Early detection of drought stress in potato (*Solanum tuberosum* L.) and grapevine (*Vitis vinifera* L.) crops through multifractal analysis applied to remotely sensed data

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Abstract

Drought stress is a growing agricultural concern, since climate variability and change are affecting the water cycle worldwide, and an increment of drought periods in some regions of the world have been forecasted for the upcoming years. Several methodologies are being developed for assessing, monitoring, and managing water availability in order to supply the accurate water amount to crops attaining the highest possible water use efficiency. In order to determine the ability of remote sensing for identifying and monitoring drought stress in potato and grapevine crops, continuous measurements of multispectral reflectance of plant canopies have been analyzed, and compared with measurements of physiological parameters simultaneously registered using gas exchange techniques, and existing spectral vegetation indices. The results evidenced that multifractal analysis of reflectance data in potato did discriminate between the Control (well irrigated) and Drought treatments around 6 days before the conventional gas-exchange assessment detected any difference. The difference was also shown by the split-reflectance spectrum. In grapevine, multifractal analysis did discriminate among treatments around 2 days prior to its discrimination by either gas-exchange or sap-flow. Therefore, multifractal analysis applied to multispectral remotely sensed data might become a useful tool for early drought stress detection to improve the water use efficiency by crops.

Keywords: Multispectral reflectance, Potato crop, Grapevine crop, Drought stress, Multifractal analysis, Wavelet transform.

Introduction

Several attempts have been carried out in order to find a reliable methodology to assess drought stress in plants, since some common methods are becoming old-fashioned e.g. validity of the Scholander pressure chamber awaits new experimental evidence (Cochard *et al.*, 2001). Gas-exchange techniques have demonstrated high success in measuring the main physiological parameters of plants such as stomatal conductance (*g*) and photosynthesis (A_N), but their disadvantage is the requirement of expensive complex equipments and well-trained technicians. Remote sensing, largely improved during the Second World War (Campbell, 1996; Lillesand *et al.*, 2004), has been used for monitoring the status of crops and natural vegetation on the basis of changes in plant reflectance patterns.

Fast metabolic changes occurring in plants, alter the reflectivity and propagation of solar radiation inside plant tissues, where a fraction is absorbed and other reflected in all directions (Gilabert *et al.*, 1997). Remote sensing captures details of reflectivity and absorption of light which are linked to the biochemical and structural components of the plant, such as chlorophyll, other pigments, water content, proteins, leaf thickness, cellular structure and cell wall and other biochemical materials (Ritchie, 2003; Blackburn and Ferwerda, 2008). All these components are affected by stresses, resulting in differences in the spectral signature of healthy and stressed plants that can be assessed through reflectance measurements (Chávez *et al*, 2009a). However, interpreting these patterns is not an easy task since most of the compounds of plant tissues act as radiation filters affecting the resultant spectrum, thus converting the reflectance response into a complex signal. Most of the meaningful spectral information is contained in small non-visible changes of the signal that conventional analysis had always obviated since they just focus on a small number of optimal wavebands while discarding the majority of the spectrum. Therefore, mathematical tools such as wavelets and multifractal analyses have the potential to capture much more of the information contained in reflectance spectra.

The aim of this work was to assess the value of remotely sensed reflectance as a reliable technique for early detection of metabolic changes caused by drought in plants, instead of destructive conventional methods. Also, the relevance of multifractal analysis to improve the accuracy and earliness of reflectance data for drought-stress diagnosis is presented.

Materials and methods

Plant material and treatments

Potato. Two experiments with potato (*Solanum tuberosum* L.) plants were carried out in a greenhouse in Mallorca, Spain, during the winter and autumn of 2006. Thirty plants of the cv. Marfona for the first experiment and 18 plants cv. Marie Spears for the second one, were grown in 10 litres pots of vegetal substrate (90%) and perlite-stone (10%). Three watering treatments were applied: *Control* (Ctrl), 100% of the daily measured evapotranspiration (dme), *moderate-drought* (D75), accounting for 75% of dme, and *severe-drought* (D50), 50% of dme. Management was similar for all plants. The treatments were initiated some 2 weeks after emergence. Thereafter, pots were weighted every day to determine water availability from evapotranspiration and irrigation was applied according to the treatment of every potted plant. Leaves from the same canopy strata were detached from sampled plants and their respective fresh weight (FW) registered immediately. Leaves were then kept in distilled water during 24 hours for determining their turgid weight (TW). Their dry weight was obtained after 48h at 60ºC in a forced air oven. The relative water content was determined as RWC = [(fresh weight – dry weight) / (turgid weight – dry weight)]x 100. Four replicates per treatment were obtained from different individuals.

Grapevine. An experiment was performed in six-year old grapevine (*Vitis vinifera* L.) plants under outdoors conditions, in Mallorca, Spain, during the summer of 2006. 12 plants cv. Tempranillo grafted on R-110 rootstock were grown in 60 litres pots containing a 20:80 v/v mixture of organic matter and sandy loam soil. Plants were irrigated daily until the end of July, and gas-exchange parameters and reflectance of solar radiation were measured. Plants were then submitted to three treatments (4 per each one) that lasted until the second week of September. The drought treatments were caused by stopping the irrigation of plants during 5 days per week. For treatment 1) *severe-drought* (D1), irrigation was re-initiated at the evening of the 6th day; and for treatment 2) *moderate-drought* (D2), irrigation was re-initiated at the early-morning of the 6th day. The *Control* (Ctrl), was normally irrigated at field capacity. Management was similar for all plants before application of the treatments. A 2cm layer of perlite and a thermal-keeper film was extended over each pot to diminish direct soil evaporation.

Reflectance measurements

During the first indoor experiment with potato, canopy reflectance was measured with a USB2000 spectrometer (Ocean Optics, USA) covering the 350-800nm wavelength region, with a resolution of 0,5nm. During the second potato experiment and the grapevine experiments, a high resolution spectrometer HR-2000 (Ocean Optics, USA) covering the 180-1100nm wavelength region, resolution 0.065nm, was used. In both experiments, a white Spectralon® panel was used for calibration measurements, resulting in relative reflectance values. Three reflectance measurements per sample were taken and averaged to estimate its spectral variability. Measurements were performed every 1 or 2 d for grapevines and 2 or 3 d for potatoes, at noon, when solar radiation was projected vertically to the Earth. So, reflection was measured under an angle of 15º from nadir, minimizing shadow effects. The aperture angle of the fore optics of the spectrometer was 25°, placed at a distance of 2 cm from the leaf surface, resulting in a projected Field of View of around 1cm in diameter.

Gas exchange measurements

In both potato and grapevine, gas exchange measurements were performed using a portable infrared gas analyzer (IRGA) Li-6400 (Li-Cor Inc., Lincoln, USA).

Sap flow measurements

Sap flow measurements were made using the thermo heat balance method (THB), which applies external heat into one point of the conductive xylem and sensed internal temperature in another near point of the same branch. Then, the sap flow was calculated according to the heat losses and flux-gradient theory (Lindroth *et al.*, 1995).

Wavelet and multifractal data analysis

The analysis described by Chávez *et al.* (2009b) was slightly modified by splitting the spectra into 4 bands to mimic those of Landsat TM: blue (450-520nm), green (520-600nm), red (630-690nm) and NIR (760-900nm). The proportion of the reflected radiation per band was calculated as a function of time (growth), resulting in heterogeneous reflectance spectra displaying singularities through time. This assessment was carried out to contrast the information it provided against the results of the wavelet-multifractal analysis of the entire spectra.

A software that describe the canonical method of wavelet multifractal modulus maximum (WTMM), originally implemented by McAteer *et al.* (2007) was modified to analyze the reflectance data. The methodology described by Arneodo *et al.* (1988) was followed and adapted to run in IDL6.3 for Windows. Data were processed with the Continuous Wavelet Transform (CWT) and the WTMM. The mother wavelet analyser used was the second derivative of the Gaussian function (Mexican hat). For more details, review Arneodo *et al.* (1995), McAteer *et al.* (2007) and Chávez *et al.* (2009b).

Data pre-processing

The pre-processing consisted of two sequential steps. In the first one, a background correction was required to account for variations in the signal due to both natural changes in the atmosphere within a sampling date. In the second step, the anomalies over moving averages of 41 wavelengths (see equations 2 and 3) were calculated. Anomalies reduce the signal to noise ratio thus making small fluctuations in the physiology of the plant more perceptible to the analyses. The background correction of the first step was performed by adjusting the measured response (G) of individual plant signals to a reference response through fitting a linear regression (Equation 1; Yarlequé, 2009).

Background correction: $S(t_i) = A.G(t_i) + B$ (1)

Where, $A = \frac{(G_{max} - G_{min})}{(G_{Total\ max} - G_{Total\ min})}$; in this equation the numerator is measured from every plant, while the denominator is the difference between maximum and minimum values of recorded reflectance among all the plants (Ctrl and PYVVi) in one determined date; and *G* and *S* are the measured and estimated passive reflectance according to *t* wavelength (nm), respectively. Finally, $B = G_{min} - A.G_{Total\ min} = G_{max} - A.G_{Total\ max}$

Moving average: $\hat{S}(t_i) = \sum_{k=i-20}^{i+20} \frac{S(t_k)}{41},$ (2)

where *t* is the wavelength (ranging from 390 to 1020 nm), *i* is the time at the middle of the analyzed moving window, and *k* is the counter that allows the analyzed window to move from -20 to +20 .

Anomalies: $S'(ti) = S\ (ti) - \hat{S}\ (ti)$ (3)

Statistical analysis

Differences between treatments means were analyzed with time-repeated measurements analyses performed with the SPSS 12.0 software package (SPSS Inc., Illinois, U.S.A.).

Results and discusion

Multifractal spectra of reflectance

The raw reflectance spectra measured from plants did not allow a clear discrimination of the treatments, although some regions of the spectra regions demonstrated different reflectance values. However, discrimination was noticeable after pre-processing and multifractal analysis of data from the 8th day post treatments application (dpt) (data not shown) for the first experiment, and from the 6th dpt for the second experiment (Figure 1), i.e. 7 and 5 days earlier than the gas-exchange measurements, respectively.

In grapevine, the resultant multifractal singularity spectra did show that after only 6 dpt, both D1 and D2 were completely differentiated from the control (Figure 2), and differences among treatments were held throughout the rest of the experiment. Differences were noticed 2 days earlier than showed by the gas-exchange measurements.

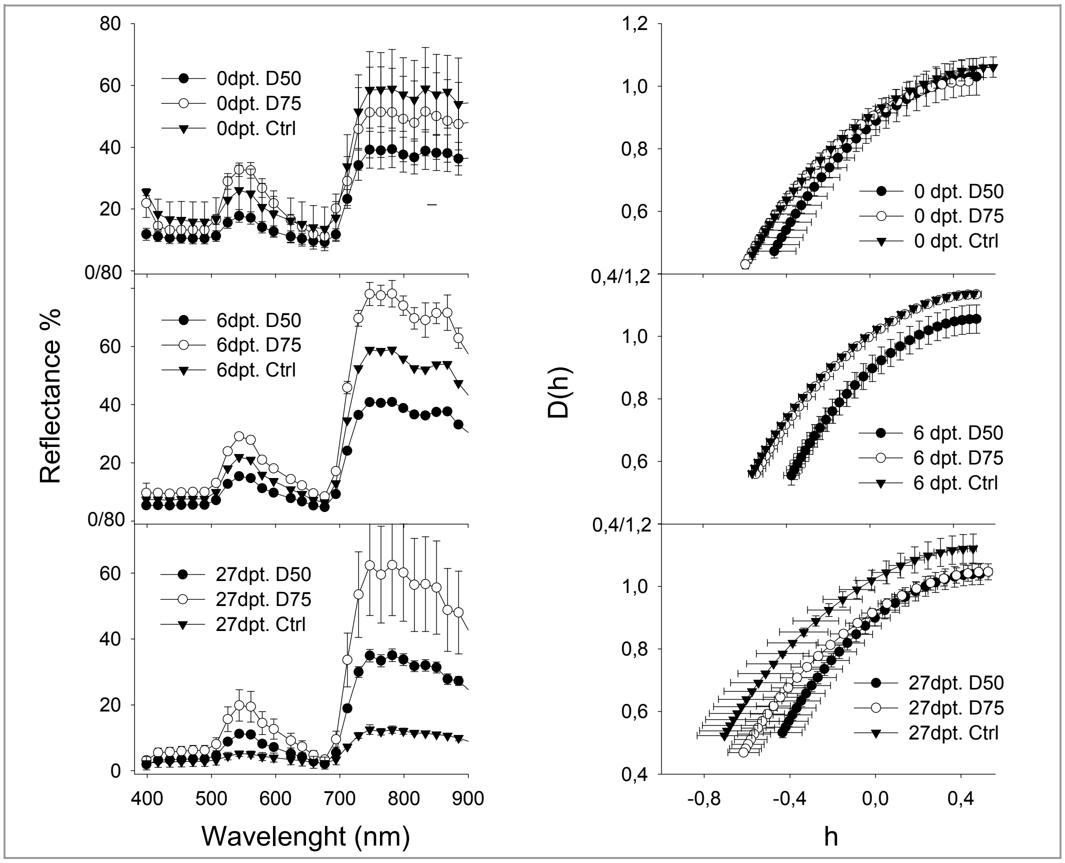

Figure 1. Passive reflectance of potato plants (*left*) obtained by the high resolution spectroradiometer HR2000, and their correspondent multifractal singularity spectra (*right*). Second experiment

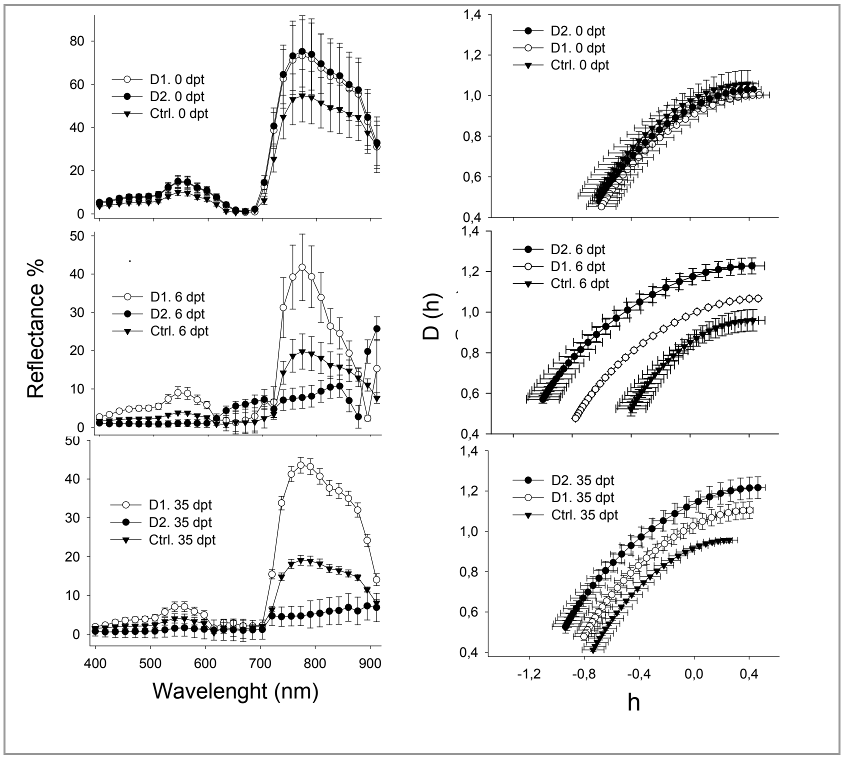

Figure 2. Passive reflectance of grapevine plants (*left*) obtained by the high resolution spectroradiometer and their correspondent multifractal singularity spectra (*right*)

Reflectance spectrum by regions and Vegetation Indexes

In potato, stress in plants was evidenced by their distinct reflectance pattern from around 7 and 5 dpt for the first and second experiment, respectively ($P<0.05$) (Figure 3). In grapevine, such differences were evident around 7 dpt ($P<0.05$). The main bands for detecting drought stress were the blue ($P<0.01$) followed by NIR, red and finally the green region of the electromagnetic spectrum ($P<0.05$).

In contrast, the spectral vegetation indexes (SVI) tested did show an inconsistent response (data not shown), even those specifically developed to retrieve water content of plants. SVI demonstrated to be an unreliable method for real-time monitoring of water status at whole plant level, probably due to the focusing on a few optimal wavebands while discarding the majority of the spectrum (Blackburn and Ferwerda, 2008). These findings confirm the statement of Gamon *et al.* (1990), Peñuelas *et al.* (1995) and Dobrowski *et al.* (2005), that SVIs are less useful for this dynamic monitoring.

Gas exchange measurements

The first potato experiment showed differences among treatments for photosynthesis rate (A_N) and stomatal conductance (*g*) from the 8th and 10th dpt, respectively ($P<0.01$) (Figure 4). During the second experiment, daily A_N and *g* did not show a clear trend. In grapevine, daily gas-exchange measurements did show rates with differences among treatments for A_N and *g* from the 8th dpt ($P<0.01$).

Potato Relative Water Content (RWC)

During the first experiment the RWC for D50 was lower than for both D75 and Ctrl ($P<0.05$) at the 6th dpt, reaching their maximum difference at 13th dpt and onwards ($P<0.01$). During the second experiment, differences among treatments occurred at 10th dpt ($P<0.05$). However, at the 17th dpt RWC appeared similar ($P\geq0.05$) probably due to a sudden increase of relative humidity (%), but later turned different again until the end of the trial, 26th dpt ($P<0.01$).

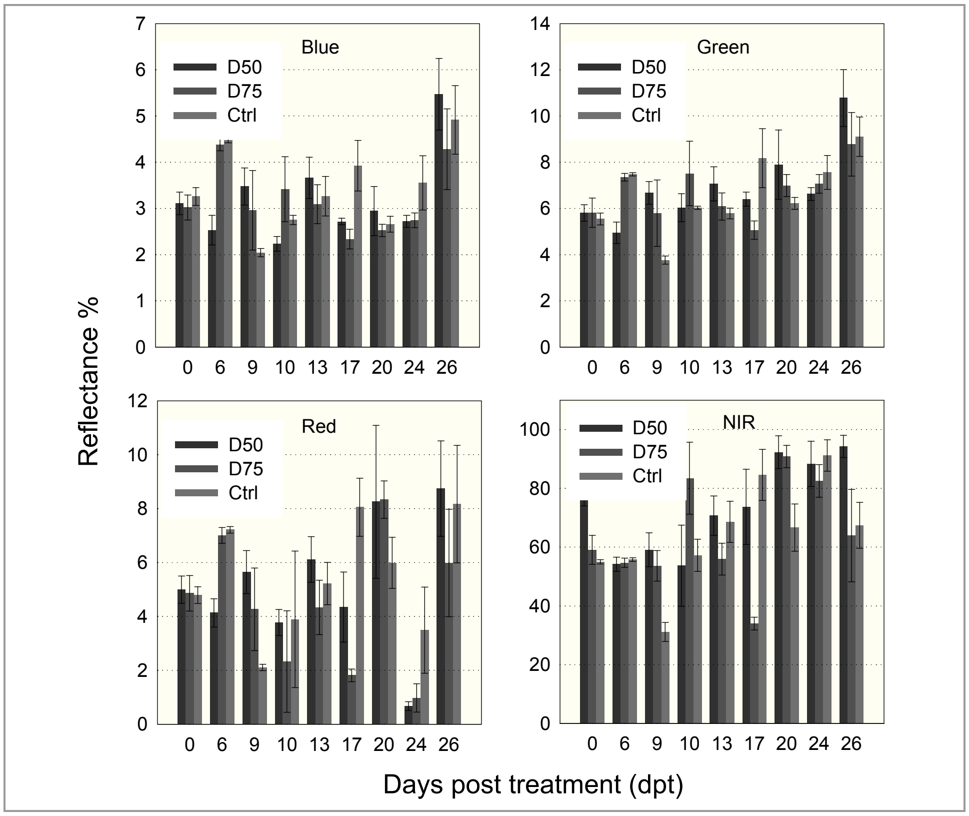

Figure 3. Reflectance of potato plants (2nd experiment) divided according to the bands of satellite Landsat TM

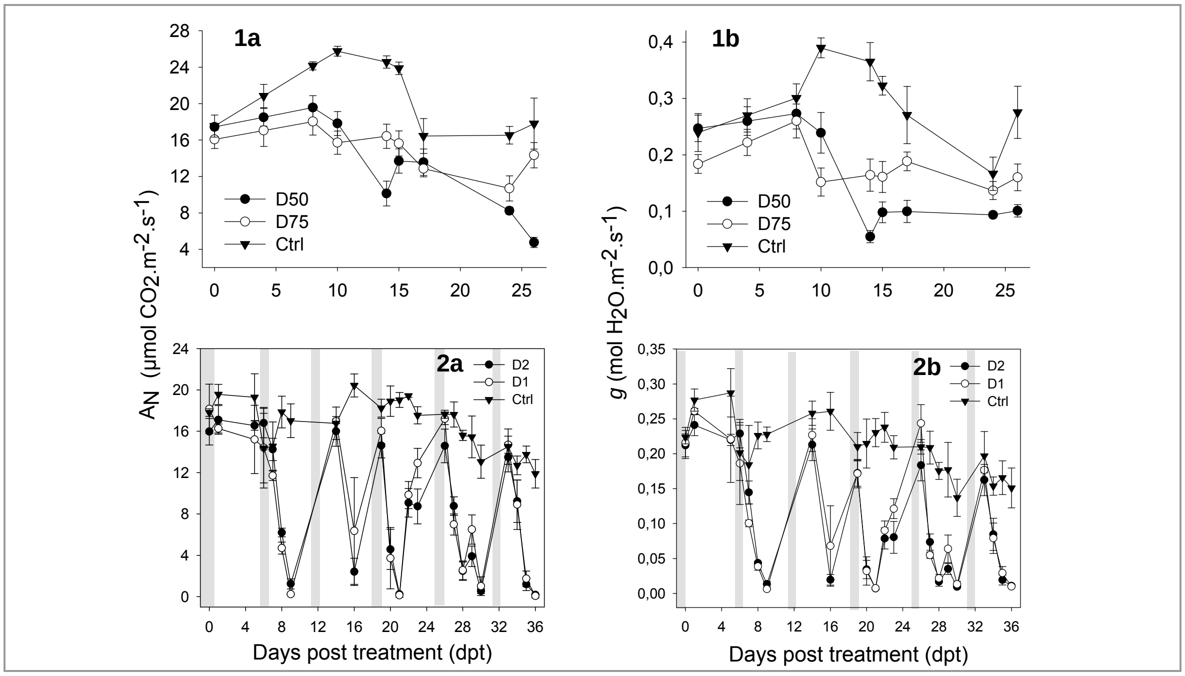

Figure 4. Gas exchange rates of plants of the potato (1) and grapevine (2) experiments. Daily photosynthesis (*a*) and stomatal conductance (*b*)

Sap flow measurements

A high similarity was observed between daily transpiration rates measured by the sap flow and gas-exchange in grapevine. During a typical sunny day (about 1500 µmol.m^{-2}.s^{-1}, average temperature at midday around 33ºC and 55% average of relative humidity), a high slope of sap flow was observed during the first hours in the morning. As the drought stress augmented, the sap flow slope progressively decreased day by day. Then, a continuous cycle of watering followed by progressive dehydration was observed weekly (Figure 5).

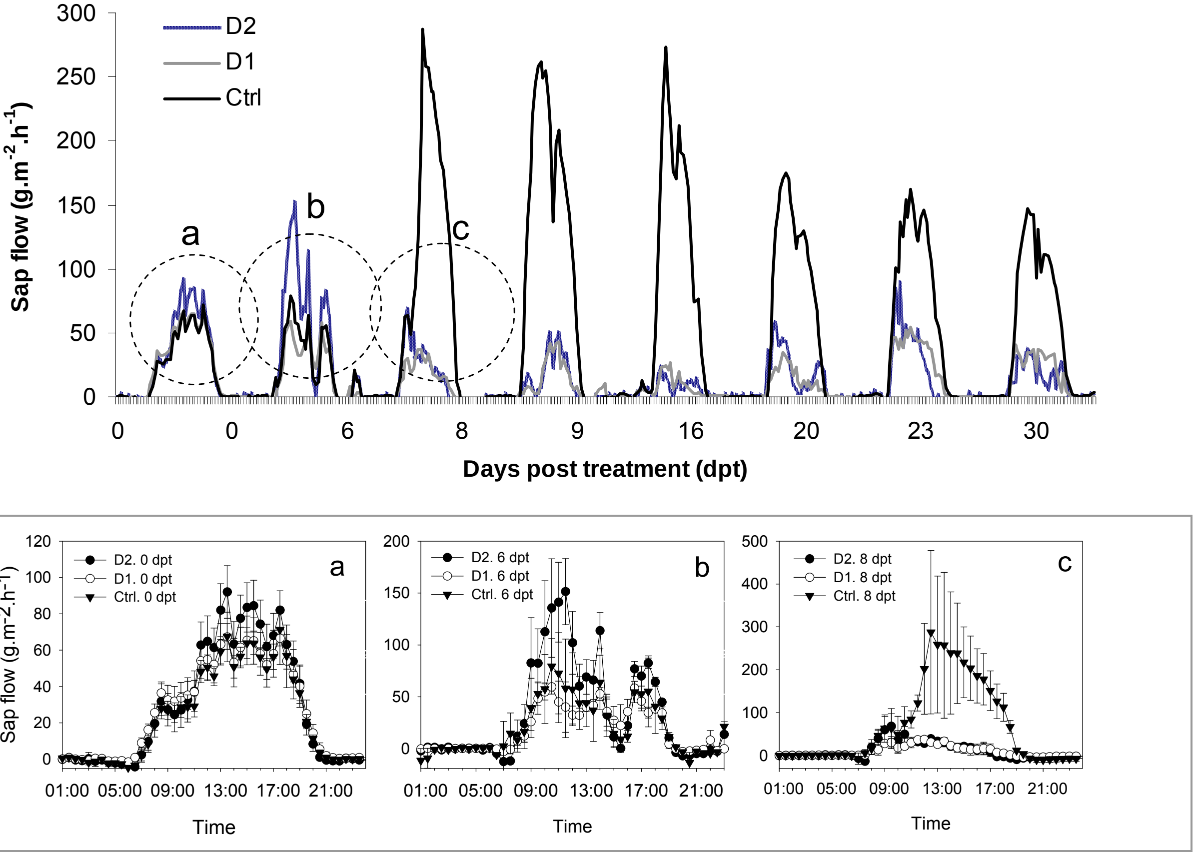

Figure 5. Continuous records of sap flow and details of readings. At the beginning of the experiment, detail in *a* indicates that there are not differences among treatments. Similar behaviour is shown in *b*. In contrast, difference among control and drought treatments is observable in *c* from day 8 post treatment and onwards

Tuber yield

Potato is sensitive to drought with reductions in yield (Jefferies, 1993; Gregory and Simmonds, 1992; Jefferies and Mackerron, 1987; van Loon, 1981). However, our results indicated that potato was almost insensitive to moderate drought. Indeed, Ctrl and D75 obtained the same production per plant during the first experiment (0.79±0.05 kg plant^{-1} for Ctrl and 0.80±0.05 kg plant^{-1} for D75). On the other hand, plants under D50 did reduce their production (0.66±0.03 kg plant^{-1}). Likewise, during the second experiment there was a similar trend among treatments (0.74±0.17 kg plant $^{-1}$ for Ctrl and 0.725±0.031 kg plant $^{-1}$ for D75) whereas D50 presented the lowest yield (0.653±0.054 kg plant $^{-1}$). Jefferies (1995) has pointed out that yield reduction under drought stress is mainly due to a reduction in canopy expansion, which may delay tuber initiation and bulking.

Conclusions

Multifractal analysis of plant reflectance data enhanced the precision and earliness of diagnosis of drought stress compared to gas-exchange and relative water content measurements. The average difference for potato and grapevine was 4 days.

Multifractal analysis provided more reliable and stable results than splitting the spectrum into the main regions. Spectral vegetation indexes (SVI) were ineffective indicators for water stress in plants.

These findings show the advantage of using multifractal techniques to extract features from the complex signals generated by potato and grapevine plants under water stress. The challenge is to replicate these findings under commercial field conditions.

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