Visible and Near-Infrared Reflectance Assessment of Salinity Effects on Barley
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ABSTRACT
We studied the effects of a soil salinity gradient (0.8-1.9 dS m⁻¹ ECA) on spectral reflectance of 10 genotypes of barley (Hordeum vulgare L.) to determine the efficacy of reflectance as a tool for assessing the responses of barley to salinity. NDVI [normalized difference vegetation index, \( (R_{900\ \text{nm}} - R_{680\ \text{nm}})/(R_{900\ \text{nm}} + R_{680\ \text{nm}}) \)] and WI (water index, \( R_{970\ \text{nm}}/R_{900\ \text{nm}} \)) were the reflectance indices used. In response to increasing salinity, near infrared reflectance decreased and visible reflectance increased, thereby lowering NDVI from 0.85 to 0.4, in parallel with decreases in biomass (from 2500-500 g m⁻²) and yield (from 900-50 g m⁻²). NDVI was, thus, a good indicator of biomass and yield. WI increased from 0.73 to 0.95, 8T (canopy temperature minus air temperature) increased from -2 to 7°C, and ¹³C discrimination (Δ¹³C) increased from 10 to 14.5 with increasing salinity. WI was, thus, related to crop water status response to salinity. NDVI and WI were, therefore, useful for measuring agronomic responses of barley to salinity.

In saline areas or in areas irrigated with saline waters, the yields of most crop species are reduced. Barley is one of the few commercial cereal crops which is relatively tolerant to salinity. Research programs were started in California 20 yr ago to screen and breed barley for salt tolerance (Norlyn, 1980). However, there are very few salt tolerant genotypes (Blum, 1988) even though saline areas represent about one third of irrigated areas on Earth (Maas and Hoffman, 1977). Because classical screening of yield response is very expensive, breeders search for indirect parameters that are easy and rapid to use so that many genotypes can be screened in relatively short time.

Salinity decreases both growth and net photosynthesis, which in turn reduces biomass and yield of higher plants (Long and Baker, 1986); remote sensing of biomass is a possible salt-response screening tool. For crops such as cereals, biomass may be a screening parameter of interest even in non saline conditions because it may be difficult to achieve further improvements in harvest index after a century of continuous achievements (Austin et al., 1980, Elliott and Regan, 1993). In the future, genetic improvements in grain yield may depend more on increasing biomass rather than harvest index (Austin et al., 1980). Under Mediterranean conditions, winter and early spring rainfall maximizes biomass production at the pre-anthesis and anthesis stages (Turner, 1982). Since the harvest index is approximately constant, yield is generally proportional to biomass at anthesis.

Superior genotypes have been identified by biomass determination through destructive sampling (Regan et al., 1992) but such sampling is unfeasible in large breeding trials because of the high labor requirement and the large sampling errors (Whan et al., 1991). The measurement of reflectance spectra by ground-based remote sensing techniques has the potential to provide an accurate, non-destructive estimate of plant biomass through the widely used NDVI (Tucker, 1979; Peñuelas et al., 1993a).

Salinized plants often exhibit symptoms of water deficit, especially under conditions of high evaporative demand (Blum, 1988). Therefore, salinity response may also be characterized by remotely sensing plant water status. Sensing the thermal radiation emitted by the canopy is one way of assessing water stress (Peñuelas et al., 1992; Araus et al., 1993). As water becomes limiting, leaf temperature increases above air temperature because transpiration is reduced (Raschke, 1960; Nobel, 1983; Berliner et al., 1984).

Another method of assessing plant water status depends on absorbance of light by water at certain near-infrared wavelengths. The higher the tissue water content the greater the absorbance, and consequently the lower the reflectance. Methods for indirect measurement of plant water stress based on leaf and canopy reflectance have been developed by several researchers, but there is controversy over their utility (see references in Bowman, 1989; Shibayama et al., 1993). However, Peñuelas et al. (1993b) showed that the ratio of reflectance at 970 nm, one of the water absorption bands, to reflectance at the reference 900 nm wavelength (\( R_{970}/R_{900} \)) closely followed the changes in relative water content (RWC), leaf water potential, stomatal conductance, and the foliage temperature minus air temperature differences when plant water stress was well developed. Sensitivity of the spectral reflectance trough at 970 nm seems to be due to the greater penetration of radiation into the canopy at 970 nm, compared with longer water absorption wavelengths (Bull, 1991).

This study was conducted to investigate the relationship between reflectance, biomass production, and plant water status under different salinity levels in barley field trials. We focused on reflectance spectral indices indicative of biomass (e.g., NDVI, Peñuelas et al., 1993a;)

Abbreviations: NDVI, normalized difference vegetation index; WI, water index; 8T, temperature difference between the air and the plant; Δ¹³C, carbon isotope discrimination.

Gamon et al., 1995) and water status (e.g., WI, Peñuelas et al., 1993a,b) as possible tools for assessing crop variation in response to salinity.

**MATERIALS AND METHODS**

**Experimental Setup**

Ten genotypes of barley with different salt tolerance were sown in a gradient of soil salinity in an experimental field. The experiment was carried out in Zaragoza (Ebro Valley, Spain, UTM coordinates: 30 T XM x = 815, y = 216, z = 225). Each of the 10 barley genotypes was sown on 19 Nov. 1993 in plots six rows wide and 1.26 m long. Drill spacing was 0.21 m, and plant density was 250 plants m⁻². A triple line source sprinkler system was used to create a soil salinity gradient in 10 plots of each genotype according to the methodology proposed by Aragüés et al. (1992) and Royo and Aragüés (1993). The triple line source consisted of three parallel sprinkler lines separated by a distance equal to the sprinkler's wetted radius. An equal quantity of water was applied through each line, but a saline solution was injected into the center line. The result was a continuous gradient of salinity with the same volume of applied water between each lateral pair. The first saline irrigation was applied on 31 January, when the plants were at the three leaf phenological stage. The last saline irrigation was applied on 31 May. A total of 28 irrigations (13 mm, two or three times each week) were applied.

The total water received (irrigation + rainfall) was 469 L m⁻². Evapotranspiration of well irrigated barley measured the same season with a lysimeter located in the experimental field was 271 L m⁻². Thus, water deficit stress was likely not present in this study. The average salinity of applied water varied from 1.7 to 15.2 dS m⁻¹. Soil apparent electrical conductivity (ECa) was measured six times during the growing season using an electromagnetic sensor (Model EM-38 of Geonics, Ltd., Ontario, Canada) that integrates 1-m depth salinity. The average value of the six measurements was used to characterize the salinity of each plot. Coefficient of variation of the six measurements was lower than 5%. The Geonics sensor EM-38 allows a quick, non-destructive measurement of soil salinity in the field (Rhoades and Corwin, 1981; Díaz and Herrero, 1992). Soil samples were taken for measurement of electrical conductivity in the saturation extract (ECe) the 25 March in order to establish the relationship between ECa and ECe. The calibration curve was as follows:

\[
ECe(0 - 50 \text{ cm}) = -0.86 + 5.08 \text{ ECa} \\
(n = 13, r^2 = 0.94).
\]

The range of salinity calculated as ECe, from -0.532 to 8.79 dS m⁻¹ ECe, included the threshold salinity levels previously reported to reduce barley growth (Maas and Hoffman, 1977; Richards et al., 1987). The average gravimetric soil water content (0- to 50-cm depth) was 21% with a saturation percentage of 55%. Plant spectral reflectance and the following biophysical parameters were measured 167 d after sowing, on April 27, corresponding to the booting phenological stage.

**Biomass and Yield Measurements**

Just before harvest, when plants were completely air dry, total above-ground biomass was measured in a control (non saline treatment, ECa = 0.8 dS m⁻¹), intermediate (ECa = 1.26 dS m⁻¹), and saline treatment (ECa = 1.72 dS m⁻¹) in all the genotypes evaluated. All plots were mechanically harvested on 22 June 1994 to obtain the grain yield.

**Chlorophyll Analysis**

At the same time that the spectral reflectance determinations, a SPAD chlorophyll meter from Minolta (model SP02, Minolta, Japan) was used to measure leaf chlorophyll concentration (Monje and Bugbee 1992) of six flag leaf blades per treatment plot. The relative units were previously calibrated measuring leaf chlorophyll concentrations by shaking fresh leaf discs in N,N'-dimethylformamide (Porra et al., 1989).

**Carbon Isotope Discrimination**

About 300 mature kernels taken from at least 10 spikes in each plot were dried at 60°C and ground to a fine powder. The ¹³C/¹²C ratios were determined by mass spectrometry by Isotope Services Inc. (Los Alamos, NM). The standard for comparison was a secondary standard calibrated against Peedee belemnite (PDB). Precision of the analysis was less than 0.1‰. ¹³C Discrimination was expressed as Δ following Farquhar et al. (1989).

\[
\Delta = \left( \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right) \cdot 10^3 \\
\delta_{\text{air}} = 8 \%o \\
R_{\text{sample}} = \frac{1}{\Delta} \cdot \left( 1 + \delta_{\text{sample}} \right) \\
\delta_{\text{sample}} = \delta_{\text{air}} - \delta_{\text{standard}}.
\]

In C₃ cereal grain, D provides an integrative measurement of WUE during the grain-filling period (Farquhar and Richards, 1984; Araus et al., 1993) and has been proposed as a possible screening method for identifying variation in WUE in barley (Hubick and Farquhar, 1989).

**Infrared Thermometry**

Canopy temperatures were measured at booting stage in all genotypes, with a Raynger II-PAG model infrared thermometer (Raytek Inc., Santa Cruz, CA) having a 15 degree field of view. The average temperature of each replicate was obtained by holding the thermometer about 0.7 m above the canopy and aiming it to the South at an angle of about 20 degrees from the horizontal. Under these viewing conditions a canopy area 0.2 m in diameter was measured. Plant canopy components filled the instrument's field of view.

**Spectral Reflectance Measurements**

Canopy radiance was measured with a narrow-band-width visible/near-infrared spectroradiometer fitted with 15 degree field-of-view optics (model SE590 with detector model CE390WB-R, Spectron Engineering, Inc., Denver, CO), and was expressed as spectral reflectance after standardization by radiance of a leveled reference standard (LabSphere Inc., North Sutton, NH). The instrument detects 252 spectral bands approximately evenly spaced between 390 and 1100 nm. When data were collected, the sun was at 40 to 50° solar zenith angle. All spectral measurements were made with the spectroradiometer pointed vertically downward (nadir) in the principal plane of the sun from 2-m height by mounting it on a boom. Four scans were averaged for each measurement (genotype × salinity treatment combination). Reflectance standard measurements were made immediately before and after the canopy spectral measurement. The measurements were made at booting or at the beginning of ear emergence stage depending on genotype, in cloudless conditions. The biomass reflectance index, NDVI, was calculated as \( R_{900} - R_{680} / R_{900} + R_{680} \) (Peñuelas et al. 1993a), and the water status reflectance index, WI, as \( R_{970} / R_{900} \) (Peñuelas et al., 1993b).
Fig. 1. Spectral reflectance in the visible and near-infrared for three different levels of salinity (measured as ECₐ) in the genotype Alpha. Error bars are ± SEM. Genotype Alpha response is depicted as a typical example of the response of the tested barley genotypes to salinity.

Data Analysis

All data on crop and reflectance parameters were analyzed by means of conventional statistical analyses. The relationships among reflectance and biological parameters were examined by correlation analysis. Principal component analyses were also conducted. SPSS version 4.0 (SPSS Inc., Chicago), Systat version 5.2 (SYSTAT Inc., Evanston, IL) and Igor (WaveMetrics, Lake Oswego, OR) were used for data analysis.

RESULTS

Reflectance spectra differed among salinity treatments in both the visible and in the near-infrared (Fig. 1). The greater biomass in low salinity plots decreased visible reflectance and increased NIR reflectance and therefore increased NDVI (Fig. 2). WI increased with salinity (Fig. 2) indicating a poorer water status. Significant differences in both NDVI and WI were found mainly between the two highest salinity levels and the other eight levels (Fig. 2).

Both biomass and yield were logarithmically related with NDVI (Fig. 3). NDVI was linearly related (P < 0.01) with biomass and yield across genotypes under only the high salinity treatment (ECₐ = 1.72 dS m⁻¹), corresponding to smaller values of NDVI in Fig. 3. The parameters related to water status, δT, Δ¹⁸C, and yield were significantly correlated with WI (Fig. 4). NDVI was not correlated with δT as a measure of water status and WI was not correlated with chlorophyll content (data not shown). There was only a slight decrease in chlorophyll content at highest salinities (about 10% for the most sensitive genotypes, data not shown).

Multivariate principal component analysis including both biological and optical parameters produced three independent factors that explained 56, 26, and 7.1%, respectively, i.e., 89% of the total variance. They were identified as indicators of salinity, barley variety, and chlorophyll content (Fig. 5).

DISCUSSION

Biomass per unit ground area is the main determinant of the amount of leaf tissue in the field of view of the reflectance sensor. The large decrease in biomass increased reflectance in the visible and decreased it in the NIR (Fig. 1), in agreement with previous work relating visible reflectance with green biomass (Peñuelas et al., 1993a, 1996; Gamon et al. 1995). Thus, NDVI became a good indicator of barley biomass (Fig. 3). There were good relationships between biomass and yield, and therefore between yield and NDVI (Fig. 3).

The reflectance spectral measurements were made during the late growth period when biomass is more important as a possible criterion for salt tolerance screening. Under Mediterranean conditions, the higher the biomass production by anthesis, the higher the final yields.
(Turner, 1982). In fact, measurement of growth rates is the method currently used to screen plants for salt tolerance (Munns, 1993). Differences in growth rates among different barley genotypes may be evaluated quickly with the NDVI. However, once the biomass exceeded 1500 g m\(^{-2}\), there was no variation in NDVI (Fig. 3), suggesting that this index should be used at high levels of salinity or during early growth stages, when the biomass is lower and the differences in NDVI are higher. NDVI was linearly related to biomass and yield for the different genotypes for the high salinity treatment alone (smaller values of NDVI in Fig. 3). It could thus be used for screening barley for salt tolerance. The reflectance measurements should also be useful for non saline conditions if made before above ground biomass reaches about 1500 g m\(^{-2}\), when reflectance measurements asymptote and become insensitive to biomass.

WI (R970/R900) increased with salinity, especially at salinity levels higher than 1.6 dS m\(^{-1}\) (Fig. 2). Since WI is based on the water absorption band at 970 nm, it indicates a lower water content in the field of view of the spectroradiometer at higher salinity. WI was related with \(\Delta^{13}\text{C}\), \(\Delta T\), and yield (Fig. 4). \(\Delta^{13}\text{C}\) and canopy minus air temperature were negatively correlated, which is consistent with the positive correlation between \(\Delta^{13}\text{C}\) and stomatal conductance (Farquhar et al., 1989). Similar correlations have been reported previously for wheat (Araus et al. 1993). Moreover, positive correlations have been reported between kernel \(\Delta^{13}\text{C}\) and either biomass or grain yield in barley (Romagosa and Araus, 1991), when grown under favorable soil water conditions. Reflectance measurements were made at midday, when water demand is maximal. Because all treatments had an adequate water supply, the WI response may be attributed to the effect of salinity on plant water status. The differences in WI are notable since the water supply was good in all treatments. The correlation between WI and \(\Delta^{13}\text{C}\) is also notable because WI was measured at a single point in time whereas \(\Delta^{13}\text{C}\) is an integrative measure of WUE during the grain filling period. The results showed that WI is a good indicator of plant water status in response to salinity and confirm recent results showing that the reflectance at 970 nm may allow the detection of plant and canopy water status (Shibayama et al., 1993, Peñuelas et al., 1993b, 1996). The WI could also improve the methodology of salt tolerance screening by reflectance techniques.

In summary, this study has shown that barley salinity response can be assessed by the reflectance indices NDVI and WI, based on the biomass and water status, respectively, or a combination of both. The use of spectral reflectance should provide a sensitive method for screening different barley genotypes in response to salinity,
especially during early growth or under high salinity treatments when biomass and yield are reduced.

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