO₄³⁻, 1 mM SO₄²⁻, 20 µM Fe²⁺, 2.5 µM B³⁺, 2 µM Mn²⁺, 2 µM Zn²⁺, 0.5 µM Cu²⁺ and 0.5 µM Mo⁶⁺; maintained between pH 5.5 and 6.5 by routine replacement of the hydroponic solution. After 14 days, plants with a similar development were selected and placed in containers (5 plants per container) using 15 plants per treatment. Four salt treatments were then applied; a control treatment without NaCl (Ct) and three levels of salt through the addition of different concen-
trations of NaCl to the nutrient solution: S1 (50 mM NaCl), S2 (100 mM NaCl), S3 (150 mM NaCl). The application of salt treatments was conducted for a total of 10 days. During the experiments, the average electrical conductivities (EC) of the nutrient solution were 1.23 dS/m, 6.09 dS/m, 10.77 dS/m and 14.95 dS/m, for Ct, S1, S2 and S3, respectively. The experiments were carried out inside a controlled growth chamber, with a temperature (T) of 23 °C/18 °C (day/night), a photoperiod of 16 h/8 h (light/darkness) and a photosynthetically active radiation of 400 µmol•m²/s. At the end of the experiments, leaves were harvested and a sample of 10 leaves per treatment, with similar size, was selected for the acquisition of hyperspectral images.

After image acquisition, all of the leaves harvested, water content and osmotic potential were determined. Leaf water contents were analyzed following the method described by Agüero et al. (2011) with modifications. 20 g of leaf pieces per treatment were taken, homogenized with a commercial grinder, and divided into three samples of five grams per treatment. The samples were dried in an oven (Thermocenter T C40/100, Salvis Lab, Rotkreuz, Switzerland), at 65 ºC during 48 hours, until constant weight. Moisture content (WC) was calculated on wet basis.

For the calculation of the osmotic potential ($\Psi_s$), three samples of five grams of leaf pieces per treatment were collected and frozen at -20 ºC. The samples were centrifuged at 2800 g (9.8 m/s²) during 15 minutes (Centronic centrifuge, J.P. Selecta, Barcelona, Spain). The supernatant was analyzed with a micro-osmometer (Roebeling 13DR, Löser Messtechnik, Berlin, Germany) to determine the osmolarity. The osmotic potential ($\Psi_s$) was calculated with the equation of Van’t Hoff: $\Psi_s = -R \times T \times c_s$. Where $R$ is the ideal gas constant (m³·Pa·mol⁻¹·K⁻¹), $T$ is the temperature (K) and $c_s$ is the osmolarity (Osm·m⁻³).

2.2 Hyperspectral images

Hyperspectral images of the 40 selected leaves were taken with a hyperspectral vision system consisting in a CCD camera and a VIS-NIR spectrometer (Headwall Photonics HyperspecTM) working in the range of 400 – 1000 nm. 189 wavelengths were considered along the range, obtaining a spectral resolution of 3.2 nm. The spectrometer was equipped with a progressive line-by-line scan spectrograph with a slit of 25 µm. The horizontal and vertical spatial resolutions were 0.26 mm/pixel and 0.1 mm/pixel respectively. A halogen lamp was used for the illumination. Specific software, Headwall HyperespecTM, was used to control the equipment. Samples were scanned acquiring the whole surface of the leaf (scan length = 100 mm). A hypercube dataset was obtained from each image. Relative reflectance hyperspectral images were computed simultaneously to the acquisition, by the software of the camera. White reference (barium sulphate) and dark current signal (acquired with the objective of the camera covered by a black tap) were acquired before each batch of images. Then, each line of the image was corrected pixel by pixel subtracting the dark current and dividing this result by the white reference minus the dark current.

The hyperspectral images of lettuce leaves were randomly divided in two different groups: a calibration set, with seven leaves per treatment (28 leaves in total), used to generate the models; and a validation set, with the three remaining leaves of each treatment (12 leaves in total), used to test the models.

The mean spectrum of the surface (excluding the midrib) of each leaf was computed. For processing the second derivative and a normalization by means of standard normal variate algorithm were applied to these mean spectra. On the calibration set of mean spectra a principal component analysis was performed. From the hyperspectral images, adequately processed and projected onto the principal component, were obtained the corresponding images of scores. Complementary an index based on only three wavelengths was proposed and applied to the hyperspectral images to obtain the corresponding virtual images. Analysis of variance and multiple comparison were performed on the 28 mean spectra of the calibration set. The index model was also applied on the leaves of the validation set.
3 Results and discussion

3.1 Analytical results

Water content and osmotic potential data are shown in Table 1. Water content of the leaves decreases progressively when saline concentration increases. The same trend can be observed with osmotic potential of the leaves. This fact suggests that lettuce plants growing in saline conditions have more difficulties to acquire water from the soil and keep less water on their tissues, which is in accordance to previous researches (Eraslan et al., 2007).

<table>
<thead>
<tr>
<th>Saline Treatment</th>
<th>Water content (%) Mean ± STD</th>
<th>Osmotic potential (MPa) Mean ± STD</th>
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<tbody>
<tr>
<td>Ct</td>
<td>94.53 ± 0.09</td>
<td>-0.65 ± 0.04</td>
</tr>
<tr>
<td>S1</td>
<td>94.54 ± 0.12</td>
<td>-1.08 ± 0.09</td>
</tr>
<tr>
<td>S2</td>
<td>93.15 ± 0.18</td>
<td>-1.49 ± 0.11</td>
</tr>
<tr>
<td>S3</td>
<td>92.70 ± 0.07</td>
<td>-2.06 ± 0.09</td>
</tr>
</tbody>
</table>

3.2 Spectral features

The mean spectrum of each saline treatment was computed (calibration set, n= 7 leaves per treatment). Figure 1 (A), shows these mean spectra. It can be observed that Ct global reflectance is lower than the other ones, mainly in the infrared range. This fact can be related to some physical properties inducing lower scattering in the leaves of Ct than in the other leaves.

In water stress conditions, crops often have a higher NIR reflectance level (Tucker, 1980; Clevers et al., 2010; Harris et al., 2006) due to the air replacing water between cells, that produces optical discontinuities and scattering. Blum (1988) states that crops under salt stress conditions usually show the same symptoms than under water stress conditions. Salinity affects the osmotic potential of the plants and to the water availability in the soil, therefore, it affects the water absorption capacity (Yamaguchi and Blumwald, 2005). As it has been seen previously on table 1, in these lettuce leaves the osmotic potential and water content decrease with the increase of salinity. This fact could induce water stress in the plants that could justify the higher NIR reflectance observed in the leaves S1, S2 and S3. This and other effects of the salinity affect the scattering process, which can induce a multiplicative effect on the spectra in the NIR region. For reducing the multiplicative effect Standard Normal Variate process was applied to the raw spectra. Figure 1 (B) shows the normalized spectra.

![Figure 1: A) Mean relative reflectance spectra of the leaves of each saline treatment. Calibration set (4 saline treatments x 7 leaves. B) Same spectra corrected by SNV normalization to eliminate multiplicative effect. C) Zoom in the visible range of SNV corrected spectra](image-url)
absorbencies in the blue and red regions. This fact causes that the spectra become more flat and, therefore, a decrease in the height of the reflectance peak around 550 nm. It could be observed a progressive increase of the reflectance in the bands of absorption of chlorophyll (480 and 680 nm) and a progressive decrease in the peak at 550 nm for higher levels of salinity. It could indicate a slight loss of chlorophyll in the leaves under salinity.

3.3 Principal component analysis on processed spectra

A Principal Component Analysis (PCA) was computed on the processed spectra (Figure 2, top). The first principal component (PC1) was retained as the most related with the changes on salt concentration of the leaves. On Figure 2 (bottom left), the PC1 loadings are shown. The projections of the mean spectra on the PC1 (scores) for each of the four salinity groups are presented in Figure 2 (bottom right).

Two regions of high values of loadings can be observed (Figure 2, bottom left), corresponding to the main regions with differences in the processed spectra: one is located around 500-600 nm and the other is placed on the red edge region. The remaining wavelengths have values of loading close to zero.

PC1 computes the slope between 550 and 510 nm, so as salinity level increases, the slope decrease, and this fact is in accordance with the evolution of the scores. In addition, processed spectra present a displacement to the right at the red edge region. PC1 also computes the curvature of the processed spectra at 710 nm. When the spectra move towards the right, the curvature increases and therefore the scores also increase. Scores progressively evolve towards high values with increasing levels of salinity. Therefore, both mentioned regions could be related to the salt concentration of the leaves. These observations are in accordance with the changes observed in the spectra.

Figure 2: Top: mean processed spectra of the leaves of calibration set for each saline treatment. Bottom left: PC1 loadings from PCA applied on processed spectra. Bottom right: PC1 scores of mean spectra of the leaves of calibration set for each saline treatment (Ct, S1, S2 and S3 treatments), mean values of each group are shown in red.

Figure 3 shows the images of scores obtained by the projection of the hyperspectral images of the leaves from calibration set, corrected by second derivative and Standard Normal Variate algorithm, on the PC1. Clearly, the differences between Ct and S3 leaves can be
appreciated, evolving from deep blue pixels (low score value) to orange and red pixels (high score value) when salinity concentration increases.

Figure 3: Images of scores obtained by projection of the hyperspectral images from calibration set, on PC1. Top: Ct treatment. Bottom: S3 treatment

3.4 Index on the red edge region

As it has been explained previously, PC1 loading shows the red edge region as the most sensitive area to the changes in the spectra induced by the salinity. Consequently, it is proposed an index (LSI) based on the most relevant wavelengths of PC1 loading. This LSI index is an approximation to the second derivative at 710 nm and it is related to the red edge displacements: \( \text{LSI} = \left(\frac{R675 + R745}{2}\right) - R710 \). LSI was applied to each pixel of the hyperspectral images of the calibration set, in order to generate the corresponding virtual images (Figure 4).

Figure 4: Virtual images obtained computing LSI index on the hyperspectral images of the calibration set leaves. Top: Ct treatment. Bottom: S3 treatment.
On Figure 4, it can be observed clear differences between the LSI values from the pixels of the leaves corresponding to the control treatment (Ct) and the pixels of the leaves corresponding to the highest salinity treatment (S3). The results of the analysis of variance and multiple comparison on the LSI values calculated on all the pixels of the 28 leaves of the calibration set, showed significant differences among the salinity levels, with a continuous and progressive trend (Figure 5).

![Figure 5: Analysis of Variance of the values of all the pixels of the leaves for each saline treatment applying the LSI index. Calibration set, n= 28 leaves (4 saline treatments x 7 leaves, 32000 pixels/leaf). Horizontal lines represent the mean ± the standard error.](image)

![Figure 6: Virtual images of LSI index of leaves belonging to validation set.](image)

The LSI index was applied to the leaves of the validation set. Figure 6 shows the results; it can be observed the differences between the leaves of Ct treatment (left) and the leaves of S3 treatment (right), similarly to the results obtained in the calibration set.

### 4 Conclusions

Hyperspectral images were applied to sense non-destructively the effect of salinity in the leaves of just harvested “baby” lettuce. These images were employed for searching the best combination of wavelengths regarding salinity sensing. One index, based on the combination of three wavelengths of the red edge region allowed identifying differences among the leaves under different saline treatments. Additionally, hyperspectral images allowed observing the distribution of the salinity effects on the surface of the leaves, which are more intense in the areas distant from the veins.

### 5 Acknowledgements

The funding of this work has been covered by the MICINN with the project Multihort (AGL2008-05666-C02-01). LPF-TAGRALIA is part of the CEI Moncloa Campus of Excellence, UPM-UCM.
6 References


