ASSESSING WATER STATUS IN WHEAT UNDER FIELD CONDITIONS USING LASER-INDUCED CHLOROPHYLL FLUORESCENCE AND HYPERSPECTRAL MEASUREMENTS

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ABSTRACT

Classical measurements for estimating water status in plants using oven drying or pressure chambers are tedious and time-consuming. In the field, changes in radiation conditions may further influence the measurements and thus require fast measurements. The possibility to detect drought stress in wheat with laser-induced chlorophyll fluorescence and hyperspectral measurements at canopy level under temperate field conditions was tested in this work. Three drought scenarios were established by using a removable rainout shelter. A laser-induced chlorophyll fluorescence sensor and a hyperspectral sensor were mounted on a metal carrier about 2 m above the canopy. Our results show that the canopy water content (%) was closely related to chlorophyll fluorescence at 690 nm, 730 nm and to the biomass index for all cultivars (R^2- values) with $R^2 \geq 0.82$***. A closer relationship between spectral measurements and canopy water content and canopy water mass was found than with leaf water potential. Selected spectral indices were significantly related to canopy water content and canopy water mass (R^2= 0.53*** – 0.96***). Two spectral indices and the fluorescence intensity at 690 nm were significantly related to the leaf water potential (R^2= 0.58* – 0.83** and $R^2 \geq 0.62$). Our data show that non-destructive methods may enable to assess water status information.

Keywords: maize, phenotyping, reflectance, spectrometer, water status, wheat.

INTRODUCTION

Drought is the most important limiting factor for crop production and it is becoming an increasingly severe problem in many regions of the. A rapid assessment of the plant water status is useful for irrigation management purposes and would also allow an efficient screening of large populations of plants as part of a high-throughput system to precisely evaluate the phenotype for breeding purposes (Schmidhalter, 2005a; Winterhalter *et al*., 2011a).

Whereas spectral relationships at various wavelengths have frequently been described for parameters like whole plant water content (Liu *et al*. 2004), less information is available regarding their ability to estimate the leaf water potential (LWP). Measuring LWP is desirable because it directly reflects the effects of drought, whereas plant (canopy) water content (CWC) can also be reduced by many other stresses or factors that decrease biomass (e.g., nutrient deficiency, soil aeration status, soil resistance or pathogens) and thus the CWC. Drought reduces plant water content, but it may also be decreased during plant development due to changes in the amount of structural tissues being formed. Given the potential advantages of LWP as a more direct indicator of the leaf water status and its attendant benefits for irrigation scheduling, simple, efficient methods to characterize LWP are highly desirable.

The spectral reflectance of wheat leaves has been reported to be closely related to changes in LWP under growth chamber conditions, with the best correlation of LWP to reflectance being found for the normalized difference vegetation index (NDVI; global $R^2 = 0.81$), but also at wavelengths of 1450 nm ($R^2 = 0.92$) (Ruthenkolk *et al.* 2001**)**. In addition, Gutierrez *et al*. (2010) found that the normalized water index $(R_{970} - R_{880})/ (R_{970} + R_{880})$ was significantly related to LWP of wheat ($R^2 > 0.6$-0.8) across a broad range of values (-20 to -40 bar).

Instead, proximal remote sensing systems depending on LICF show great promise in detecting water stress and nitrogen levels (N) in crops (Apostol et al., 2003; Zhang et al., 2005; Bredemeier and Schmidhalter, 2005; Thoren and Schmidhalter, 2009) due to the inherent competition between chlorophyll fluorescence and both photochemistry (PSI and PSII) and heat dissipation. Hence, any change in the yield of these two processes will lead to a corresponding change in the fluorescence yield (Lichtenthaler and Rinderle, 1988). This is true even in light of the fact that the intensity of the fluorescence emission is <3% of that of the absorbed light (Stober and Lichtenthaler, 1993); fluorescence emission remains a standard method for detecting plant stress (e.g., water deficit, temperature stress, nutrient deficiency, polluting agents, and attack by pathogens) (Stober et al., 1992). Fluorescence has successfully been used to detect water stress (Günther et al., 1994; Apostol et al., 2003; Bredemeier and Schmidhalter, 2005; Zhang et al., 2005) in plants. However, most of these studies were done at the leaf level and under controlled conditions, thereby limiting their practical application to field studies.

Surprisingly few studies have examined the relationship between water status (water content and leaf water potential) and chlorophyll fluorescence, the exceptions being Hsiao et al. (2004) and Schmuck et al. (1992). The former study, albeit under controlled conditions, revealed that the water content and water potential of vegetable plug seedlings were related to several measurements and indices of chlorophyll fluorescence at 720 nm. The latter study echoes these findings, indicating that both the variable chlorophyll fluorescence at 690 nm, and 730 nm are good indicators of the water content and water potential of maize.

However, spectral reflectance and LICF is also affected by many factors under field conditions. It depends on complex interactions between several internal and external factors. For instance, spectral reflectance is influenced not only by the plant water status, but also by leaf thickness (Ourcival *et al*. 1999), differences in leaf surface properties (Grant *et al*. 1993), soil background, and non-water stress related variation in leaf angle, canopy structure (Asner 1998), leaf area (Sims and Gamon 2003), canopy architecture, measuring angle, solar zenith and row spacing (Jackson and Huete 1991).

The aims of this study were (i) to study the stability of newly developed high throughput precision technique to detect water status in plants by measuring the leaf water potential, which can reflect the water status of plants in a short time scale, or the canopy water content and the canopy water mass which can reflect the water status of plants in the longer run under temperate environmental conditions; (ii) to estimate the most suitable spectral reflectance indices or LICF parameter which can be used for high throughput precision phenotyping or irrigation scheduling to detect the water status in wheat.

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MATERIALS AND METHODS

Experimental setup

The experiments were conducted under field conditions at the research station of the Chair of Plant Nutrition of the Technische Universität München in 2005 and 2008. Four cultivars of wheat (Ludwig, Ellvis, Empire, and Cubus) were sown on October 6, 2004, at a seeding rate of 300 seeds per m^2 and two cultivars (Cubus and Mulan) were sown mid of October, 2007. Residual soil nitrate levels of 42 kg NO_3-N ha^{-1} were detected by a quick-test procedure (Schmidhalter, 2005b). Liquid urea ammonium nitrate fertiliser was split into two portions of 80 kg N ha^{-1} in BBCH 20 and 40 kg N ha^{-1} in BBCH 30. Phosphorus and potassium supplies in the soil were adequate, requiring no further applications. The soil at the research station is characterised as 60% silt, 25% clay, and 15% sand with pH ($CaCl_2$) 6.2. The water holding capacity of the soil is high (250 mm down to 1.2 m depth).

The experiment was a two-factorial setup with different winter wheat cultivars (four in 2005 and two in 2007) and four water treatments (rainfed, irrigated control, early water stress and late water stress). To control the amounts of water applied to the plants, an automatic removable rain-out shelter platform was used together with spray irrigation. The experiment included 28 plots, each 4 m long and 1.8 m wide. Each regime was applied to two plots in 2005 and four plots in 2007 for each cultivar, except for the rainfed treatment, which had only a single replicate. First, the plants were grown under rainfed conditions and then, early stress (withholding water from begin of May to mid of June) and late water stress (withholding water from end of May 28 to begin of July) were applied.

Sensor measurements

For fluorescence measurements, a fluorescence sensor developed by Planto GmbH (Leipzig, Germany) connected to a portable computer (Thoren and Schmidhalter, 2009) was used and mounted on a self-moveable metal carrier. The sensor was mounted at a height of 3 m with a zenith angle of 45° above the plant canopy. The sensor used a pulsed laser beam (λ 640 nm, 3 mm in diameter) to induce chlorophyll fluorescence emission, which was measured at 690 nm (F690) and 730 nm (F730) 2000 times per second and averaged to one single value per second.

For spectral reflectance measurements, a passive reflectance sensor for measuring at wavelengths between 300 - 1100 nm connected with a portable computer and Geographical positioning system (GPS) was used. The sensor consists of one optics and an automatic reference white plate to measure subsequently canopy reflectance and sun reflectance in around 15 sec. The optics was positioned at a height of 2 m above the plants in the nadir direction. The aperture was 12° and the size of the field of view was 0.42 m^2. With the readings from the spectrometer unit the canopy reflectance was calculated and corrected with a calibration factor estimated from a reference white standard.

In this study, we calculated and tested reflectance indices as $(R_{780} - R_{670})/(R_{780} + R_{670})$, $(R_{410} - R_{780})/(R_{410} + R_{780})$, $(R_{490} - R_{780})/(R_{490} + R_{780})$, $(R_{510} - R_{780})/(R_{510} + R_{780})$ and R_{600}/R_{780}.

Sensor measurements were taken several times during the growing period from stem elongation until ripening (Table 1). The different sensor values were related to classically-determined values of CWC, canopy water mass (CWM) LWP.

To measure LWP, a pressure chamber (PMS Instrument, Corvallis, OR, USA) was used (Schmidhalter et al., 1998). Pressures were read within one minute of leaf removal from the plant and LWP was determined as the average value from six separate readings of fully-expanded flag leaves.

Growth stage	Date	Sensor mesurements Time of day	Physiological parameters measured
Stem elongation	May 25, 2005	12:45 – 13:19	Leaf water potential
Stem elongation and inflorescence emergency	June 1, 2005	12:46 – 13:40	Leaf water potential
Inflorescence emergence and anthesis	June 8, 2005	14:11 – 15:04	Canopy water content, canopy water mass
Anthesis and milk development	June 21, 2005	13:41 – 14:14	Leaf water potential, canopy water content, canopy water mass
Anthesis and milk development	June 23, 2005	13:40 – 14:15	Leaf water potential
Milk development and ripening	July 4, 2005	10:42 – 11:10	Leaf water potential, canopy water content, canopy water mass
Heading	June 2, 2008	13:38 - 14:30	Leaf water potential, canopy water content, and canopy water mass
Heading and flowering	June 10, 2008	13:25 - 14:20	Leaf water potential
Milk and dough	July 2, 2008	14:56 - 15:50	Leaf water potential, canopy water content, and canopy water mass

To determine aboveground aerial biomass, plants were cut above the ground within a 0.22 m^2 area (A). A representative subsample was then dried in an oven (105°C) until there was no change in dry weight (DW). Canopy water content (in

Table 1. Sensor measurements and physiological parameters at different growth stages, dates and times.

%) was calculated as CWC = (FW – DW)/FW * 100. In addition, canopy water mass (in g/m^2) was calculated as CWM = (FW – DW)/area.

RESULTS

The relationship between canopy water content and spectral indices of two wheat cultivars subjected to four watering regimes

The coefficients of determination for the relationships between five spectral indices and canopy water content of Cubus and Mulan at the individual measurements and combining data for each cultivar are given in Table 2. As well as, the relationships between four indices and canopy water content of Cubus at the individual measurements and the combined data for each cultivar are shown in Figure 1. The results demonstrated that all spectral indices were significantly related with canopy water content of Cubus and Mulan at individual measurements and across all measurements for two harvest times, except one measurement day for Mulan at June 2 ($R^2 \geq 0.75$; $p \leq 0.001$). Positive relationships between spectral indices and canopy water content of two cultivars, except R_{600}/R_{780} were found. The highest coefficient of determination could be shown between $(R_{510} - R_{780})/(R_{510} + R_{780})$ and canopy water content of Cubus ($R^2 = 0.96$; $p \leq 0.001$) with the combined data in Figure 1.

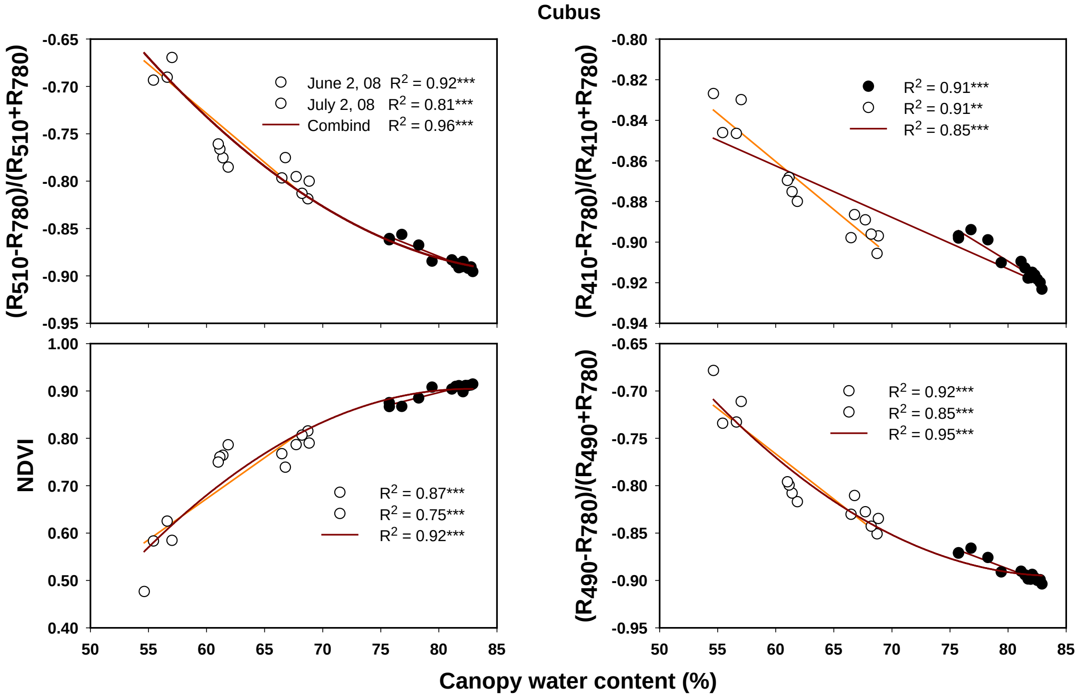

Figure 1: The relationship between canopy water content and four spectral indices for Cubus subjected to four watering regimes. Data were pooled across four watering regimes. Measurements were taken at two dates and the regressions over all were fitted.

Table 2: Coefficients of determination of the relationship between canopy water content and spectral indices for two winter wheat cultivars subjected to four watering regimes at two dates.

Cultivas	Dates	NDVI	$(R_{510}-R_{780})/(R_{510}+R_{780})$	$(R_{490}-R_{780})/(R_{490}+R_{780})$	$(R_{410}-R_{780})/(R_{410}+R_{780})$	R_{600}/R_{780}
Cubus	June 2, 08	0.87***	0.92***	0.92***	0.91***	0.87***
	July 2, 08	0.75***	0.81***	0.85***	0.91***	0.76***
	combined data	0.92***	0.96***	0.95***	0.85***	0.93***
Mulan	June 2, 08	0.11	0.05	0.08	0.01	0.02
	July 2, 08	0.83***	0.82***	0.83***	0.84***	0.81***
	combined data	0.94***	0.93***	091***	0.50***	0.94***
All cultivars	combined data	0.93***	0.89***	0.89***	0.82***	0.92***

*, **, *** Statistically significant at $P \leq 0.05$; $P \leq 0.01$ and $P \leq 0.001$, respectively

The relationship between canopy water mass and spectral indices of wheat cultivars subjected to four watering regimes

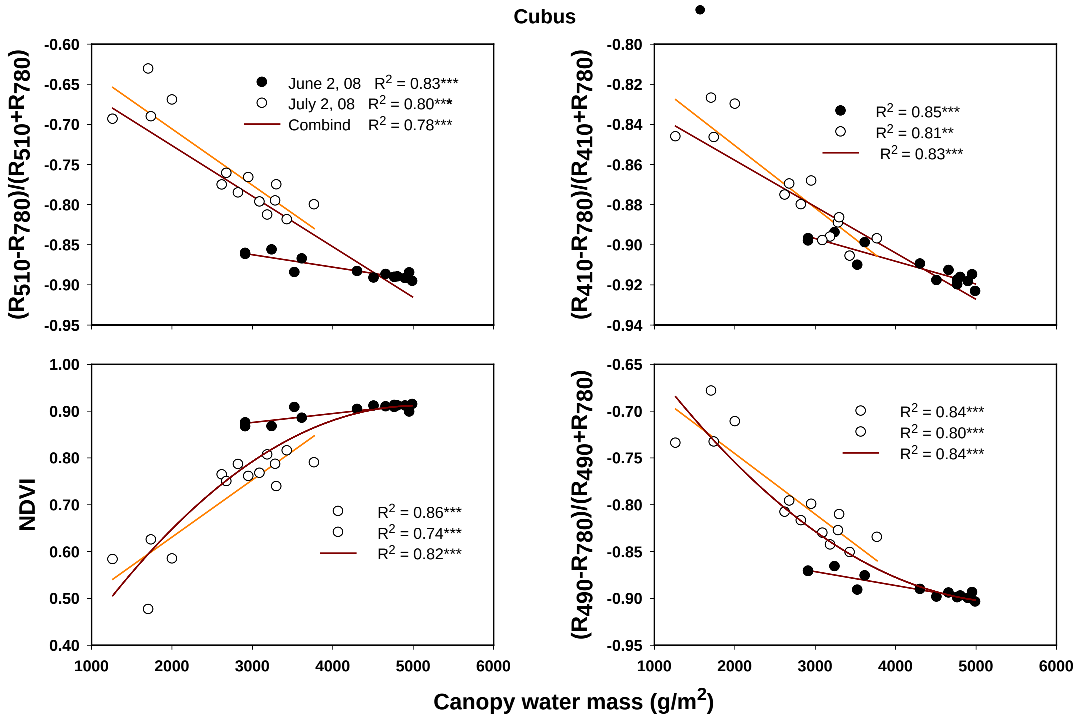

Figure 2: The relationship between canopy water mass and four spectral indices for Cubus subjected to four watering regimes. Data were pooled across four watering regimes. Measurements were taken at two dates and the regressions over all were fitted.

Table 3: Coefficients of determination of the relationship between canopy water mass and spectral indices for two winter wheat cultivars subjected to four watering regimes at two dates.

Cultivars	Dates	NDVI	$(R_{510}-R_{780})/(R_{510}+R_{780})$	$(R_{490}-R_{780})/(R_{490}+R_{780})$	$(R_{410}-R_{780})/(R_{410}+R_{780})$	R_{600}/R_{780}
Cubus	June 2, 08	0.86***	0.83***	0.84***	0.85***	0.76***
	July 2, 08	0.74***	0.80***	0.80***	0.81***	0.75***
	combined data	0.82***	0.78***	0.84***	0.83***	0.80***
Mulan						
	June 2, 08	0.14	0.07	0.11	0.01	0.01
	July 2, 08	0.85***	0.86***	0.87***	0.88***	0.85***
	combined data	0.74***	0.76***	0.81***	0.53***	0.73***
All cultivars	combined data	0.78***	0.78***	0.79***	0.79***	0.76***

*, **, *** Statistically significant at $P \leq 0.05$; $P \leq 0.01$ and $P \leq 0.001$, respectively

Close relationships between all spectral indices and canopy water mass of Cubus and Mulan were found at the individual measurements and across all measurements for two harvest dates, except one measurement day for Mulan at June 2 ($R^2 \geq 0.53$; $P \leq 0.001$) shown in Figure 2 and Table 3. The highest coefficient of determination was recorded between $(R_{410} - R_{780})/(R_{410} + R_{780})$ and canopy water mass for individual measurements of Mulan at July 2 ($R^2 = 0.88$; $P \leq 0.001$).

The relationship between leaf water potential and spectral indices of two wheat cultivars subjected to four watering regimes

Leaf water potential showed good relationships with mainly two spectral indices $(R_{410} - R_{780})/(R_{410} + R_{780})$ and $(R_{490} - R_{780})/(R_{490} + R_{780})$ (Fig. 3). However, the relationships were affected by the date (ambient temperature and radiation condition) and it was not possible to fit one single regression curve across all measurements. Significant relationships between leaf water potential and two spectral indices for Cubus and Mulan varied between $R^2 = 0.58$* to 83**. The two spectral indices were negatively related to the leaf water potential.

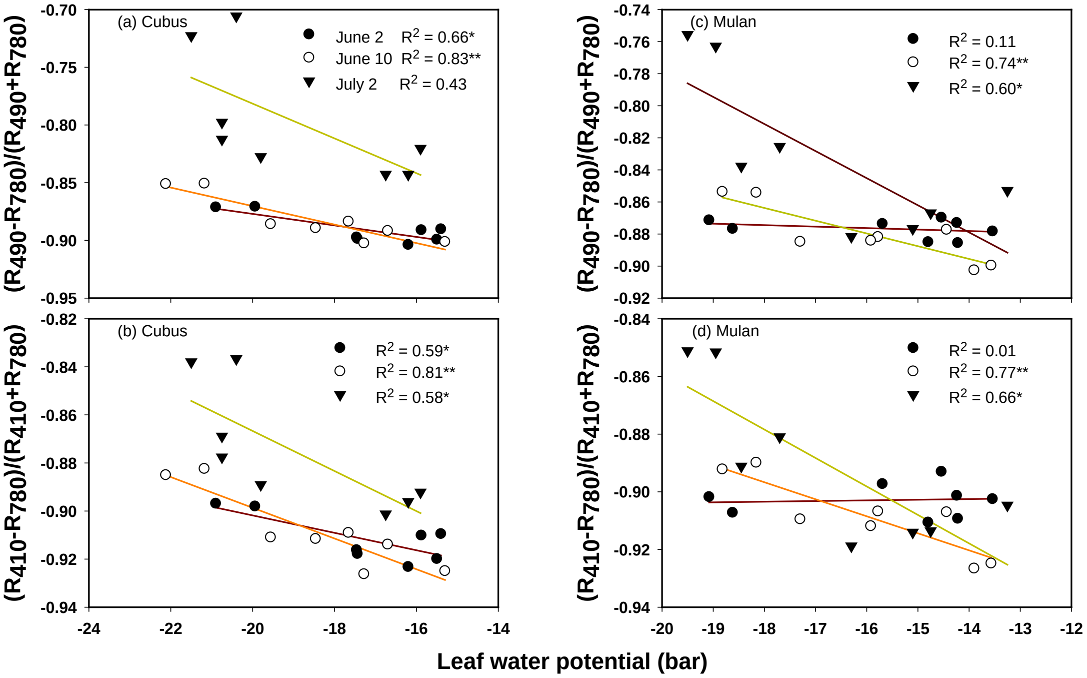

Figure 3: The relationship between leaf water potential and two spectral indices of (a & b) Cubus and (c & d) Mulan subjected to four watering regimes.

The relationship between canopy water content and each of the fluorescence intensity at 690 and 730 nm

The relationship between CWC and several fluorescence parameters were determined across three biomass harvests from BBCH 57 to 90 (Fig. 4). The fluorescence intensity at 690 nm and 730 nm increased with increasing CWC. The fluorescence intensity at 690 and 730 nm behaved differently among all the cultivars, although three of them (Empire, Ellvis, and Cubus) behaved similarly.

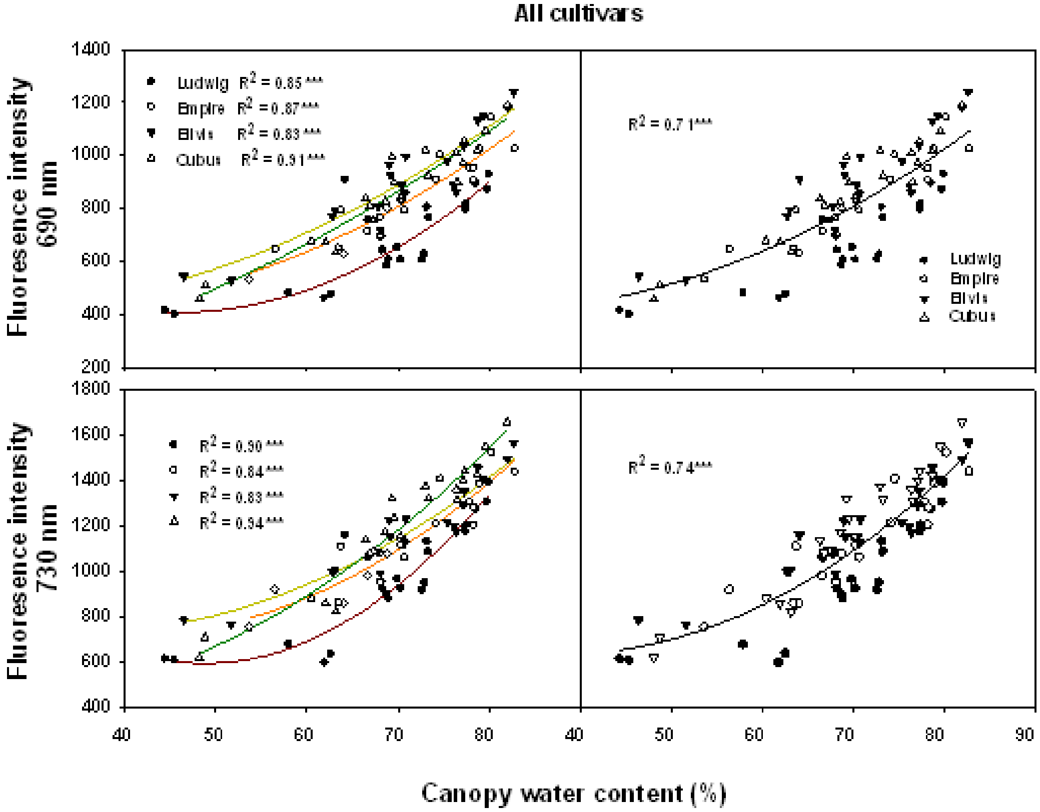

Figure 4: The relationship between canopy water content and fluorescence intensity at 690 and 730 nm. Data were pooled across four watering regimes and all measurements dates and presented for each individual cultivar (left) and for all cultivars together (right).

The relationship between canopy water mass and each of fluorescence intensity at 690 and 730 nm

The relationship between CWM and several fluorescence parameter were determined across three biomass harvests from BBCH 57 to 90 (Fig. 5).CWM shows a linear relationship with all fluorescence parameters (when significant) (Fig.5). Except Empire, the results largely mirrored those for CWC. Significant positive relationships were found between CWM and chlorophyll fluorescence at each of 690 nm and 730 nm, both for each individual cultivar and across all cultivars pooled together.

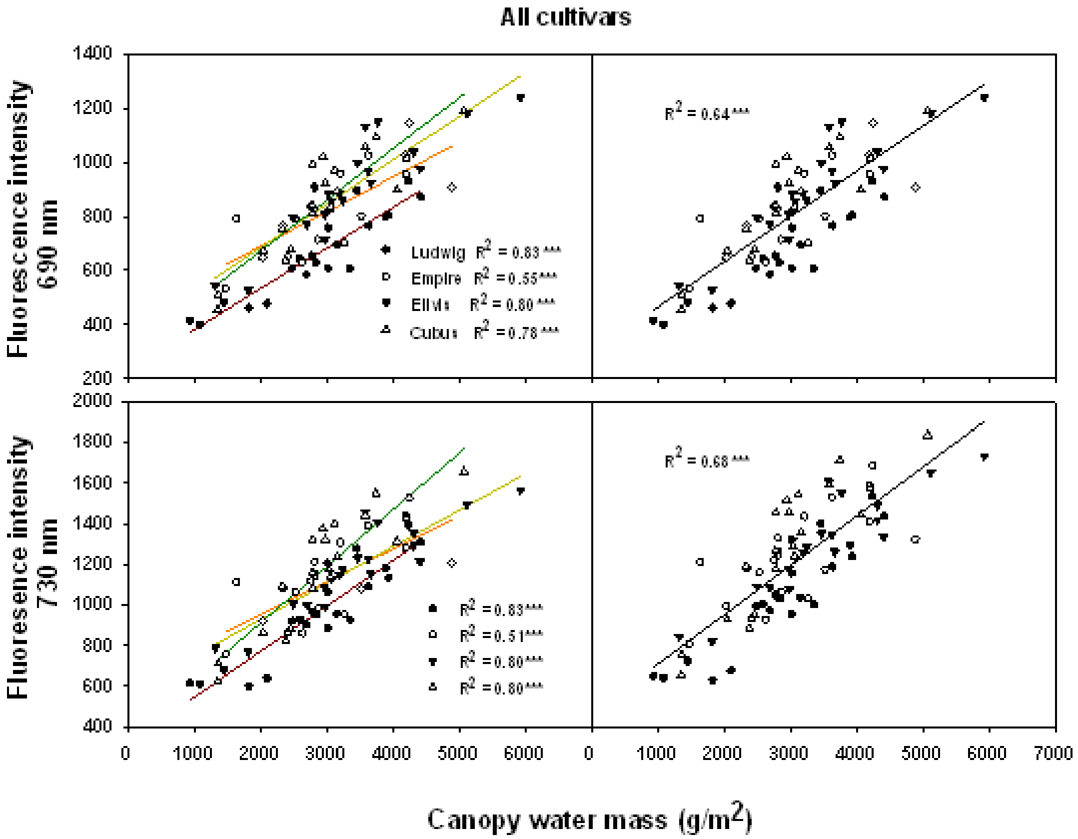

Figure 5: The relationship between canopy water mass and fluorescence intensity at 690 and 730 nm. Data were pooled across four watering regimes and all measurements dates and presented for each individual cultivar (left) and for all cultivars together (right).

The relationships between leaf water potential (bar) and each of fluorescence intensity at 690 and 730 nm

Linear relationships between leaf water potential with fluorescence parameters are shown in Fig. 6. Slightly lower values were observed for the relationship between leaf water potential (bar) and fluorescence intensity at 690 nm for each cultivar.

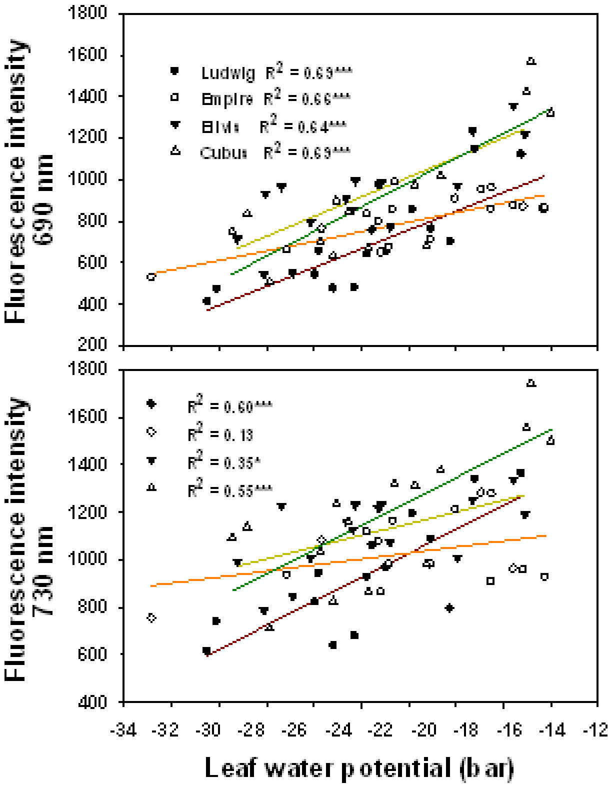

Figure 6: The relationship between leaf water potential and fluorescence intensity at 690 and 730 nm. Data were pooled across four watering regimes and all measurements dates and presented for each individual cultivar.

DISCUSSION AND CONCLUSIONS

In this study high-throughput sensors were examined to access short term drought stress and long term drought stress (CWC, CWP). LWP is the best short term water stress predictor. Under mild climate conditions the plants become fully rehydrated overnight and so the plants start without stress every morning. In this experiment sensor values correlated better with CWC than with LWP. These results are in contrast to Elsayed et al. (2011), where the spectral measurements correlated best with LWP. But in this latter study only light conditions were changed, all the other environmental effects were eliminated. This demonstrates that sensors may be well suited to detect short term drought stress under such conditions, but this may be more difficult to achieve under field conditions with a single measurement because plants with lower LWP under field conditions show usually also some long term drought stress effect like changes in biomass, leaf structure or in canopy architecture. Influence on sensor values described for leaf thickness (Ourcival *et al*. 1999), differences in leaf surface properties (Grant *et al*. 1993), soil background and non-water stress related variation in leaf angle, canopy structure (Asner 1998), leaf area (Sims and Gamon 2003), canopy architecture, measuring angle, solar zenith and row spacing (Jackson and Huete 1991). Such effects may have influenced the measurements in these experiments and resulted in best correlations to CWC for all sensors.

Our results show the closest relationships of the fluorescence intensity at 690 and 730 nm to CWC. Hsiao et al. (2004) showed that the water content of *Brassica oleracea* seedlings was related to F_m/F_s ($R^2 = 0.91$) and R_{Fd} ($R^2 = 0.98$) with a fluorescence image system at 720 nm under controlled conditions (where F_m, F_s, and R_{Fd} are the maximum, steady state, and variable chlorophyll fluorescences, respectively). In contrast, Schmuck et al. (1992) found that the variable chlorophyll fluorescence R_{Fd} in the 690 nm and 730 nm regions, used to detect changes in water content, depended on species. In wheat plants, the water stress treatment had no influence on the R_{Fd} values in either wavelength region, whereas a clear trend was observed in maize plants.

The fluorescence intensity decreased with increasing canopy water content and canopy water mass, as well as with decreases in leaf water potential; these results agree with findings by Theisen (1988), who reported that when drought stress became visible, strongly reduced fluorescence intensities at 685 nm and 730 nm were observed. Günther et al. (1994) found that the fluorescence intensities at 685 nm and 730 nm of stressed oak tree branches (*Quercus pubescens*) were strongly reduced in comparison to those of the healthy branches. In addition, Apostol et al. (2003) found that the emission of the fluorescence intensity of plants with 25% and 50% leaf water deficit was lower than for irrigated plants. In contrast, Lichtenthaler and Rinderle (1988) found that the fluorescence intensity increased with increasing water stress.

The inverse relationship between the stress level and the fluorescence index points to reduced photosynthesis of photosystem II because of closed stomata. Hence, the non-photochemical quenching is increased to prevent PS II photooxidation (Baker, 2008), and this leads to an increase of the yield of heating and decreases the fluorescence intensity by dissipating energy as heat. The competition between chlorophyll fluorescence and both photochemistry (PS I and PS II) and heat dissipation will lead to a corresponding change in the fluorescence yield.

Our assessment of reflectance indices as a method to measure the water status in wheat demonstrated that the selected five indices such as $(R_{410} - R_{780})/(R_{410} + R_{780})$, $(R_{510} - R_{780})/(R_{510} + R_{780})$, $(R_{490} - R_{780})/(R_{490} + R_{780})$, NDVI, and R_{600}/R_{780} are apparently useful for describing water status of wheat canopies by using canopy water content and canopy water mass regardless of growth stage at individual measurements and across all measurements. This shows, that indices are not only useful for irrigation scheduling when data are combined across all measurements in one year, but might also be useful for evaluating the phenotype for breeding purposes with individual measurements.

Some studies such as Liu et al. (2004) found that there were no relationships between WI, NDWI, red edge position, and red edge position with plant water content of wheat for most of the individual measurements. Our results are in an agreement with Graeff and Claupein (2007), who found that the wavelength ranges 510 - 780 nm ($R^2 = 0.79^{***}$), 540 - 780 nm ($R^2 = 0.79^{***}$), are most suitable to describe the water content in wheat. The increase in reflectance at these spectral regions may be attributed to a compound effect of a change in the internal leaf structure and to a change in light absorption by photosynthetic pigments due to altered photosynthetic activity. In additional, Winterhalter et al.

(2011b) found that the index R_{850}/R_{725} was significantly related to canopy water mass of maize (0.72***).

The index NDVI is associated with the leaf area index (LAI) according to Aparicio et al. (2002) and with both chlorophyll content and LAI according to Eitel et al. (2008). In this experiment, NDVI is strongly related to canopy water content and canopy water mass at individual measurements and across all measurements for each cultivar and all cultivars. But the relation may depend on the LAI also.

The leaf water potential undergoes rapid temporal fluctuation as a function of environmental conditions (Jensen et al., 1990). The results show that changes in leaf water potential can reliably be detected by using spectral measurements under field condition. LWP was related to two spectral indices $(R_{410} - R_{780})/(R_{410} + R_{780})$, and $(R_{490} - R_{780})/(R_{490} + R_{780})$ at most of the individual measurements. Global spectral relationships measuring LWP probably cannot be established across plant development. Even so, spectrometric measurements supplemented by a reduced calibration data set from pressure chamber measurements might still prove to be a fast and accurate method for screening large numbers of cultivars

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